Chapter 2
The Prediction of Shelf Life Values in Function of the Chemical Composition in Soft Cheeses

Abstract The determination of shelf life of food products is one of the most important problems in the modern industry. With reference to this argument, the list of scientific papers is long enough. It should be considered that the food production is subdivided into a number of different fields and subsectors, depending on the typology of raw materials, intermediates, finished products and by-products. In addition, every class of food product can be easily expanded in comparison with the classification of the Codex Alimentarius Commission due to the presence of industrial imitations. Several of these industrial products may be perceived as 'ameliorated' versions of the original prototype. The prediction of food durability is certainly influenced by several parameters: the typology of process, the packaging system, the choice of the correct storage condition and other factors. Moreover, the function of food additives should be discussed. The food producer is always responsible for the correctness of nutritional data and other information related to the food product, including the durability. The aim of this paper is to review previous theories and related calculations for the preventive determination of shelf life values in cheeses, especially soft products. These calculations are based on the approximate chemical formulation of the final product. Authors have also discussed the possible modification of predictive equations for a peculiar *pasta filata* product, the so-called '*mozzarella* cheese in water'.

Keywords Casein · Hydrolysis · Moisture · *Mozzarella* cheese · Shelf life · Yeasts and moulds

2.1 The Food Durability: An Overview

The determination of shelf life (SL) of food products is one of the most important problems in the modern industry. With reference to this argument, the list of scientific papers is long enough [1, 2].

It should be considered that the food production is subdivided in a number of different fields and subsectors, depending on the typology of raw materials,

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intermediates, finished products and by-products. In addition, every class of food product can be easily expanded in comparison with the classification of the Codex Alimentarius Commission [3] because of the presence of industrial imitations. Moreover, several of these industrial products may be often perceived as 'ameliorated' versions of the original prototype by consumers [4–7].

The prediction of food durability is influenced by several parameters: the typology of process [8], the packaging material and related packing systems [9], the choice of the correct storage condition [8] and other factors. In addition, the function of food additives should be considered [10]. The food producer is always responsible for the correctness of nutritional data and other information related to the food product, including the durability.

The aim of this paper is to review several theories and related calculations for the preventive determination of SL values in cheeses, especially soft products. These calculations are based on the approximate chemical formulation of the final product. Moreover, the possible modification of predictive equations has been discussed for a peculiar type of *pasta filata* product, '*mozzarella* cheese in water'.

From a general viewpoint, it can be affirmed that the SL of foods and beverages is strictly related to the typology of food product; on these bases, food durability may be considered as a sort of 'fingerprint' for the peculiar food and might be used for identification and classification purposes. Two or more similar products may have the same SL; consequently, the concept of 'fingerprint' may be questionable [8]. On the other hand, the SL may be considered as a performance indicator for foods and beverages. In effect, the simple colorimetric modification of certain products may signal that the related food is or appears expired [9, 11]. As a result, the exact determination of the temporal period between the production of foods and the 'unacceptable' modification of sensorial features may be expressed as a simple number: in other words, every food may be classified by means of this 'performance value' [9, 12]. According to the 'principle of food degradation' [8], all edible products are exposed to the continuous and unstoppable transformation of chemical, physical, microbiological and technological features during time, in all possible storage conditions.

Moreover, the estimation of food durability values needs solid mathematical bases because the 'true' SL has to be centred into a numerical range with maximum and minimum limits [13]. However, this requirement—the existence of mathematical restrictions—might be the cause of problems when related equations have to be solved and obtained results have to be verified in the real world. One of the most interesting approaches may be the predictive estimation of a minimum SL with accessory 'positive' errors [1]. On these bases, the minimum SL would be always reliable while the mathematical error should influence only the maximum value.

The following list contains main factors affecting SL values:

(a) Food or beverage type (category). Examples: meat and meat-based products; fruits and vegetables; milk and milk products; seafood; industrial preparations
(b) Food or beverage sub-class. A useful reference is the 'Codex General Standard for Food Additives', Annex B, Parts I and II [3]

(c) Processing technology, from raw materials to finished products
(d) Packaging procedures, including the preliminary evaluation of packaging materials
(e) Storage conditions with peculiar reference to temperatures and logistics
(f) Every parameter (microbiological counts, chemical contents, etc.) with influence on sensory features of food products.

From a general viewpoint, the problem of the correct and reliable determination of SL values may be difficult and 'thorny'; related responsibilities are ascribed to the food producer [13].

In detail, the determination of food durability values can be complex because of a number of different factors; the subdivision of foods and beverages in peculiar categories is helpful. A typical example is related to the production of cheeses: with concern to these products, SL depends basically on the following points [14]:

- Cleaning methods and sanitisation procedures for pipelines and milk collection equipment.
- The chemical and microbiological condition of used raw milks.
- Storage conditions, with peculiar reference to thermal values.

The 'Hazard Analysis and Critical Control Points' (HACCP) approach implies that the production of hygienically sure and compliant foods cannot be demonstrated when the above-mentioned basic information and other data are not considered by cheesemakers [15].

Other important points are:

- The influence of food packaging materials (FPM) on the SL of the packaged cheese [16, 17]
- The use of food additives, antimicrobial agents and other chemicals for production of 'ameliorated' and 'imitation' cheeses. For example several additives—nisin, sodium propionate, potassium sorbate—may be used because of their notable action against moulds in analogue products [7].

However, the category of cheeses may appear extremely variegated and the determination of food durability values cannot be discussed from a general viewpoint. As a result, authors have decided to mention the peculiar class of '*pasta filata*' products and the related sub-category of *mozzarella* cheeses (MZR) with the aim of giving useful demonstrations and examples. In addition, abundant scientific literature is available about *mozzarella* cheeses.

2.2 Cheeses and Food Durability

The category of *pasta filata* cheeses is included in the general macrogroup of rennet-coagulated cheeses [5]: this list concerns 10 different cheese subclasses at least. One of the main features of *pasta filata* cheeses is substantially related to the distribution of the amorphous ‘paracasein’: this name means a small group of milk proteins. Paracaseins are generally rearranged and aligned into roughly parallel fibres [18] by means of a peculiar technique of cheese production. In detail, the original raw milk (different types are used, but most known applications are related to cow’s and buffalo’s milks) is coagulated with the use of animal, vegetable or microbial enzymes to obtain the so-called ‘curd’. This intermediate material is subsequently ‘cooked and stretched’ in hot water. The final result is a peculiar protein matrix containing also fat globules (variable dimensions). As a consequence, the heterogeneous quasi-laminar structure of caseins determines peculiar features of *pasta filata* cheeses. For example the exceptional stretchability is dependent on the behaviour of proteins. Caseins may be considered as organic textile fibres: consequently, should the curd be drawn in a single direction with the addition of hot water, these protein chains would easily dispose themselves in parallel and roughly ordered lines.

Naturally, good products need adequate raw materials and correct conditions: the right acidity of raw milks and the resulting curd, the correct quantity of calcium, the high water temperature and the speed of mixer screws [18].

The main and most known *pasta filata* product in the world is probably *mozzarella* cheese. A number of different versions and ‘ameliorated’ foods with this name are available at present. The low-moisture *mozzarella* cheese (LMMC) is a good example [18].

From the technical viewpoint, MZR are not ripened products [19]: these foods can be heated or used without other processes a few hours after the production. However, ripening modifications can occur in MZR during their SL period: in fact, it may be affirmed that *pasta filata* cheeses tend to change their original composition and the microbial population into the packaging container. This situation corresponds to a series of different reactions and microbial fermentations: normally, ‘fresh’ cheeses may show these anomalies when the related food durability exceeds 15 days.

For this and other reasons, microbiological limits for this type of cheese appear very rigorous: legally, the European Regulation (EC) No 2073/2005 and subsequent amendments has defined strict requirements. Another example can be shown: the french *Fédération des Entreprises du Commerce et de la Distribution* (FCD, French Retail and Wholesale Federation) has forced cheese producers to consider additional parameters with reference to unripened cheeses from pasteurised milk [20]. With reference to yeasts and moulds, the following rules are mandatory:

- 100 colony-forming units (CFU) per gram at the arrival near mass retailers.
- 100 CFU per gram at the end of SL periods (expiration or ‘best before end’ date).

These requirements, valuable for French mass retailers only, are not absolute: yeasts and moulds can reach 5,000 CFU/g at the reception near mass retailers for *mozzarella* and similar traditional cheeses, while 50,000 CFU/g can be tolerated at the end of SL [20].

This simple example shows how the management of *pasta filata* cheeses may be difficult from the microbiological viewpoint. Basically, the FCD recognises that the limit of yeasts and moulds may notably increase. As an implicit result, SL values can be dependent on the variable behaviour of these life forms into cheeses.

Moreover, the peculiar subclass of MZR is composed of various versions: there is no real possibility of defining one SL value only for all existing products with this name. For example, the same *mozzarella* cheese can be packed into plastic containers of different types with or without water: the first group concerns products with notable weights (400- 1,000–2,000 g, etc.), while the second category is normally produced as little spheres ('cherries') with reduced weight: 5, 6, 8, 10, 100 or 250 g. It has to be highlighted that these two classes of *mozzarella* cheese show dissimilar behaviours during their SL. Naturally, the microbial spreading should be easily predicted and notably accelerated when cheeses are immersed in water, and the possible modification of sensorial features is further increased because of the small dimension of products (lower weights = higher contact surface between cheeses and water). Additionally, yeasts and moulds are non-pathogenic agents but cheeses can be excellent 'culture media' for different degrading life forms [1]: this risk has to be carefully taken into account when speaking of cheeses in water.

2.3 Soft Cheeses and Cherries in Water: Predictable Differences

As above mentioned, the group of *pasta filata* cheeses comprehends different products: most known cheeses are *mozzarella*, *provolone*, *kashkaval* [4, 21] and *halloumi* [22]. MZR are particularly studied because of the absence of ripening periods. However, several modifications—hydrolysis, expulsion of proteolysed substances and fatty molecules dissolved in water, enhanced fermentation by *Lactobacillaceae* and *Streptococcae*, microbial spreading—may occur within 15 days after the initial production into the final packaging [23].

When MZR are packed without water, the sum of the above-mentioned transformations can be easily identified with a sort of undesired 'maturation' of the initial cheese into the container. The casein matrix tends to remove the exceeding water (the original aqueous content and the result of the additional hydrolysis) and different dissolved molecules: calcium and sodium salts, proteose-peptones, organic acids (by microbial fermentation), etc. It has to be noted that this exceeding water is not allowed to drain unless perforated packages are used. Consequently, packaged MZR are essentially metastable products because of the presence of contaminated and exceeding hydrolysis water at the food/packaging interface. Generally, MZR tend to show excessive softness [24] with reference to external layers of the product (surfaces are clearly the softest section). In addition, pH values tend to increase and oxidation-reduction (redox) potentials may turn to negative values [25]. The same phenomenon has been observed in different cheeses produced from sheep milk [26, 27].

As a consequence, the microbial spreading by yeasts and other degrading life forms can become important because of one or more of the following conditions:

- Negative values of redox potentials.
- Increase of water and degraded substances.

Should this situation be observed, the packaged *mozzarella* cheese would become superficially degraded and non-edible: in other words, an unpredictable reduction of the declared SL would be noted [1].

Apparently, the above described phenomenon might appear 'silent' when speaking of MZR in water. For example, *mozzarella* cherries in water show a peculiar behaviour. In detail, this product is initially able to absorb a certain amount of 'external' water into the packaging material [28] depending on:

- The degree of proteolysis of para-κ-caseins (Sect. 1.3), and
- The fat content on dry matter (FDM) amount [29].

As a clear and macroscopic consequence, MZR tends to increase their weight compared with the initial value. Modest dimensions of cheese spheres (cherries) are also a distinctive advantage: the higher the global surface (cheeses are subdivided in small pieces), the higher the observable absorption.

This important step should occur on the first day after the initial production. However, high FDM values or advanced proteolysis can diminish the predictable absorption of water. Subsequently, *mozzarella* cherries tend to release progressively water. As above mentioned, this water is composed of two fractions:

1. The initial absorbed water on the first day after the final packaging (variable amount) and the original aqueous amount of cheeses.
2. The total quantity of exceeding water (cause: partially degraded caseins become more and more unable to absorb water depending on the degree of proteolysis and the reduction of molecular weights).

Additionally, the microbial spreading is really enhanced in the external and constantly wetted layers of *mozzarella* cherries. The visual detection of spoiled MZR may be difficult because of non-transparent packages and the suspension in water. On the other hand, the odour of contaminated waters can be important because of the probable emission of 'rotten eggs' smells [30]; this defect is caused by the abundant presence of free sulphur amino acids.

Substantially, cheeses in water tend to spoil with increased speed compared with normal *mozzarella* cheeses. The following sensorial features should be attentively considered because of their strong connection with the real acceptability (from the viewpoint of food safety):

- Softness, gumminess, stickness
- Colourimetric modifications: from white to yellow or brown colours [30]
- Superficial defects (microblisters) by yeast fermentation
- Textural modifications
- Red pinpoint colonies on cheese surfaces by pigment producers such as *Serratia marcescens* [1]

- Excessive pH variations
- Excessive moisture values (drained cheeses)
- Unacceptable increase of the microbial spreading
- Detection of pathogen agents
- Detection of microbial toxins
- Packaging failures.

2.4 Predictive Models for Shelf Life of Cheeses

Basically, the preventive estimation of durability is mandatory from the legal viewpoint [8, 13], and the food producer or packer is entirely responsible for the correct determination of this important value. However, the evaluation of food durability values depends on various factors, including packaging materials; consequently, the HACCP approach is surely needed.

From a general viewpoint, the preventive estimation of remaining shelf life (RSL) for food and beverage products was initially carried out with the predictive microbiology. However, this peculiar approach tends to examine systematically the probable behaviour of specific spoilage life forms and the temporal advance of related processes in edible products [31]. On the other hand, the prediction of RSL should include the problem of the microbial ecology in food systems and the evaluation of peculiar chemical and physical indicators [32]. Consequently, many techniques can be used including electronic temperature integrators, data loggers and dedicated databases.

The weight of the predictive microbiology on the prediction of RSL has been predominant [31]: different mathematical models have been created and developed with the aim of describing and predicting microbial growth curves [33]. On these bases, different software products have been also created for predicting the durability of peculiar foods. Several of these programs use microbial models and chemical information at the same time.

Two examples are the Seafood Spoilage and Safety Predictor (SSSP) with reference to seafood products [34, 35] and the more general Pathogen Modeling Program (PMP). Actually, the validation of obtained predictions in 'true' conditions should be always recommended because mathematical elaborations may be related to in vitro experiments.

On the other side, RSL may be estimated by means of mathematical equations with 'chemical' inspiration like the Arrhenius law [13, 36].

In the specific cheese sector, many applications have been published. Several of these approaches are based on empiric equations: these expressions may calculate SL values of whole or portioned cheeses (different packaging materials) on the basis of chemical, physical and microbiological data [1, 2, 37].

The creation of artificial neural networks (ANN) has made possible the creation of peculiar algorithms for the prediction of SL values of processed cheeses [38]. Once more, different inputs have to be processed: yeast and mould (YM) count, pH, total viable count, soluble nitrogen content, sensory evaluations…

The prediction of SL appears correlated to many factors. This reflection may highlight the role of sensorial evaluations: the correlation between organoleptic data and the initial amount or several chemical (or microbiological) variables may be useful. In other words, the aim should be the possible creation [8] of a coherent and reliable system for the estimation of RSL on the basis of a collection of apparently disconnected variables (microbial counts, chemical compounds, sensorial scores, etc.). This approach has to discriminate received inputs: probably, much of the available information may be irrelevant, misleading or simply redundant [1]. Moreover, processing and storage conditions have to be examined: the simple record of storage temperatures can determine the validity of analytical results in terms of food hygiene [39].

Section 2.5 is dedicated to the practical application of a peculiar predictive approach. This empiric method may 'design' the SL of *pasta filata* cheeses on the basis of a few parameters: raw materials, environmental hygiene, etc. This procedure was created for *pasta filata* products in normal conditions with the exclusion of MZR in water. However, similar methods may be corrected when different products, processing and/or storage conditions are applied. Should this possibility be verified, the original approach could furnish reliable results even with relation to highly perishable cheeses.

2.5 A Peculiar Approach: Cheeses in Water

With reference to cheeses, the empiric approach to the prediction of RSL can also be used for different goals. Generally, food technologists would be able to modify cheese performances—including RSL—on the basis of the original formulation. However, several cheesemakers prefer to modify a small number of 'main' parameters (curd, salt, rennet) without the addition or subtraction of 'secondary' additives (lactic acid, etc.).

This section is dedicated to the prediction of RSL for MZR in water by means of a predictive formula for packaged *pasta filata* cheeses [1]. The related approach has been included in a free software: the 'Deductive Evaluation of Shelf-Life: Cheeses' 1.0 (DESC 1.0). Obtained results are reliable on condition that following conditions are verified [9]:

- The 'moisture on free fat basis' (MFFB) index of packed soft cheeses is >70 %.
- Packed semi-hard cheeses have MFFB $\geq$63 %.
- Anyway, cheeses are stored at 2 $\pm$ 2 °C (a second version of the method allows 10 $\pm$ 2 °C).

Otherwise, obtained results have to be validated [1, 2]. Moreover, following products should not be considered [9]:

- Cheeses in water
- Other cheeses under peculiar conditions: sliced cheeses, products packaged under modified atmosphere, smoked cheeses, etc.

With reference to DESC 1.0, the predictive equation is [1]:

$$[SL]_{2^\circ C} = -1.6 \times MFFB + 29.9 \times pH - 10.9 \times \log_{10} YM \quad (2.1)$$

where $[SL]_{2^\circ C}$ = shelf life at 2 ± 2 °C and $Log_{10}YM$ = decimal logarithm of YM count. Actually, DESC 1.0 may use other similar equations:

$$[SL]_{10^\circ C} = -0.7 \times MFFB + 11.6 \times pH - 2.6 \times \log_{10} YM \quad (2.2)$$

$$[SL]_{FR} = \frac{1}{3} \times [SL]_{2^\circ C} + \frac{2}{3} \times [SL]_{10^\circ C} \quad (2.3)$$

where $[SL]_{10^\circ C}$ = shelf life at 10 ± 2 °C and $[SL]_{FR}$ means the value of RSL according to the french AFNOR V01-003 norm. The aim of this study is to calculate the food durability at 2 ± 2 °C by means of the Eq. 2.1.

As above declared, the predictive approach has been specifically elaborated for *pasta filata* cheeses but *mozzarella* cherries in water are not considered [1]. However, the chemical composition and the technology of these products are substantially identical. As a consequence, authors have decided to test the performance of the Eq. 2.1 for *mozzarella* cherries in water.

The study has been carried out near a cheesemaking industry: different productions of MZR in water have been sampled. Basically, MZR have been subdivided into two distinct typologies:

(a) A first group, named MZR-100: net weight, 100 g; gross weight, 180 g; weight of water: 80 g
(b) A second group, named MZR-250: net weight, 250 g; gross weight, 500 g; weight of water: 250 g.

All described cheeses have been produced by means of the normal ‘stretching method’ for MZR in hot water [18]; the final packaging has been realised with the addition of simple pasteurised water. Subsequently, produced cheeses have been sampled in the following way:

- MZR-100: five different lots, five samples per lot.
- MZR-250: five different lots, five samples per lot.

As a result, 10 different production lots have been sampled: 50 cheeses have been considered for the study and stored at 2 ± 2 °C. Chemical and microbiological analyses have been carried out in the following way.

First of all, 10 stored samples (temperature: 2 ± 2 °C) have been analysed: five lots per MZR-100 and five lots per MZR-250 cheeses, storage time: 24 h after the production date. Following analyses have been carried out:

- Moisture content (MC) of drained cheeses according to an internally validated thermogravimetric method [40].
- Fat content (FC) of drained cheeses according to the AFNOR NF V04-287 norm.
- pH value of drained cheeses with a calibrated pH-meter.

- YM count of drained cheeses according to the ISO 21527-1:2008 norm.
- Total coliform (TC) count of drained cheeses according to the AOAC 991.14 protocol.

The remaining MZR-100 and MZR-250 samples (40 total products, 20 cheeses per group, four products per lot) have been subdivided into four different groups containing one cheese per lot. The first group has been considered for analytical controls 7 days after the production date; other groups have been considered after 14, 21 and 28 days, respectively. This time, the following data have been obtained:

- pH value
- TC count of drained cheeses.

With reference to the positive or negative evaluation of cheeses in this preliminary study, acceptable products cannot show deviations from the below mentioned list of parameters:

1. Absence of unpleasant odours.
2. Absence of unusual colours.
3. Anomalous texture for the drained cheese. The product should be able to sustain the pressure of one packaged product (same weight) without ruptures for 60 s; subsequently, the cheese should return to the original shape.
4. Absence of blisters, holes, ruptures, other similar defects.
5. Absence of gaseous fermentation into the bag with consequent dilatation.
6. pH values > 6.3.
7. TC counts $\geq$ 1,000 CFU/g.

Tables 2.1 and 2.2 display all obtained results for MZR-100 and MZR-250 groups, respectively (average data). Additionally, MFFB values have been added.

Table 2.1 Analytical data for MZR-100 samples

Number of days after the production	MZR-100 samples, five different lots					
	MC	FC	MFFB index	YM count	pH	TC count
1	60.2	18.7	74.0	1.1	5.87	1.2
7					6.02	1.5
14					6.12	2.0
21					6.19	2.5
28	*Please note* expired products because of following failures: blisters and anomalous odours				6.26	2.9

All displayed data are average results. MC is for moisture (g/100 g). FC is for fat content (g/100 g). MFFB is for ‘moisture on free fat basis’. YM and TC are for ‘yeast and mould’ and ‘total coliform’ respectively. The estimated shelf life (SL) at 2 ± 2 °C has been 45 days. The observed SL at 2 ± 2 °C has been 21 days

Table 2.2 Analytical data for MZR-250 samples

Number of days after the production	MZR-250 samples, five different lots					
	MC	FC	MFFB index	YM count	pH	TC count
1	59.7	19.5	74.2	1.2	5.92	1.0
7					6.07	1.6
14					6.16	2.2
21					6.24	1.8
28	*Please note* expired products because of following failures: blisters, anomalous odours, textural defects, pH values > 6.3, TC counts > 1,000 CFU/g				6.32	3.3

All displayed data are average results. MC is for moisture (g/100 g). FC is for fat content (g/100 g). MFFB is for 'moisture on free fat basis'. YM and TC are for 'yeast and mould' and 'total coliform', respectively. The estimated shelf life (SL) at 2 ± 2 °C has been 46 days. The observed SL at 2 ± 2 °C has been 21 days

Two simple deductions may be inferred on the basis of Tables 2.1 and 2.2:

1. Expired MZR-100 cheeses have shown sensorial failures, but the increase in pH and TC values is interesting and should be correlated to organoleptic defects.
2. Observed blisters and anomalous odours are surely caused by mixed fermentation, but the role of coliforms—proteolytic bacteria—should be attentively evaluated: generally, unpleasant smells were associated to the presence of sulphur amino acids.

On the other side, it has to be noted that the predictive Eq. 2.1 has calculated high SL values in comparison with the real expiration date: both MZR-100 and MZR-250 cheeses can be considered expired after 21 days, while the real date cannot be inferred (between 21 and 28 days). Consequently, the best strategy is the definition of a 'minimum' and sure RSL value: 21 days.

Differences between estimated and real RSL values were expected. However, theoretical results (45 and 46 days for MZR-100 and MZR-250 cheeses, respectively) may be mathematically corrected by means of a correction factor. In fact, real RSL correspond to 46.7 and 45.7% of calculated and erroneous RSL for MZR-100 and MZR-250, respectively. In other words, the estimated RSL may be multiplied by a number between 0.457 and 0.467 (best choice: average value, 0.462) with the aim of obtaining the real value.

Moreover, the ratio between the net weight of cheeses and the related gross weight is approximately 0.5 for MZR-100 and MZR-250: this number is similar to the supposed correction factor of 0.462.

Consequently, the original Eq. 2.1 may be corrected for MZR in water on condition that a correction factor = 0.5 is used. The chemical meaning should be evident: the higher the amount of free (and bioavailable) water, the higher the expiration of cheeses in water in comparison with normal products. More research is needed, but this hypothesis could confirm the connection between RSL of cheeses in water and the original composition of products after the production. This limitation is already evident in Eq. 2.1 because of the MFFB index [1], but the presence of free water in excess tends to reduce predictable RSL values.

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