

Chapter 2

Evolutionary Profiles of Caseins and Degraded Proteins in Industrial High-Moisture Mozzarella Cheeses. A Simulative Approach

Abstract This Chapter evaluates the consequences of protein modifications in cheeses, with special emphasis on mozzarella cheeses. Basic features of modern and historically relevant cheeses depend on the chemical and physical state of main components. The palatability and consumeristic acceptance strongly depend on the flavour and taste features of the fat phase in foods. On the other side, the modification of proteins is interesting. With specific reference to caseins, the main nitrogen-based structure of the final cheese product, many factors influence protein degradation. Because of the needed bioavailability of free water, the amount of moisture becomes important enough. Unfortunately, higher moisture contents may mean lower shelf-life values and enhanced proteolytic degradation. The aim of this Chapter has been to show analytical results of an emulation study carried out on different industrial high-moisture mozzarella cheeses during storage. Obtained data and calculated results seem to suggest that the amount of small molecules increases globally during time, but demolition is mainly ascribed to medium and large protein molecules.

Keywords Absorption • Casein • Cow's milk • CYPEP:2006 • Refrigerated storage • Low-moisture mozzarella cheese • Moisture • Emulation

Abbreviations

A%	Apparent hydric absorption
CYPEP:2006	Cheesemaking Yield and Proteins Estimation according to Parisi:2006
DP	Degraded protein
FC	Fat matter
HAC	High-absorption casein
HMM	High-moisture mozzarella
MC	Moisture
MW	Molecular weight
PA	Protein
SimP	Simulated polypeptide

2.1 Cheese Proteins and Caseins. Chemical Degradation

Cheese is an ideal substrate for amines production, because of the tripartite composition of this product and its microbial ecology [1–4]. In fact, each typology of cheese from goat, cow, or sheep milks is approximately represented with a three-components formulation concerning moisture content (it is the estimated water amount in the product), lipids (also named ‘fat matter’), and proteins, with a minor presence of carbohydrates (residual lactose is the main fraction) and salt (sodium chloride, calcium salts) [5, 6]. Cheeses may be considered the result of the dissolution of solid compounds in aqueous media. On the other side, solid compounds—lipids, proteins, salts, carbohydrates, etc.,—correspond to the solid matter of cheeses, and are generally defined ‘dry matter’. Fat matter and non-protein molecules are trapped into a solid matrix made of proteins with various molecular weights and different spatial arrangements.

The comprehension of cheese structures may be more complicated than the above-described system because of the nature of proteins in cheeses. These proteins, with variable dimensions and physical-chemical features depending on the peculiar type of milk (cow milk is the most used raw matter, but other milks can be considered), should be further subdivided in three categories:

- (a) Caseins (with relation to cow milk, four different casein molecules are known and described)
- (b) Lactoglobulins
- (c) Lactoalbumins.

In general, the production of cheeses is based on the precipitation of a ‘caseous’ matrix from the original milk at acid pH values, trapping all other solid substances with certain molecular weights. This simple descriptions highlights the role of ‘caseins’, while other proteins are not mentioned explicitly [7]. In fact, caseins only can be modified chemically and physically in this way in synergy with water (because of the formation of different hydrogen bonds). On the other side, lactoalbumins and lactoglobulins cannot coagulate at acid pH values; the result of initial cheese productions is always the intermediate agglomeration of caseins and other solid substances (with the exclusion of low-sized molecules) with a considerable water amount. On the other hand, remaining proteins are ‘filtered off’ (actually, expelled) from the new caseous matrix with residual carbohydrates (too low molecular weights) and different organic or inorganic compounds.

Basic features of modern and historically relevant cheeses depend on the chemical and physical state of main components: Certainly, the modification of fat matter after lipolysis and other chemical mechanisms is important by the marketing and technological viewpoints at least. The palatability and consumeristic acceptance strongly depend on the flavour and taste features of the fat phase in foods [7]. On the other side, the modification of proteins may appear more interesting because of direct and indirect influences on the yield of cheesemaking procedures and the safety (hygienic profiles) of the final product and the related process.

With specific reference to caseins, the main nitrogen-based structure of the final cheese product, many factors influence protein degradation. Generally, these reactions are ascribed to microbial life forms: proteolytic microorganisms. Because of the needed bioavailability of free water, the amount of moisture becomes important enough. When speaking of cheesemaking yields, the higher the moisture, the higher the resulting weight of recovered curds (and the higher the final quantity of obtained cheese, with or without ripening).

Unfortunately, higher moisture contents mean also lower shelf-life values and enhanced proteolytic degradation (with additional demolition of organic molecules such as residual lactose and lipids). In addition, water absorption may be lowered depending on the amount of fat matter, the quantitative and qualitative composition of nitrogen-based molecules (after decomposition of caseins), and the quantity of cations with peculiar binding properties [8, 9]. On the other side, aqueous amounts may be overestimated because moisture measures do not concern water only: actually, 'moisture' means all non-solid matters that can be removed under high-temperature treatments (example: 102 °C) after a defined temporal period [10].

The study of molecular profiles of nitrogen-based molecules, caseins above all, is extremely important when speaking of cheeses, in particular packaged products. The normal detection and quantification of these special molecules is carried out in the industry (and in official laboratories by means of direct experimental methods. The main systems are [11, 12]:

- (a) **The Kjeldahl method** This system is widely recognised, applicable to all possible food products, relatively cheap, and accurate. On the other side, it measures all organic nitrogen-based molecules, while protein contents might be overestimated. Moreover, the analytical result concerns only nitrogen (in grams); the determination of correlated molecules has to be made by conversion with adequate factors (example for cheeses: 6.25)
- (b) **The Dumas approach** This method is very rapid and it can be performed by means of automated systems. On the other side, it can be expensive enough. In addition, it measures all organic nitrogen-based molecules, while protein contents might be overestimated
- (c) **Infrared spectroscopic systems** There are different approaches depending on the used infrared spectroscopic range. Generally, these procedures are expensive enough and related equipment has to be calibrated.

On the other hand, the proteolysis of caseins in cheeses may be carried out by means of indirect systems. One of these has been discussed in recent years for a peculiar typology of cheeses: the 'Cheesemaking Yield and Proteins Estimation according to Parisi: 2006' (CYPEP:2006) method [3]. This system aims to give a simulated composition of the sampled product on the basis of two parameters only: moisture (MC) and fat matter (FC).

The aim of this Chapter is to give a description of the proteolysis evolution in selected cheeses by means of this system. A dedicated study has been carried out

within a cheesemaking industry of this purpose. However, because of the important influence of water (moisture) on obtained results, this Chapter discussed only the proteolysis evolution in high-moisture cheeses only: the chosen category is mozzarella cheese obtained by cow milk curd. Other cheeses can be analysed in the same way: Chaps. 3 and 4 are dedicated to low-moisture and diced mozzarella cheeses respectively. It should be considered that diced mozzarella cheeses are normally low-moisture products; however, their moisture content appears to increase during time. Consequently, their behaviour is peculiar.

Anyway, fat matter and proteins are modified because of a notable number of chemical and biochemical reactions, in accordance with the First Law of Food Degradation [13].

2.2 Protein Evolution in High-Moisture Mozzarella Cheeses Under Refrigerated Conditions

2.2.1 Materials

Nine different productions (lots) of industrial high-moisture mozzarella cheeses have been sample for this study near a single Producer. These lots have been stored under refrigerated conditions (temperature: 2 ± 2 °C; four 400.0 grams- samples per lot) and subsequently re-sampled after seven and 14 days of storage. Consequently, six samples have been obtained for each mozzarella production (total: 54 samples). All mozzarella cheeses have been found to be vacuum-packaged with thermosealable films, generally polyamide/polyethylene plastic matters.

With reference to this study, one single high-moisture mozzarella (HMM) sample per lot has been named 'HMM-nnn' ('nnn' is an acronym used for the representation of the lot) and immediately analysed after 24 h (two samples per lot, two analyses). The remaining four samples have been named 'HMM-nnn-x' ('x' means seven or 14 days after the production) and analysed after seven and 14 days: consequently, two of them have been presented for analyses after 7 days, and the remaining cheeses have been analysed after 14 days. As an example, the third sample group for mozzarella cheeses, lot 010, analysed after 7 days, has been named 'HMM-010-7'.

All sampled cheeses have been analysed according to the above-mentioned schedule. MC, FC and proteins (PA) have been evaluated for all sampled products.

2.2.2 Analytical Methods

MC and FC have been obtained with:

- (a) Infrared thermo-gravimetric method, for MC determination;
- (b) AFNOR NF V04-287, for fat matter determination.

The most probable amount of proteins (PA) has been indirectly calculated by means of the CYPEP:2006 indirect method [3, 9]. All results have been calculated as the average of two data per sample.

2.2.3 Results and Discussion

Table 2.1 shows obtained average data (MC, FC and PA) for sampled cheeses in function of storage days after the production (1, 7 and 14 days). Obtained data correspond to the average value of the whole group of samples for MC, FC, pH and PC respectively.

Substantially, the initial composition of sampled cheeses is variegated enough, although MC is always in the range 58.4–60.6% 24 h after production. In detail (Table 2.1):

- (1) MC average value is 59.5%
- (2) FC value is 19.0%
- (3) PA average result, obtained by means of CYPEP:2006, rigorous method, is 17.8%.

After 7 days, analytical data show a little MC augment while FC appear to be essentially unchanged; PA values seem to decrease (Table 2.1). In particular:

- (a) MC increases from 59.5 to 60.8% after 7 days
- (b) FC appear to remain constant: 18.7%
- (c) PA values seem to tend to the number 14.7% (original result: 18.1%).

Finally, analytical data show a different situation after 14 days (Table 2.1):

- (1) MC is 62.1%
- (2) FC value appears to be slightly decreased (average result: 17.4%)
- (3) PA indirect result is obtained as the average data of MC and FC values, in the same way of above-mentioned numbers. Interestingly, it appears unchanged: 14.1%.

Table 2.1 Chemical data for HMM samples, average data

Stored high-moisture mozzarella cheeses, refrigerated conditions (average data)			
Storage days	MC, %	FC, %	PA, %
1	59.5	19.0	17.8
7	60.8	18.7	14.7
14	62.1	17.4	14.1

MC is for: moisture, FC is for: fat matter, PA represents proteins (the most reliable amount for proteins, according to CYPEP:2006)

In general, MC profiles have shown a certain increase: from 59.5 (one day of storage) to 62.1% (14 days at 2 ± 2 °C). On the other hand:

- (a) FC decreases from 19.0 to 17.4%
- (b) PA values decrease in average (from 18.1 to 14.1%).

Consequently, the most probable amount of proteins in sampled cheeses shows a certain diminution (−0.9%) during 14 days; however, this decrease could have been more remarkable if compared with MC amounts (+2.6%). The augment of water in all samples, measurable as moisture content, can be easily correlated with two physical–chemical phenomena at least [5, 9]:

- (a) The more or less enhanced lipolytic degradation of triglycerides in cheeses, and/or
- (b) The proteolytic degradation of caseins (and the little amount of residual albumins and globulins) in the product.

The result of above-mentioned reactions may give complex situations, depending on pH, acidity values, storage conditions (high temperatures, sunlight exposure, etc.), and the involved proteolytic microflora.

The situation of packaged mozzarella cheeses (and other packaged cheeses unable to expel hydrolysis water from external cheese layers) is complex enough. The analytical composition obtained by means of CYPEP:2006 shows a tripartite emulated structure where the sum of MC, FC and PA should not exceed 100% (actually, the sum should be lower than 100 g per 100 g of sampled product).

On the contrary, examined cheeses show the following results as sum of the tripartite structure in function of storage days (Table 2.2):

- (a) MC + FC + PA after 24 h: 96.3%
- (b) MC + FC + PA after 7 days: 94.2%
- (c) MC + FC + PA after 14 days: 93.6%.

Table 2.2 High-absorption casein content, amended high-absorption casein amount, apparent hydric absorption, the sum of the main three cheese components, and degraded protein values for HMM samples, average data

	Stored high-moisture mozzarella cheeses, refrigerated conditions (average data)				
	MC + FC + PA, %	HAC, %	A%	DP	HAC _{CORR} , %
Storage days					
1	96.3	23.9	134.0	6.1	11.7
7	94.2	24.7	168.2	10.0	4.7
14	93.6	25.0	177.4	10.9	3.2

MC is for: moisture, FC is for: fat matter, PA represents proteins (the most reliable amount for proteins, according to CYPEP:2006). HAC is high-absorption protein, A% is apparent hydric absorption, DP mean ‘degraded proteins’, and HAC_{CORR} means ‘amended HAC’. HAC_{CORR} values have been calculated with the Italian patent-pending WISDOM Cheese software [14]

In other words, the sum of the main components in packaged mozzarella cheeses seems to decrease after 14 days with 2.7 g ‘lost’ per 100 g (it should be also mentioned that the labelled shelf-life of these cheeses was 28 days). Consequently, it could be inferred that the degradation of these cheeses appears to be important enough.

Moreover, the chemical structure of proteins is clearly changed during time. The CYPEP:2006 indirect method can give the simulated composition of these mozzarella cheeses including ‘high-absorption casein’ (HAC) amounts, protein contents, and other dissolved matters (salts + residual carbohydrates + organic acids are the main constituents for this parameter).

A new quantity—apparent hydric absorption (A%)—can be calculated: this variable means the ratio between absorbed water by HAC and the aqueous content absorbed by PA. Substantially, A% represents the excessive aqueous absorption in cheeses mainly ascribed to proteins. HAC is a theoretical casein molecule with molecular weight (MW): 22,296 Da without the original glycomacropeptide fraction, which is removed in the initial stages of curd production actually representing the average weight of four different casein species in cow’s milk. HAC may be apparently higher than the real PA content in cheeses; consequently, it is an apparent quantity and could be amended if needed [8, 14]. Anyway, HAC represents all caseins able to absorb water ‘at the maximum level’ [3, 8]. As a result, it should be considered that HAC and A% values in examined cheeses are different enough between 1 and 14 days (Table 2.2):

- (a) HAC and A% after 24 h: 23.9 and 134.0% respectively
- (b) HAC and A% after 7 days: 24.7 and 168.2% respectively
- (c) HAC and A% after 14 days: 25.0 and 177.4% respectively.

In other words, HAC content seems to grow up during time, while the meaning of A% clearly suggests that the apparent absorption ascribed to HAC is increased if compared with the moisture amount really inglobated in the caseous matrix.

Actually, HAC can increase during time on condition that $A\% \leq 100$ only. The right approach to this problem is to consider that a remarkable fraction of hydrolysis water (and dissolved gaseous substances) cannot be expelled from packaged cheeses; caseins could mainly absorb this quantity on condition that these proteins are the only molecules able to absorb water. Naturally, this conventional assertion should be demonstrated, but water molecules in packaged cheeses can also remain ‘free’ (without chemical bounds such as hydrogen bonds) and consequently favour protein degradation [8].

As a result, A% may be also used as a number expressing the ratio between HAC amount in the sampled cheese (based on moisture and fat matter data) and the real PA quantity. According to CYPEP:2006 method (rigorous version), $A\% = 100$ means that $HAC = PA$ in packaged cheeses because:

- (a) Water absorbed by HAC is equal to the aqueous content absorbed by PA, and
- (b) HAC is conventionally defined able to absorb 100% of the total aqueous content until a theoretical limit equal to 3.1 kg of water per kilogram of HAC [3, 9].

Actually, A% can exceed 100% because a certain amount of water can be found in cheeses without stable hydrogen bonds with protein molecules; as a result, this ‘free’ water is not considered when speaking of $A\% \leq 100$ [9].

On these bases:

- (1) A % values > 100 mean that a certain amount of water is not absorbed by caseins and proteins; at the same time, the difference ‘(A%—100)/100’ may be assumed as the percentage of degraded PA proteins (DP) with MW between 0 and 22,296 Da (Eq. 2.1). On the other side, the remaining amount is equal to the amended and reliable HAC able to absorb water ‘at the maximum level’, also defined HAC_{CORR} (Eq. 2.2):

$$DP = PA \times \frac{(A\% - 100)}{100} \quad (2.1)$$

$$HAC_{CORR} = PA - DP \quad (2.2)$$

- (2) A% values ≤ 100 means that water absorbed by HAC is lower than the total water absorbed by proteins. Consequently, PA are low-absorption proteins if compared with HAC: substantially, they correspond to the sum ‘HAC + p-HAC’ where ‘para-high-absorption casein’ (p-HAC) represents caseins with molecular weight > 22,296 Da. This p-HAC corresponds to the hypothetical HAC without the original glycomacropeptide fraction, which is removed in the initial stages of curd production, but it is not able to absorb notable water amounts [9]. p-HAC is present in the original cheese but is not able to absorb water ‘at the maximum level’; it can only evolve towards HAC. At this stage, HAC is not proteolysed; there is no necessity of calculating HAC amended amounts.

With relation to packaged cheeses, A% apparently increases (hydrolysis water cannot be expelled and analytically eliminated) and the general trend is always the augment of A% and apparent HAC. Consequently, HAC_{CORR} and DP have to be calculated if needed (Eq. 2.1 and 2.2; Table 2.2) and possibly displayed with PA vs time (Fig. 2.1). HAC_{CORR} values have been calculated with the Italian patent-pending WISDOM Cheese software [14]. In general, DP appears to augment between 1 and 14 days (initial value: 6.1%; after 7 days, 10.0%; after 14 days, 10.9%) as the consequence of hydrolysis and apparent A% increase, while HAC_{CORR} decreases: initial value: 11.7%; after 7 days, 4.7%; after 14 days, 3.2%).

This theoretical approach has to be evaluated carefully because of the involved hypotheses: more research is needed. However, an interesting development of the simulated calculation concerns more degraded proteins, and polypeptides in particular. Scientific literature reports that one of the main amino acids found in caseins is lysine [15] with MW 146.19 Da. Substantially, a polypeptide with 15 lysine units could be hypothesised for simulation purposes and named ‘simulated polypeptide’

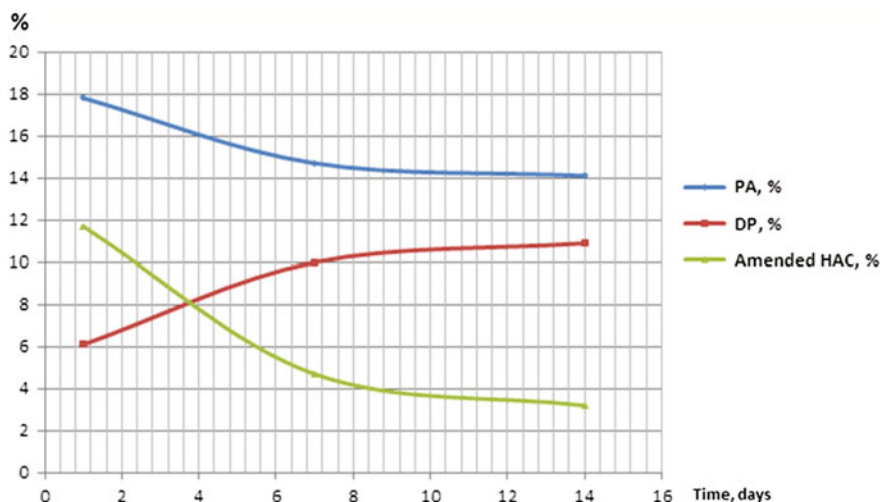


Fig. 2.1 Protein amount, degraded protein content and amended high-absorption casein in industrial high-moisture mozzarella cheeses stored under refrigerated conditions (average data) versus time (days). PA represents proteins (the most reliable amount for proteins, according to CYPEP:2006); HAC is high-absorption protein and DP mean ‘degraded proteins’. Amended HAC (also named HAC_{CORR}) values have been calculated with the Italian patent-pending WISDOM Cheese software [14]

(SimP), with MW = 2192.85 Da (very close to 2223 Da). This amount might be calculated and the related evolution could be expressed versus time.

On these bases, should the DP amount be considered for each experiment and subdivided mathematically in ten different intervals with MW of 2223 Da (the HAC MW is 22,296 Da; the interval between 0 and 22,296 Da can be subdivided in ten 2223 Da- intervals), the related probable amount could be simulated on condition that:

- (a) DP amount is considered as the sum of ten different quantities in the following way:

$$DP = \sum_{i=1}^{i=10} DP_i = DP_1 + DP_2 + DP_3 + \dots + DP_{10} \quad (2.3)$$

where DP_1 is the DP amount for the 0–2223 Da- interval, DP_2 is the DP amount for the 2224–4460 Da- interval, ... and DP_{10} corresponds to the DP amount for the final amount excluding HAC

- (b) Each DP_i quantity is obtained as the sum of 'i' fractions (F) obtained by means of Eq. 2.4:

$$F = \frac{DP}{\sum_{i=1}^{10} i} = \frac{DP}{(1+2+3+4+5+6+7+8+9+10)} = \frac{DP}{55} \quad (2.4)$$

Consequently, should DP amount be equal to a , the following results could be calculated:

- $F = DP/55 = a/55$
- $DP_1 = 1 \times F = a/55$
- $DP_2 = 2 \times F = 2a/55$
- $DP_3 = 3 \times F = 3a/55$
- ...
- $DP_{10} = 10 \times F = 10a/55$

This simulated approach can be used with the aim of displaying the theoretical degradation of PA (original HAC) during time. Figure 2.2 shows the situation for cheeses samples after 24 h, while Fig. 2.3 and 2.4 concern cheeses after seven and 14 days respectively.

Finally, the simulated low-MW-polypeptide, SimP, may be estimated (Fig. 2.2, 2.3 and 2.4): it would correspond to the first interval between 0 and 2223 Da, including all nitrogen-based organic molecules derived from proteolytic reactions in

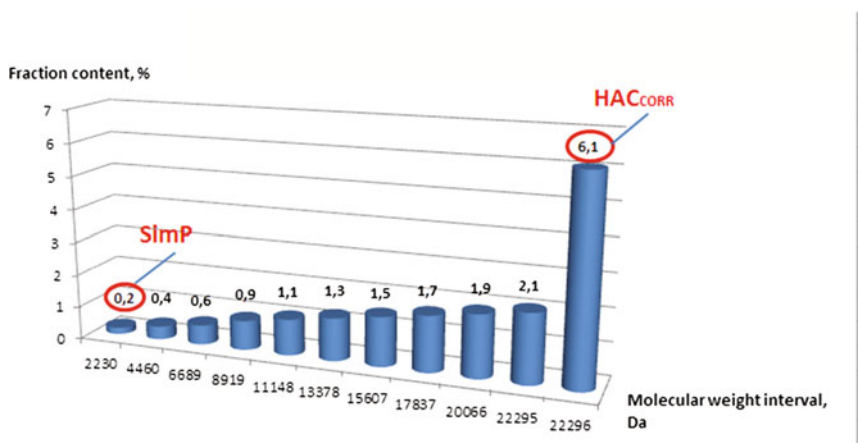


Fig. 2.2 The theoretical degradation of proteins (original HAC) in industrial high-moisture mozzarella (HMM) cheeses samples (0–4 °C) after 24 hours-storage. The lowest molecular weight interval (between 0 and 2223 Da) corresponds to the simulated low-MW-polypeptide, SimP, may be estimated and shown: it would correspond to the first interval representing all molecules in this range. Amended HAC (also named HAC_{CORR}) values have been calculated with the Italian patent-pending WISDOM Cheese software [14]

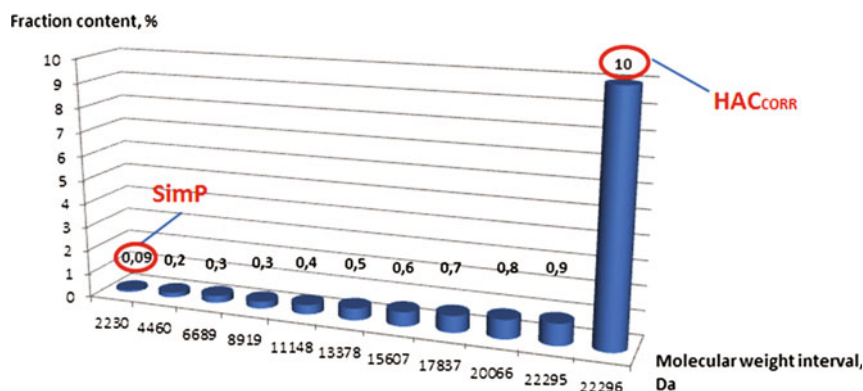


Fig. 2.3 The theoretical degradation of proteins (original HAC) in industrial high-moisture mozzarella (HMM) cheeses samples (0–4 °C) after 7 days of storage. The lowest molecular weight interval (between 0 and 2223 Da) corresponds to the simulated low-MW-polypeptide, SimP, may be estimated and shown: it would correspond to the first interval representing all molecules in this range. Amended HAC (also named HAC_{CORR}) values have been calculated with the Italian patent-pending WISDOM Cheese software [14]

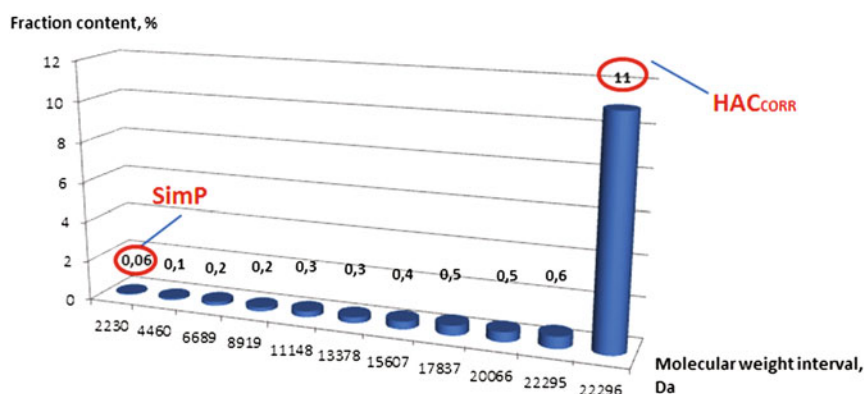


Fig. 2.4 The theoretical degradation of proteins (original HAC) in industrial high-moisture mozzarella (HMM) cheeses samples (0–4 °C) after 14 days of storage. The lowest molecular weight interval (between 0 and 2223 Da) corresponds to the simulated low-MW-polypeptide, SimP, may be estimated and shown: it would correspond to the first interval representing all molecules in this range. Amended HAC (also named HAC_{CORR}) values have been calculated with the Italian patent-pending WISDOM Cheese software [14]

this range. The emulated proteolysis is clear enough but SimP values decreased from 0.2% after 24 h to 0.09% after 7 days and 0.06% after 14 days. Substantially, the amount of small molecules increases globally during time, but demolition is mainly ascribed to medium and large protein molecules (high MW); in addition, PA decrease during time, and SimP is a protein fraction.

2.3 Conclusions

Obtained data may be interesting when speaking of high-moisture mozzarella cheeses. However, the simulation can obtain different results in other mozzarella cheeses. Chapters 3 and 4 are dedicated to low-moisture and diced mozzarella cheeses, respectively, and the same simulative approach may give different results.

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