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Research Paper / Araştırma Makalesi

Production and Physicochemical Properties of Casein-Based Adhesives

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ABSTRACT

In this study, different adhesives manufactured by using various casein powders (micellar casein, α_S-, β- and κ-casein fractions, sodium caseinate and calcium caseinate) were investigated for their properties and potential application in the food industry. Casein-based adhesives using different sources of caseins were produced and the differences in their adhesive strength were determined. For the isolation of casein fractions, the methods of selective solubility, precipitation and a decanter centrifuge (for separation of precipitated casein and supernatant) were used. Achieved purities of α_S-, β- and κ-casein fractions were higher than 25, 91 and 54%, respectively. Results showed that the type of casein raw material used in the production of adhesives had an influence on the adhesive properties and the highest adhesive strength was achieved with the enriched α_S-casein fraction and micellar casein.

Keywords: Casein, Adhesive, Food industry, Separation process

Kazein Bazlı Yapıştırıcıların Üretimi ve Fizikokimyasal Özellikleri

ÖZ

Bu çalışmada, çeşitli kazein tozları (misel kazein, α_S-, β- ve κ-kazein fraksiyonları, sodyum kazeinat ve kalsiyum kazeinat) kullanılarak üretilen farklı yapıştırıcıların özellikleri ve gıda endüstrisinde kullanım olanağı araştırılmıştır. Farklı kazein kaynakları kullanılarak kazein bazlı yapıştırıcılar üretilmiş ve yapışkan mukavemetlerindeki farklılıkları belirlenmiştir. Kazein fraksiyonlarının izolasyonu için seçici çözünürlük, çökeltme ve dekanter santrifüj (çöktürülmüş kazeinin ve süpernatantın ayrılması için) yöntemleri kullanılmıştır. α_S-, β- ve κ-kazein fraksiyonları için elde edilen saflıklar sırasıyla %25, 91 ve 54'ten büyük bulunmuştur. Sonuçlar, yapıştırıcıların üretiminde kullanılan kazein hammaddesinin türünün yapıştırıcı özellikleri üzerinde etkili olduğunu ve en yüksek yapışkan gücünün zenginleştirilmiş α_S-kazein fraksiyonu ve misel kazein ile elde edildiğini göstermiştir.

Anahtar Kelimeler: Kazein, Yapışkanlık, Gıda endüstrisi, Ayırma işlemi

INTRODUCTION

Caseins are the major proteins in milk and they consist of different polypeptide chains. They are negatively charged aggregates, also known as casein micelle, which are suspended in milk. Bovine milk contains about 2.8% (w/w) casein [1]. The natural structure of casein micelle is composed of individual casein fractions, which are α_{S1}-, α_{S2}-, β-, and κ-casein. These fractions differ in their amino acid sequence and number, and they have different features. Casein adhesives are environmentally friendly adhesives [2] based on natural protein [3]. They are made from the cow's milk protein casein [3]. For the isolation of casein factions, the raw material of casein is required to be isolated from milk as the very first step, which is most commonly done by microfiltration of skim milk [4, 5] (Figure 1). Caseinates are made by adding a salt such as sodium hydroxide (NaOH) or calcium hydroxide ($Ca(OH)_2$) to acid casein, which obtained after precipitation of skim milk.

The use of casein glues dates back to ancient Egypt [6]. But casein glues were also used by Greek, Roman and Chinese craftsmen [7]. There is a recipe from the 11th or 12th century by the monk Theophilus for the production of a waterproof glue from cheese [7] or the casein contained in it. Casein glues have been used for thousands of years, although their detection is difficult because they are degraded by microorganisms [3]. Their large-scale production began in the 19th century in Germany and Switzerland [8].

The areas of application of casein adhesives are diverse. They are mainly used in the wood industry and as labelling adhesives in the beverage industry [2]. In addition, casein adhesives were used as an adhesive for aircraft propellers during the First World War [9], as well as for the production of water-resistant plywood for aircraft [7].

For the technical use of casein adhesives, their productions are said to be inexpensive as well as having a long service life [8]. When used as a labelling adhesive in the beverage industry, casein adhesives [8] are also said to have high ice water resistance [2] as well as a fast label removal time [10]. Casein adhesives produced in the industry typically consist of the ingredients water, casein, calcium hydroxide [11], borax [12], urea [13] and alkalis such as sodium hydroxide [7]. The addition of the chemical components leads to a solubility of the casein, a water resistance of the casein adhesive [11], the adjustment of the pH value or the flowability [13].

The purpose of this study was to isolate micellar casein and casein fractions using raw milk and to produce casein based adhesive using different sources of casein as well as to determine the structure or type of casein that is responsible for the adhesive strength of the casein-based adhesive. In addition, it was aimed that a literature survey for the analytical methods for the characterization of the adhesives was conducted and the area of application of the casein adhesives was discussed.

MATERIALS and METHODS

Production of Micellar Casein Powder and Individual Casein Fractions (α_s-, β- and κ-casein)

The manufacture of the model substrate, micellar casein, was performed using raw milk. Raw bovine milk was supplied from the research station Meiereihof (University of Hohenheim, Stuttgart, Germany). After pasteurisation (74ºC, 30 s) and separation of fat at 55ºC (SA 10; Frautech S.r.l., Schio, Italy), the pasteurised skim milk was standardised to a protein content of 3.4%, as described by Koerzendoerfer, Noebel, and Hinrichs [14]. Micellar casein was separated from the pasteurized skim milk using a cross-flow membrane filtration unit (Model TFF; Pall GmbH, Dreieich, Germany) equipped with multichannel gradient of permeability (GP) ceramic membranes (7P19-40 GP Membralox Module, 99.7% a-alumina; Pall Exekia, Bazet, France) with 4-mm-diameter channels, a cut-off of 0.1 mm and an effective filtration area of 1.69 m^2 as described in detail in one of our previous studies [5]. In order to reduce content of whey protein, the skim milk retentate was subjected to a microfiltration (MF) in the diafiltration (DF) mode using demineralised water (20 kg) as described by Schaefer, Schubert, and Atamer [15]. From the obtained micellar casein, α_s-, β- and κ-casein fractions were isolated (Figure 1) according to the method described by Schubert et al. [16]. For the isolation of the fractions, specific properties of individual casein fractions such as isoelectric pH, calcium and temperature sensitivities were used to adjust different features of casein such as dissociation, solubility and precipitation behaviours. Since α_S-, and β-casein are sensitive to calcium ions, unlike κ-casein [17], the method of calcium chloride ($CaCl_2$) induced particle formation was applied. Precipitation of α_S-, and β-casein allowed the mechanical separation of these two fractions from κ-casein. The separation process (S1 to S4, Figure 1) were conducted using a decanter centrifuge (model MD 80-S, Lemitec GmbH, Berlin, Germany). The operational parameters of decanter (F_Z: centrifugal force, Δn: differential speed in decanter) for each seperation are given in Figure 1. The micellar casein and the fractions were spray-dried and the gained powders (Figure 2) as well as the liquid concentrates were used for the production of casein glue samples.

Chemical Composition of the Samples

The measurements of major constitutents of casein powders (total protein, calcium, dry matter, lactose and fat contents) were carried out according to the corresponding methods reported by Post et al. [18]. The isolated casein fractions were analysed by reversed phase high-performance liquid chromatography (RP-HPLC) according to Post, Ebert, and Hinrichs [19]. The purity of the casein fraction ($P_{n\text{-}CN}$; %) was defined as the ratio of casein fraction content ($C_{n\text{-}CN}$, fraction) to the total casein content in the isolated fraction ($C_{total\text{-}casein}$, fraction) as determined by RP-HPLC (Eq. 1).

$$P_{n-CN} = \frac{C_{n-CN,fraction}}{C_{total\ CN,fraction}} \cdot 100\% \quad (1)$$

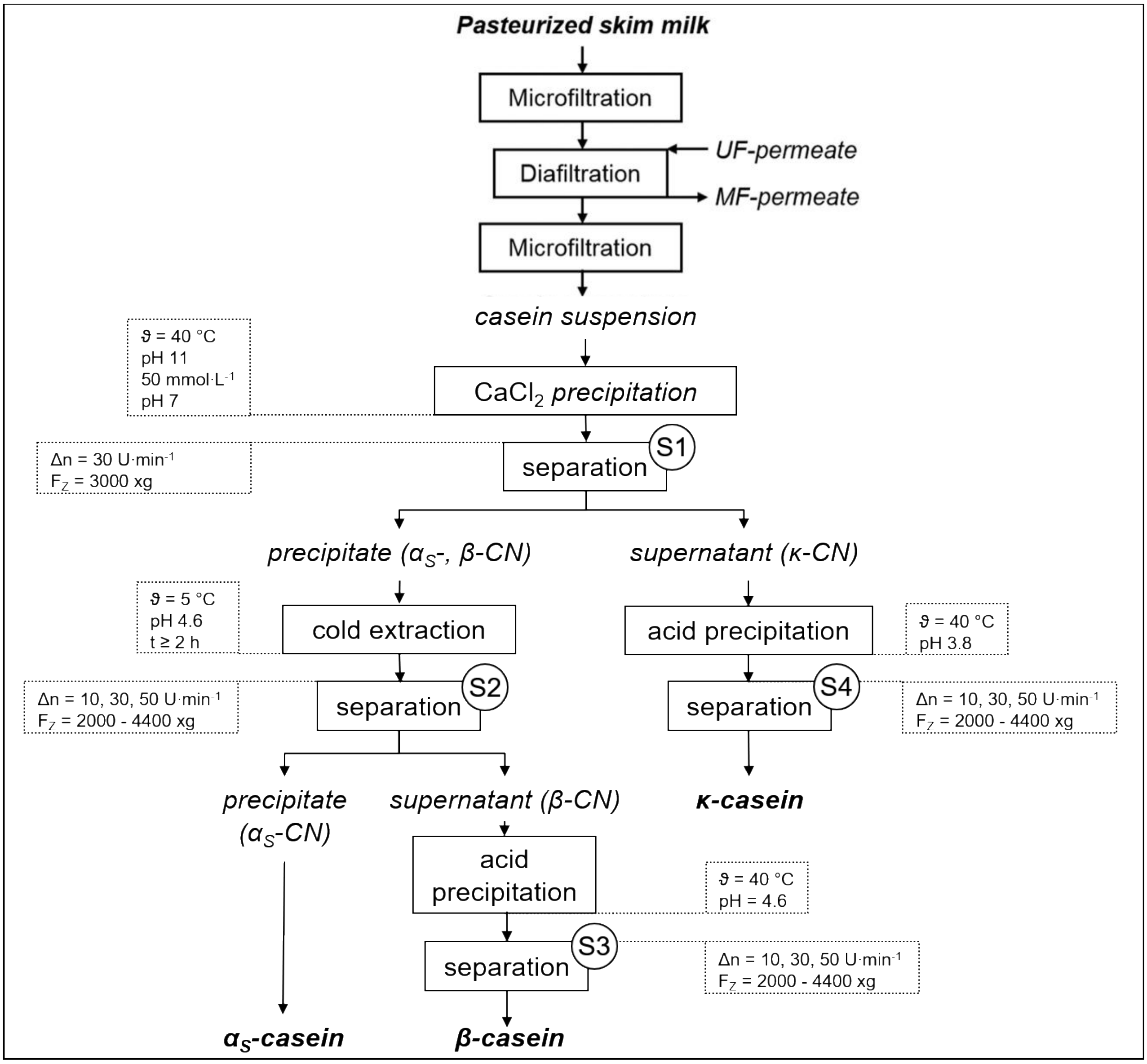

Figure 1. Process for the production of α_S-, β- and κ-casein fractions using pasteurized skim milk. CN: casein, t: time, ϑ: temperature, F_Z: centrifugal force, Δn: differential speed in decanter, UF: Ultrafiltration, MF: Microfiltration, S1 - S4: Separation 1 - Separation 4.

Casein Adhesive Preparation

The casein adhesives were produced using the obtained casein powders. Besides micellar casein and casein fractions, calcium caseinate and sodium caseinate (Meggle Group GmbH, Wasserburg am Inn, Germany) were also included as raw material of casein based glue samples for the measurements. For the preparation of the casein adhesive, mainly casein, water, borax and urea were used. The mixing ratio of the ingredients was based on the recipe of a casein adhesive from the Deutschen Kunsstoff-Institut (DKI) in Darmstadt (casein powder: 24.4 g, water: 78.0 g, borax: 6.2 g, urea: 12.0 g). Distilled water was placed in a beaker, the casein powder, borax and urea were added and mixed by hand using a hand scraper. Mixing was continued until a homogeneous mass was formed. The time taken to reach a homogeneous consistency was determined using a stopwatch. The required mixing time for each sample was around 5 min. This was followed by a 4-hour swelling and hydration period of the casein adhesive at room temperature. During this time, the samples were occasionally mixed. The finished casein adhesives were stored in the refrigerator at 2 °C until use. After 8 days at the latest, new casein adhesives were prepared.

Physical Analysis

Casein adhesive samples were compressed with a plunger speed of 0.5 mm/s using an Instron texture analyser (Instron 5944, Instron Deutschland GmbH, Pfungstadt, Germnay) equipped with a cylinder (d=5 mm). The penetration depth was 10 mm and measurement temperature was 20°C. The maximum force was recorded as adhesive strength together with adhesiveness and cohesiveness.

RESULTS and DISCUSSION

There has been a growing interest in micellar casein and casein fractions (α_S-, β- and κ-casein fractions) because of its valuable properties, such as emulsifying and foam-stabilising properties [20], bio-functional properties [21], non-food applications (e.g., coating agents and glues [6, 22]. Therefore, isolation of casein and casein fractions have been intensively studied. In a review article [4], different methods applied for the isolation of β-casein fraction and functional properties of β-casein was discussed in detail. In order to improve the purity and yield of the casein fractions different separation technologies such as nozzle centrifuges [19] and decanter centrifuges [23] have been investigated for an efficient separation of precipitated α_S - and β-caseins from the κ-casein enriched liquid phase.

In one of our recent studies, a method for the isolation of casein fractions at pilot scale was presented in detail [16]. In the method, different sources of casein such as commercial micellar casein powder, micellar casein concentrate (in house produced) and β-casein reduced micellar casein concentrate were used for the isolation of the fractions. In this current study, the same method was applied to isolate the α_S-, β- and κ-casein fractions.

As seen in Figure 1, the raw material for the isolation of casein fractions was pasteurized skim milk. The obtained powders were spray-dried and example images of manufactured micellar casein and casein fractions are given in Figure 2. Using the illustrated method, the achieved purities were > 25%, > 91% and > 54% for α_S-, β- and κ-casein fractions, respectively (see also Table 1).

The produced casein micellar powder as well as the casein fractions were used for the production of casein based adhesives. Besides these casein samples, two different caseinate samples (sodium caseinate and calcium caseinate) were also studied as casein source, which are the most common commercially found form of casein. In Table 1 compositional analysis of all the casein samples used for the preparation of the adhesives are given. Micellar casein is the native form of casein because in the production process pure physical processes (e.g., separation, membrane filtration) are applied [4]. In the production of caseinate (sodium or calcium caseinate), unlike micellar casein manufacture, the production process is a combination of physical and biochemical processes. Caseinates are produced by adding an alkali to the derived casein, which can be rennet-casein or lactic acid-casein.

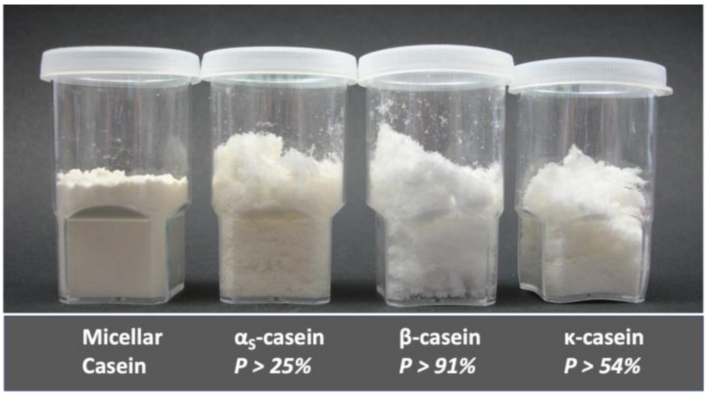

Figure 2. Example images from the manufactured casein samples (micellar casein and α_S-, β- and κ-casein fractions) after spray drying (P: purity)

In previous studies [19, 24], the focus was on the isolation of casein fractions and the functional peptides obtained using these fractions. Hence, it was not investigated, which casein fraction is responsible for the specific adhesive property of casein. Our experiments, which were conducted with six different adhesive samples produced from α_s-casein, β-casein, κ-casein, calcium caseinate, micellar casein and sodium caseinate (Figure 3), revealed that micellar casein and α_s-casein showed the highest adhesive strength in comparison to the other adhesives produced using other casein sources (β-casein, κ-casein, calcium caseinate and sodium caseinate). The results of this study arose the following three main questions: i) Why does α_s-casein fraction show such a good adhesion property? ii) How are the adhesion properties influenced by fractionation process (salts, modification of casein structure)? iii) Which molecular structures are important for the adhesion?

The usage of casein as an adhesive can be derived by the amount of patent applications per year. After a break of more than 15 years, when no further patents for casein as an adhesive were applied, casein started to be used again in the beginning of the 1990s. Then, further research was done and casein adhesives were developed, for example to allow a fast fixation on wet and cold glass bottles, without having problems of diluting the glue or slipping labels. This was interesting, as the filling and labelling speed of bottles in the food industry had increased significantly [25]. Furthermore, a more recent patent from 2013 describes a mixture of casein, water, acrylate, starch, urea, polyethylene oxide, paraffin oil, and ester which can be used for adhesion of electrical devices and laminated boards [26].

Table 1. Chemical composition of the micellar casein powder

Casein sample	Producer	Protein content [%, w/w]			Purity [%,w/w]*
		Casein	Whey protein	Total protein	
Micellar casein	In house production	80.10±0.70	0.20±0.10	82.10±0.08	n.d.
Sodium caseinate	Meggle	87.10±0.30	n.d.	91.70±0.94	n.d.
Calcium caseinate	Meggle	90.40±0.30	n.d.	92.60±0.56	n.d.
α_s-casein	In house production	78.60±4.50	n.d.	n.d.	25.6±3.8
β-casein	In house production	102.50±3.60**	n.d.	n.d.	91.7±2.8
κ-casein	In house production	76.50±7.60	n.d.	n.d.	54.9±2.7

n.d.: not determined, *: The purity of the casein fraction ($P_{n\text{-}CN}$; %) was defined as the ratio of casein fraction content ($C_{n\text{-}CN}$, fraction) to the total casein content in the isolated fraction ($C_{total\text{-}casein}$, fraction) as determined by RP-HPLC.**: indicates that impurities in analytical standards can cause a difference between actual and real casein content, leading to calculated values above 100%.

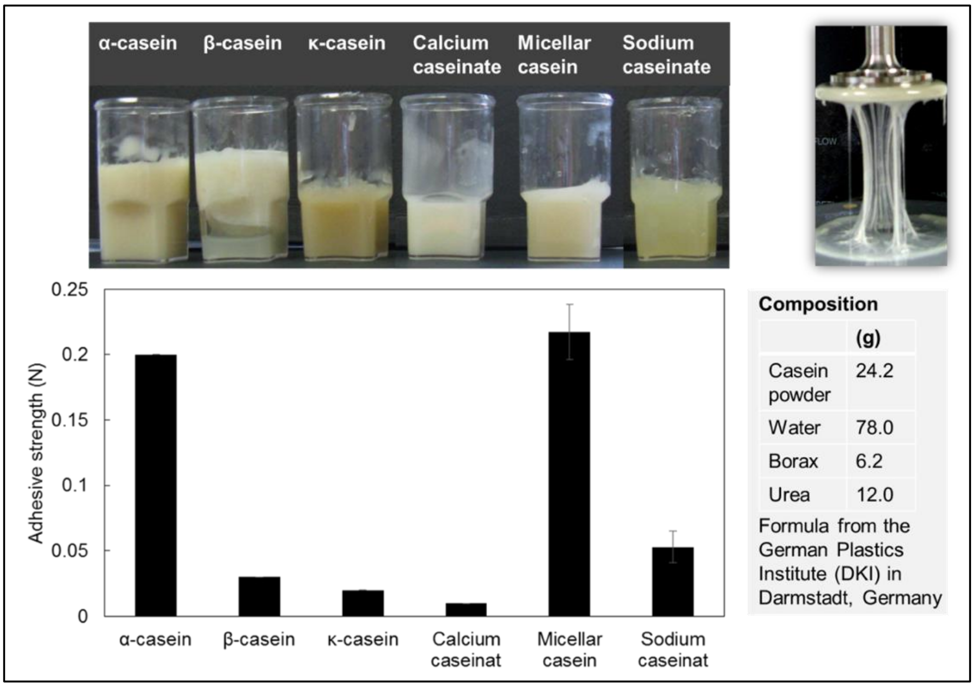

Figure 3. Adhesive strength of the samples produced from α_s-casein, β-casein, κ-casein, calcium caseinate, micellar casein and sodium caseinate. Sodium or calcium caseinate are prepared by mixing casein by either a sodium or calcium compound

The production of adhesives from proteins has a long tradition. Adhesives from casein protein has been applied for labelling of bottles in the beverage industry [3]. Besides bottle labelling, there are further areas of application for casein adhesives such as for paper coatings, paper glue and wood. Plant proteins from soy, wheat, potatoes, sugar beets and rapeseed are other possible environmentally friendly bio-based adhesives form renewable sources [27-29]. Although diverse applications for casein glue are feasible, currently, casein adhesives are mainly used to glue bottle labels, especially in the beverage industry. Its properties like the ability to glue on cold and wet materials, to be recycled and to be processed at a high production speed are favourable.

An in-depth understanding of the molecular basis of functional properties of casein fractions plays an important role as the demand for manufacturing of adhesives from naturally-produced polymers is considered. This study also evaluated the methods available for the characterization of the adhesives. The analysis methods, which are commonly applied for characterization of adhesives in the literature, can be classified into four groups: Chemical analysis, microbiological analysis, physical analysis and visual observation. Table 2 gives an overview of the corresponding methods, which were used in the studies conducted on investigation and classification of bio-based adhesives. By applying these selected methods, future work is needed to be conducted is to achieve an in-depth understanding of the molecular basis of functional properties of casein fractions. Based on the current knowledge on the structural properties of casein fractions, the working hypothesis should be that α_s-casein fraction allows easily the attachment on the hydrophilic and also hydrophobic surfaces due to its triblock-copolymer character (as illustrated in Figure 4). With a profound knowledge, the targeted proteins, protein domains and peptides having an optimized adhesive features could be produced.

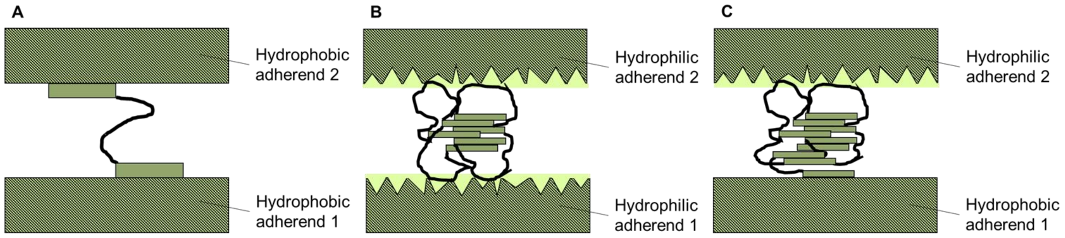

Figure 4. Hypothetical adhesion of adherends through α_s-casein. A) hydrophob-hydrophob binding due to triblock copolymer character of α_s-casein, B) hydrophil-hydrophil binding through self-assembled micelle and steric effect, C) hydrophil-hydrophob binding [30, 31].

Table 2. Applied methods for the characterization of adhesives

#	Classification	Method	Scope	Reference
1	Chemical analysis	Fourier transform infrared spectroscopy	Chemical structure	[32-34]
2		X-ray photoelectron spectroscopy	Chemical composition of adhesion surface	[32]
3		Strength temperature dependence	Storage /Keeping quality	[35]
4		Nuclear magnetic resonance	Chemical structure	[28, 36]
5	Microbiological analysis	Mold resistance test	Storage /Keeping quality	[37]
6	Physical analysis	Atomic force microscopy	Adhesion forces in interface	[38]
7		Contact angle	Wettability on surface	[22, 34]
8		Depth sensing indentation	Hardness	[39]
9		Differential scanning calorimetry	Phase transition / Thermal properties	[34, 40]
10		Peeling test	Dry strength	[41]
11		Scanning probe microscopy	Frictional force of adhesive	[40]
12		Shear impact strength	Dry strength	[42]
13		Shear strength by tensile loading	Dry strength	[27]
14		Tensile lap-shear strength	Dry strength	[43]
15		Viscosity	Flow resistance	[34]
16		Water immersion test	Water resistance	[28, 34, 44]
17		X-ray diffraction	Structure of nano composites	[27]
18		Zeta (ζ) potential	Charge stability in disperse system	[32]
19	Visual observation	Scanning electron microscope	Adhesion surface observation	[27, 32, 34]
20		Transmission electron microscopy	Adhesion surface observation	[34]

CONCLUSION

In this study, using a recently published method [16], micellar casein and casein fractions (α_s-casein, β-casein, κ-casein) were produced from pasteurized skim milk at a large scale. Selective solubility and precipitation by adding calcium chloride as well as a temperature-controlled decanter centrifuge for the separation process were applied to isolate the casein fractions. Although the method needs some improvements via optimization of the process parameters, a very highly purified β-casein fraction and enriched α_s-casein, κ-casein fractions could be achieved with the applied method. Casein adhesives were produced from various casein sources (micellar casein, α_s-casein, β-casein, κ-casein, sodium caseinate and calcium caseinate) and with the aid of physical measurements the adhesive strength of the casein-based adhesive samples was determined. It was found that the source of casein has an effect on the adhesive properties. Casein-derived adhesives are important bio-based products which have preferable characteristics, such as water resistance and simple usage, especially in the application of bottle labelling in the food industry. Although, synthetic adhesives are predominantly produced from petroleum-based polymers, from a sustainability point of view, there is still a need and demand for manufacturing of adhesives from naturally-produced polymers such as such as starch, tree gums, clays or milk proteins. Therefore, further research towards an in-depth understanding of the adhesion properties of the individual casein fractions, its independency from milk prices and a possible transfer of the adhesion mechanism to plant-based glues is necessary to fully satisfy technical, ecological, and economic requirements for a comprehensive use in the industry and households.

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