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Isolation of casein protein fractions

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Abstract: The fractions of caseins (Ŭs-, ɓ- and ə-) are widely used in food applications due to their physiochemical properties as well as their bio- and techno-functional properties. Especially, ɓ-casein fraction is a precursor having a variety of bioactive peptides such as antihypertensive and opioid. Different methods have been established in isolating and purifying the casein fractions, particularly on ɓ-casein isolation. However, the obtained yield for each fraction still requires further improvement. The aim of this study was to increase the yield and purity of the fractions by constructing a set of experiments on technical scale and determining the main process parameters. Based on our previous studies on laboratory scale, a method for the technical scale isolation of casein fractions from micellar casein powder was developed. A comparable yield and purity values for ɓ-casein were obtained.

1. Introduction

Bovine milk constitutes two main proteins, casein and serum (whey) proteins. Casein is the major fraction and constitutes about 80% of the total protein. Bovine casein consists of four individual types of casein molecules: Ŭs1-(~38%), Ŭs2-(~10%), ɓ-(~34%), and ə-caseins (~15%) *[1]*. Caseins are widely used in food and non-food applications. They have various important techno-functional properties such as solubility, emulsification, foam formation and stabilization, water-binding, gelation, heat and acid stability *[2-4]*. They also play an important role in non-food applications such as the production of plastic materials *[5]*, textile fibers *[6]* and glues *[7]*.

Recently, interest in pure casein fractions, especially ɓ-casein, has grown. ɓ-casein has good emulsifying and foam stabilizing properties because of its amphiphilic structure *[8]*. Ŭs-casein has ends with hydrophobic regions, whereas in the middle a hydrophilic region *[9]*. Due to this structural establishment, Ŭs-casein can also be used as structure formers such as stabilizers. Furthermore, Ŭs-casein is able to develop mixed micellar structures, in which active substances can be entrapped *[10]*. ə-casein is a source of physiologically active compounds, caseinomacropeptides, which occurs during hydrolysis of ə-casein with chymosin *[11]*. Caseinomacropeptides contain no

phenylalanine in their amino acid sequence, making tehm suitable as a protein source for phenylketonuria patients *[12]*.

Since the beginning of 1950s, several methods, either at small or large scale, have been developed for the isolation of casein fractions, especially ɓ-casein *[13]*. Some of the pioneering work involves ion-exchange chromatography *[14]*, electrophoresis *[15]*, selective precipitation *[16]*, gel chromatography *[17]* and liquid chromatography *[18]*. The processes for the isolation of the fractions use mainly selective solubility and precipitation as well as cross flow membrane filtration (microfiltration and ultrafiltration) *[13]*. Considerable progress has been made in isolating and purifying ɓ-casein. By applying a small-scale separation with a process volume of 5 L, a ɓ-casein purity of 90 %, a yield of 10 %, and a corresponding recovery of 22 % were achieved *[19]*. Technically fractionated caseins, to be applied in infant-formula, sport drinks or clinical nutrition are of special economic interest. Although numerous methods had been proposed, their technical recovery with a high purity and yield has not been realized. This study, therefore, aimed to further investigate the isolation method of casein fractions and to improve the isolation method.

2. Materials and Methods

2.1. Production of micellar casein

The micellar casein powder was produced using the method presented by Kersten *[20]*. In the method a combination of microfiltration and diafiltration was used. The skim milk (fat content: < 0.1%) was concentrated using microfiltration (membrane: Membralox 3272 6C, 0.1 ɛm, Pall Corporation, Bazet, France) at a constant temperature of 50 °C. Afterwards, a diafiltration process was applied using ultrafiltration permeate, which was produced from sweet whey powder (Bayolan PT, BMI, Landshut, Germany). The obtained concentrate was spray dried and the micellar casein powder was used for the fractionation experiments.

2.2. Isolation of the casein fractions

The methods of Law and Leaver *[21]* and Post and Hinrichs *[19]* were optimized to obtain the food-grade ɓ-casein as well as the other two casein fractions, Ǔs- and ə-casein. The calcium sensitive fractions, Ǔs- and ɓ-casein, were precipitated by calcium chloride (50-100 mM) and then separated by centrifugation (at 3000 *g* for 10 min at 20 °C). The main steps of the fractionation process are illustrated in Figure 1. The influence of the two main process parameters, $CaCl_2$ concentration and pH value, on the isolation method were investigated in detail in a range varying from 10 to 50 $CaCl_2$ mmol/L and from 4.0 to 4.6, respectively.

2.3. Analysis

For calculating the total protein content the nitrogen content was determined according to the Dumas method (Leco FP-528, Leco Instrumente GmbH, München-Gladbach, Germany) and was multiplied by conversion factor of 6.38 for milk and milk products *[22]*. The calcium content was determined according to VDLUFA IV C 10.6.8 *[22]* by complexometric titration. While the dry matter was analyzed according to VDLUFA IV C 35.6 *[22]*. The lactose content was determined using infrared measurements according to IDF Standard 141C:2000 (LactoScope FTIR Advanced FTA-3.0, Delta Instruments, Drachten, Nederland) *[23]*. Fat content was determined using the gravimetric method according to Weibull-Stoldt (VDLUFA VI C 15.2.3) *[22]*.

Selectively isolated casein fractions were analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC) using an Agilent Technologies 1200 System and separated on a PLRP-

S analytical column (150 x 4.6 mm, 300 A, 8 εm Agilent Technologies) under gradient conditions according to Post *[24]*.

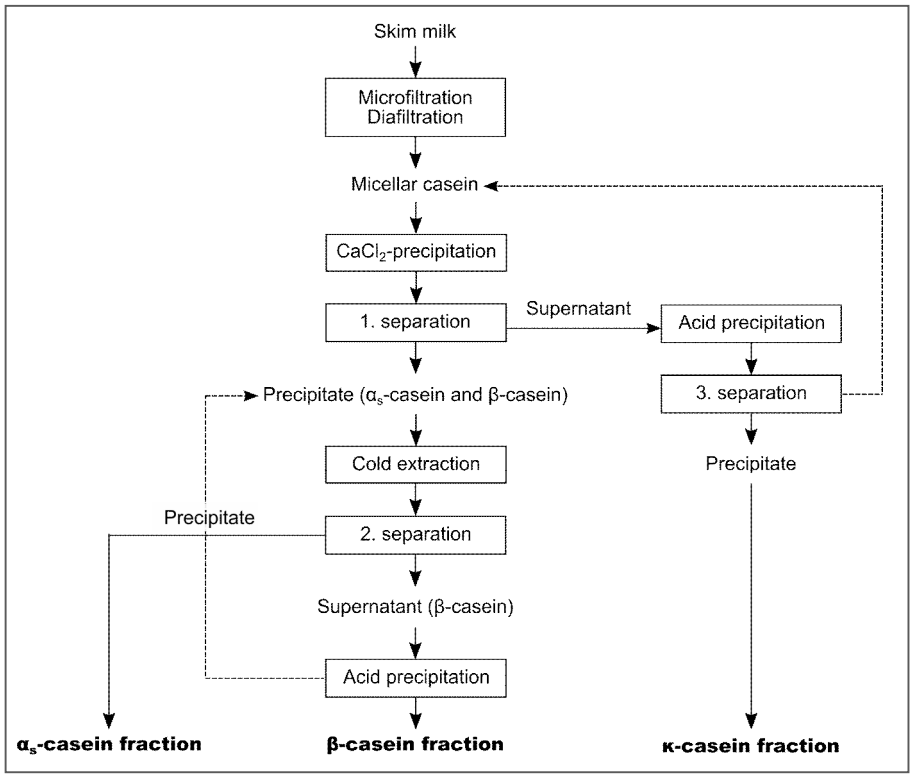

Figure 1: The main steps for the technical recovery of different casein fractions.

2.4. Calculations

The purity of a single casein fraction ($P_{n\text{-}CN}$; in %) was defined as the ratio of the single casein fraction content ($c_{n\text{-}CN,\ fraction}$) and the total casein concentration in total ($c_{total\ casein}$) (Eq. 1).

$$P_{n\text{-}CN} = (c_{n\text{-}CN,\ fraction} / c_{total\ casein}) * 100 \qquad (1)$$

The yield ($Y_{n\text{-}CN}$; in %) of each casein fraction is calculated using Eq. 2:

$$Y_{n\text{-}CN} = (m_{total\ CN} * P_{n\text{-}CN}) / (m_{MCN} * c_{total\ protein,\ MCN}) * 100 \qquad (2)$$

where $m_{total,CN}$ refers to the mass of the obtained n-casein fraction, m_{MCN} to the initial mass of micellar casein (MCN) powder used for the isolation, while $c_{total\ protein,\ MCN}$ refers to the calculated protein content of the micellar casein using the measured the nitrogen content.

The recovery ($R_{n\text{-}CN}$) of each casein fraction is calculated according to

$$R_{n\text{-}CN} = (Y_{n\text{-}CN,\ fraction} / c_{n\text{-}CN,\ MCN}) * 100 \qquad (3)$$

where $c_{n\text{-}CN,\ MCN}$ was defined as the analyzed n-casein concentration of the micellar casein powder using reversed-phase high performance liquid chromatography.

3. Results and Discussion

The chemical composition of the micellar casein powder is given in Table 1. The obtained micellar casein powder is almost free from whey proteins (0.2%, w/w) and contained the three casein fractions (Ŭs-, ɓ-, and ə-casein). The casein to total protein ratio in the powder was calculated to be 91%. The obtained chemical composition of the micellar casein powder is similar when compared to the previously obtained powder composition *[24]*.

Table 1: Chemical composition of micellar casein powder (mean of triplicate analysis).

Main component	Composition (%, w/w)
Dry matter	96.6 ± 0.2
Calcium	2.08 ± 0.04
Lactose	18.6 ± 0.2
Fat	2.4 ± 0.2
Casein/Total protein	91.1
Whey protein	0.2 ± 0.1

Skim milk is used for the extraction of casein. This can be done by applying a purely physical process or a combination of physical and biochemical processes *[13]*. Examples of the applied methods are namely: (i) precipitation by rennet (rennet-casein) *[25]*, by mineral acids such as hydrochloric or sulphuric acid (mineral acid-casein) *[16]* or using a lactic starter (lactic acid-casein) *[15]* and (ii) separation using a membrane filtration process such as microfiltration *[26]*. In this study, for the production of casein, a cross-flow membrane filtration process was used to obtain micellar casein concentrate. In comparison to other chemical and thermal processes applied for the separation of the individual fractions *[13]*, no modification of the casein micelles occurs and the functional characteristics of the casein protein does not change *[27]*. Therefore, caseins obtained by using this method can be considered as ñnativeò.

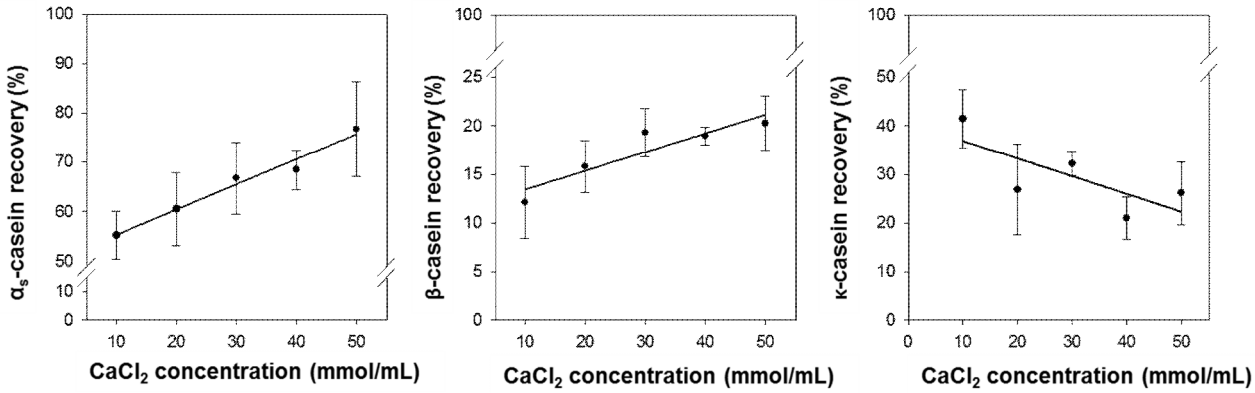

Figure 2: Recovery values of ɓ-, Ŭs-, and ə-casein fractions depending on the $CaCl_2$ concentration used for the precipitation of ŬS- and ɓ-caseins in Figure 1.

For the fractionation of the caseins, the calcium sensitive ŬS- and ɓ-caseins were precipitated by addition of $CaCl_2$-solution. The final calcium concentration were varied in the range of between 10 and 50 $CaCl_2$ mmol/L in order to investigate the influence of the calcium concentration on the isolation method. The dependency of each fraction to the applied calcium concentration in the fractionation process are illustrated in Figure 2. Variation of the $CaCl_2$ concentration had an

influence on the purity and recovery of any casein fraction. Similarly, the effect of the pH on the isolation of Ǔs- and ɓ-casein fractions can be depicted in Figure 3.

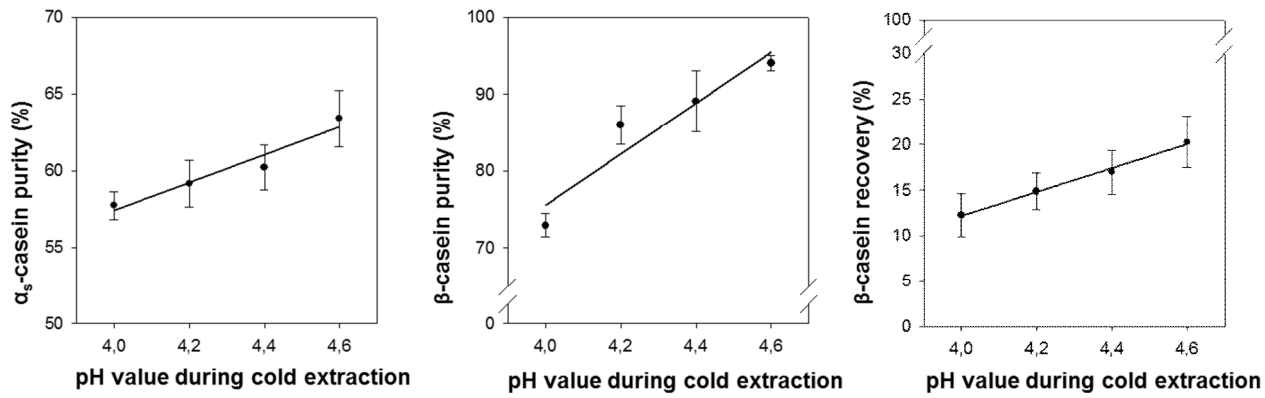

Figure 3: Purity values of Ǔs- and ɓ-casein and recovery of ɓ-casein fractions depending different pH values used in the cold extraction.

Figure 4 shows the example chromatographic profiles obtained in this study. The chromatographic profiles of Ǔs-, ɓ- and ə-casein obtained from fractionation of casein are illustrated together with the profile of micellar casein. A high purity achieving a value of 90% could be obtained for the ɓ-casein fraction at a large process scale. The obtained purity value was in close agreement with the results previously reported at small process scales *[19, 24, 28]*. Using the method proposed in Figure 1, a higher yield was achieved for the ɓ-casein fraction.

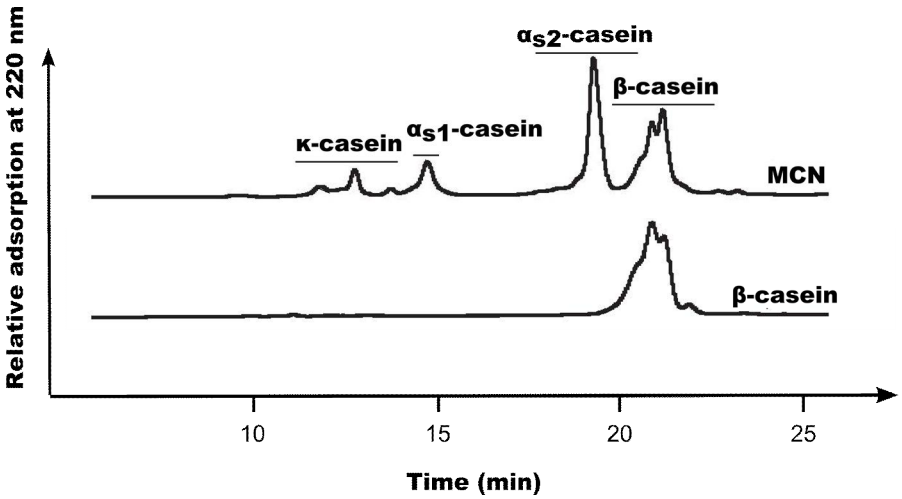

Figure 4: Chromatographic profiles of micellar casein (MCN) and ɓ-casein obtained from fractionation process according to the method illustrated in Figure 1.

4. Conclusions

Isolation of individual casein fraction is of growing interest due to their multifunctional applications and clean-label status. Although various isolation and purification methods of casein fractions have been reported, there is still a need of improvement for their isolation, especially on a technical scale. Using micellar casein obtained with the help of a membrane filtration separation process, highly

purified ɓ-casein fractions can be gained at a technical scale using the proposed method outlined in this study. The process enables achieving higher yields for ɓ-casein fractions. This process leads to highly enriched soluble Ŭs- and ə-casein fractions. The main two process parameters, the $CaCl_2$ concentration applied for the precipitation and the pH value used during cold extraction, have an influence on the isolation of the individual fractions. Further work is in progress to optimize the two fractions of Ŭs- and ə-casein.

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