AUTHORS

Adomas Petrosius
Barbara Schlicht
Chr. Hansen A/S
PerkinElmer, Inc.

LabChip® GXII Microcapillary Electrophoresis to Study Primary Proteolysis During Cheese Ripening

Introduction

Mammalian milk contains two major groups of proteins: caseins and whey proteins. Casein proteins consist of four main casein types, namely κ, α-s1, α-s2 and β. At the initial stage of cheese production, the coagulant (enzyme) cleaves κ-casein, resulting in a chain reaction of casein aggregation. Due to this aggregation a gel-like structure, known as the curd, is formed. When the gel is cut, a liquid (referred to as 'whey'), containing most of the whey proteins, is expelled and drained from the gel. The drained gel is then pressed to form the cheese. Due to this phase separation, cheese protein ends up consisting mainly of α-s1, α-s2 and β caseins.

Overtime, the caseins in cheese get degraded through a process called cheese ripening. This degradation process is regarded as primary cheese proteolysis. Casein degradation depends on the cheese type, types of enzymes and bacterial cultures used, as well as temperature, moisture and other conditions. Cheese texture and flavor differentiation are largely affected by the degree and pattern of casein degradation (Sousa, Ardö, McSweeney, 2001).

Soluble nitrogen (SN), measured by Kjeldahl nitrogen analysis, is the main primary proteolysis indicator currently used in the cheese industry. This fraction is commonly expressed as a percentage of total nitrogen (TN), yielding an expression SN/TN %. SN rises as intact caseins (insoluble protein fraction at pH-4.6) get degraded over time. Degradation products get captured in the soluble nitrogen fraction.

For the Better

A major drawback of SN/TN (%) as a primary proteolysis indicator is the fact that it does not allow to determine which of the casein types are being degraded. Relative degradation of α-(s1 and s2)- or β-casein has different consequences for cheese texture and flavor development.

Chr. Hansen A/S is a market leading innovator and supplier of ingredients for the dairy industry. We strive to supply our customers with well-designed, state-of-the-art enzyme and culture solutions for cheese production. Our customers require ingredients that lead to very specific cheese texture and flavor properties. To support the needs of our customers, we need to have a more nuanced view on how exactly Chr. Hansen ingredients impact the primary cheese proteolysis.

Using the LabChip® HT Protein Express Assay (PN 760499) in combination with the LabChip® GXII Touch™ Protein Characterization System from PerkinElmer enabled us to develop and implement a complete method for extraction and analysis of intact caseins in cheese. A set of standards of isolated α-(s1 and s2)- and β- caseins were used to establish calibration curves for these proteins. Subsequently, corresponding caseins were identified and quantified in a cheese extract (example in Figure 1) (Anema, 2009).
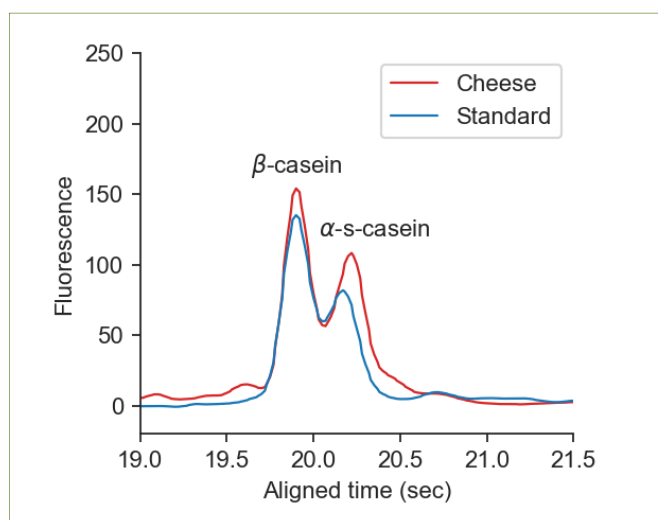

Figure 1: Overlaid electropherograms of casein standard (blue), and cheese extract (red). The α-s1- and α-s2-caseins are assumed to comigrate as a single peak and are referred to as a-s-casein.

The summed concentration of the two casein types is referred to as total casein. In cheese, total casein decreases over time as it gets degraded. As a result of this process, SN/TN (%) fraction increases. Figure 2 illustrates the inverse correlation between total casein and SN/TN (%), which holds true while the intensity of the β-casein and α-s-casein peaks are comparable. Hence, quantification of caseins allows us to evaluate primary cheese proteolysis. It also takes us an important step further: it allows us to determine if the two casein types are degraded differently. Figure 3 shows how differently the two casein types are degraded in the cheese samples from Figure 2.

Having the knowledge of the degradation pattern differences allows us to better classify the proteolytic activity of the used ingredients. Generally, degradation of α-s-casein contributes to cheese texture softening. This effect is exemplified in Figure 4. β-casein degradation, on the other hand, has implications on cheese bitterness. Bitterness, a generally undesired flavor in cheese, can be related to high presence of hydrophobic peptides often released through β-casein degradation (Sousa, Ardö, McSweeney, 2001). Ability to quantify these casein types thus enables us to improve the control of certain cheese properties.
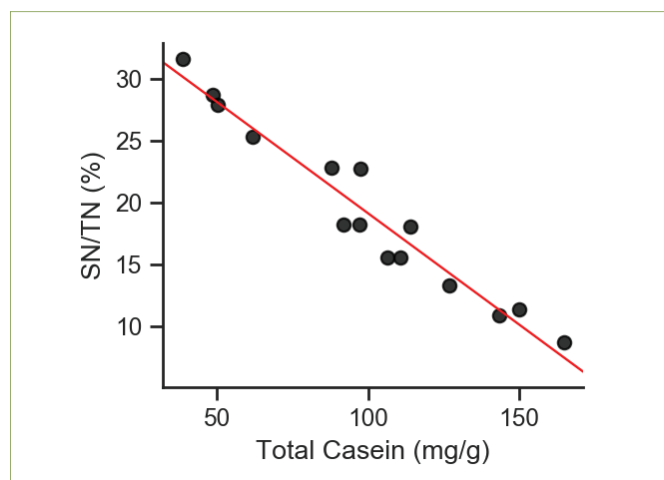

Figure 2: Relationship between SN/TN (%) (Kjeldahl analysis) and corresponding Total Casein content (LabChip analysis) of 15 individual cheese samples.
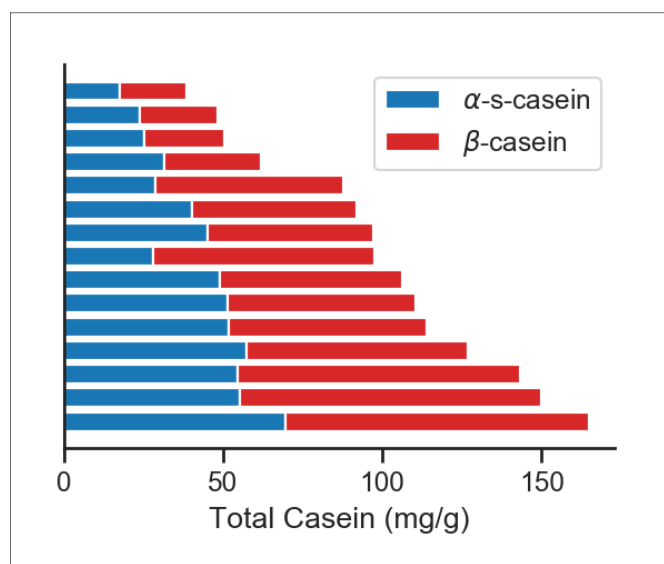

Figure 3: Fractions of α-s-casein and β-casein in the Total casein of 15 individual cheese samples.
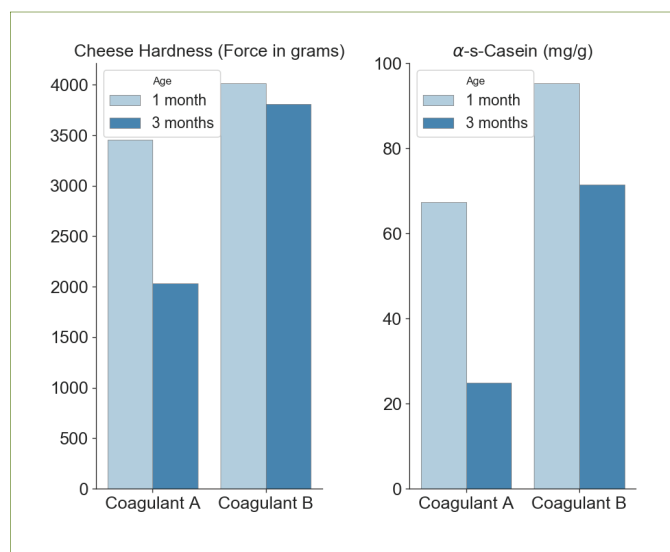
Figure 4. Cheese hardness and the corresponding α-s-casein content in two Cheddar cheeses, produced with two different coagu-lants (A and B). Coagulant A heavily degrades α-s-casein over time, in turn impacting the cheese hardness.

The high analysis throughput, sophisticated data output and minimal consumption of raw materials makes the LabChip® GXII Touch™ electrophoresis system very cost effective. Furthermore, the analysis is relatively easily applicable to a variety of sample matrices. Besides conventional cheese, we are now exploring application potential in milk, whey and other dairy products like yoghurt, quark and sour cream.

References

1. Sousa, M. J., Ardö, Y., McSweeney, P. L.H. (2001). Advances in the study of proteolysis during cheese ripening. International Dairy Journal, 11, 327-345.

2. Anema, S. G. (2009). The use of "lab-on-a-chip" microfluidic SDS electrophoresis technology for the separation and quantification of milk proteins. International Dairy Journal, 19, 198-204.

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs