PAPER

SHELF LIFE VALIDATION BY MONITORING FOOD ON THE MARKET: THE CASE STUDY OF SLICED WHITE BREAD

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ABSTRACT

The aim of this work was to develop a protocol to validate the shelf life obtained in laboratory based on the product performance on the market. Packed sliced white bread was chosen as target product. Bread shelf life was assessed by measuring the changes in firmness during storage at 25°C and by determining the firmness of bread samples directly purchased from the market with different storage life. Firmness data were modelled by the restricted Avrami equation. Results suggest that shelf life estimates obtained in laboratory should be validated by checking the evolution of product quality on the market.

Keywords: laboratory storage, market conditions, shelf life validation, white bread

1. INTRODUCTION

Shelf life can be defined as a finite length of time after production, during which the food product retains a required level of quality - the so-called acceptability limit - under well-defined storage conditions (NICOLI, 2012a). Shelf life is an important feature of all foods, including raw materials, ingredients and semi-manufactured products. Every packed food has its own shelf life and all the subjects involved in the food chain should be aware of it. Shelf life assessment of foods is a vital process for food companies not only to comply with the legal obligations, but above all to maintain their brand reputation on the market avoiding product recalls. Indeed, the challenge of every food business operator is to ensure that the product characteristics fulfil the requirements of consumers whenever they buy and eat the food.

The adoption of a systematic approach is always necessary to get reliable shelf life data. Shelf life assessment is generally performed under laboratory conditions by developing experimental storage trials, as extensively described by many authors (FU and LABUZA, 1993; ROBERTSON, 2009; GALIC *et al*., 2009; NICOLI, 2012b). The preliminary steps of any shelf life assessment imply the identification of the most critical chemical, physical or biological event leading to the product quality depletion. A suitable quality decay indicator should be then selected and the relevant acceptability limit defined. The next step is the evaluation of the changes of the quality indicator as a function of storage time under laboratory conditions, either mimicking the most probable conditions experienced by the product on the market (real-time shelf-life testing) or able to accelerate deteriorative reactions (accelerated shelf life testing - ASLT) (LABUZA and SCHMIDL, 1995; MIZRAHI, 2000; ROBERTSON, 2009; NICOLI, 2012a). Finally, kinetic data are modelled to estimate reaction rate. The latter is used to compute the shelf life, which is the time needed to reach the acceptability limit (LABUZA and SCHMIDL, 1985; VAN BOEKEL, 1996; CALLIGARIS *et al*., 2012).

Shelf life assessment under laboratory conditions is applied in different stages of the product life-cycle (FU and LABUZA, 1993; NICOLI, 2012b). It cannot be disregarded during the development of new products to assign a suitable food dating. Moreover, it is employed for the on-going shelf life validation of consolidated products. The drawback is that the shelf life values, commonly obtained in laboratory, hold only when the product, in its real life, experiences the same environmental conditions suffered during the shelf life experiments. Obviously laboratory shelf life does not take into account any changes in environmental conditions (e.g. temperature, light) during distribution and storage. In addition, laboratory shelf life tests should be run on a number of different product batches to take into account production variability (CALLIGARIS and MANZOCCO, 2012). It is a matter of fact that the number of batches adopted should be increased proportionally to the lack of production standardization.

Although the discrepancy between laboratory and market shelf life is recognized as a critical issue, there is a lack of information about the possible error deriving from attributing to a product a shelf life based only on laboratory tests. In the light of the continuous improvement process required to increase food industry competitiveness, it could be very profitable to verify if the laboratory shelf life estimates really account for the shelf life of the product on the market shelves.

To our knowledge this study is a first attempt to develop a useful protocol to validate the shelf life obtained in laboratory based on the product performances on the market. To this purpose, packed sliced white bread was chosen as target ambient stable product since it represents a widespread bakery good. The shelf life attributed by the producers to this food category is about 2-3 months. As well studied, sliced bread quality depletion during storage is mainly affected by the crumb firmness increase (HE and HOSENEY, 1990; GIL *et* *al*., 1999; RONDA *et al*., 2011). The latter was thus chosen as critical indicator to estimate the product shelf life. The research was divided in two parts. In the first one, bread shelf life was assessed by means of a typical laboratory shelf life test by measuring the changes in firmness of sliced bread during storage at 25°C. Firmness data were then modelled by the restricted Avrami equation and used to estimate shelf life based on reasonable acceptability limits. The shelf life laboratory test was replicated four times by using bread from different production batches. In the second part of the experimental activity, a high number of bread samples, each belonging to different production batches and having different storage life, were directly purchased from the shelves of stores with different size. Also in this case, bread firmness was evaluated and used to estimate shelf life following the same modelling procedure applied in the laboratory shelf life tests. Discrepancies between laboratory and market shelf life estimates were then discussed.

2. MATERIALS AND METHODS

2.1. Laboratory shelf life test

Freshly made commercial sliced white bread was kindly provided by an Italian factory. According to the producer indications, the shelf life of bread was 75 days, bread slices were superficially treated with ethanol and packed in conventional atmosphere. The original polypropylene bags of bread slices contained 285 g of product corresponding to 16 bread slices having dimensions 9x9x0.9 cm. Twenty-five original packages, all from the same batch, were stored just after production at 25°C in a SMD34 thermostated cell (Dexion, Brescello, Italy). At increasing storage time up to 72 days, two sample packages were removed from the thermostated cell and bread slices were analyzed for firmness. The storage test was replicated four times using different bread batches produced between January and June 2013. The overall number of samples considered in the laboratory shelf life test was equal to 100.

2.2. Market shelf life test

Sliced white bread samples analogous to those considered in the laboratory shelf life test (same producer, production plant, number and dimensions of slices) were purchased on the market by visiting 35 different stores located in the north-eastern part of Italy in the period from November 2012 to April 2013. Selected stores were grocery stores serving a limited number of persons in a small area (small stores); supermarkets with a larger number of customers than traditional groceries (medium stores) and hypermarkets of well-known retailer brands (large stores). The ratio among the number of the small, medium and large stores was 1:1:1. Each sample (two bread packages) was from a different production batch as declared on the label. The storage life after production was calculated from the production date declared by the producer on the product bar code.

2.3. Analytical determinations

2.3.1 Firmness

Firmness of bread crumb was measured by a puncture test using an Instron 4301 (Instron LTD., High Wycombe, UK). The instrumental settings and operations were accomplished using the software Automated Materials Testing System (version 5, Series IX, Instron LTD., High Wycombe, UK). Four slices were taken from each bread package. The central part of the slice (4.5x4.5x0.9 cm) was sampled by manual cut with a sharp knife. A uniaxial compression test was performed at ambient conditions (20±2°C, ambient humidity). Samples were penetrated using a cylindrical probe of 12.7 mm diameter mounted on a 100 N compression head. Crosshead speed was set at 5 cm/min. Force-distance curves were obtained and firmness was taken as the force (N) required to compress the bread crumb by 0.3 cm. Five measurements were performed in different places of each bread slice.

2.3.2 Moisture content

Moisture content was determined by AOAC gravimetric method n. 925.09-1925 (AOAC, 1980) by drying the samples in a vacuum oven (1.32 kPa) at 75°C until a constant weight.

2.4. Data analysis

The results reported in this work are the average of at least twenty firmness determinations for each bread sample. All determinations are expressed as the mean ± standard error (SE).
The changes in bread firmness were analyzed by the Avrami equation (AVRAMI, 1939; 1940; COLWELL *et al*. 1969):

$$\theta_t = \frac{F_{\max} - F_t}{F_{\max} - F_0} = e^{-kt^n} \quad (1)$$

where θ_t is the non-firmed fraction of bread at time *t*, F_{max} is the maximum firmness, F_0 is bread firmness at time 0, F_t is bread firmness at time *t*, *n* is the Avrami exponent and *k* is the rate constant having units depending on the value of *n* ($days^{-n}$). F_0 was assumed to be the lowest firmness value observed in just prepared bread (0.49 N), while F_{max} was taken as the highest firmness value (3.05 N) observed in bread stored for the longest time (72 days). The restricted Avrami equation considering n=1.0 was also applied.
The Avrami equation was fitted using non-linear regression. Parameter estimates relevant to bread from different batches were compared using *t* test with Bonferroni adjustment for multiple testing. Shelf life estimates and related confidence intervals were first computed on the log scale for better normal approximation and delta method was used to compute standard errors. Statistical analysis was performed by using R v. 3.0.2 (The RStudio Foundation for Open Access Statistics).

3. RESULTS AND DISCUSSION

3.1. Laboratory shelf life test

The first step in facing a shelf life assessment test is the definition of the critical indicator to be used to monitor product quality decay during storage. In this work, the evolution of bread quality was monitored by assessing the changes in crumb firmness. This parameter was chosen in accordance with literature data, indicating that crumb firmness is well related to the product rejection by consumers (GAMBARO *et al*., 2004; GIMENEZ *et al*., 2007; GIL *et al*., 1999; RONDA *et al*., 2011).

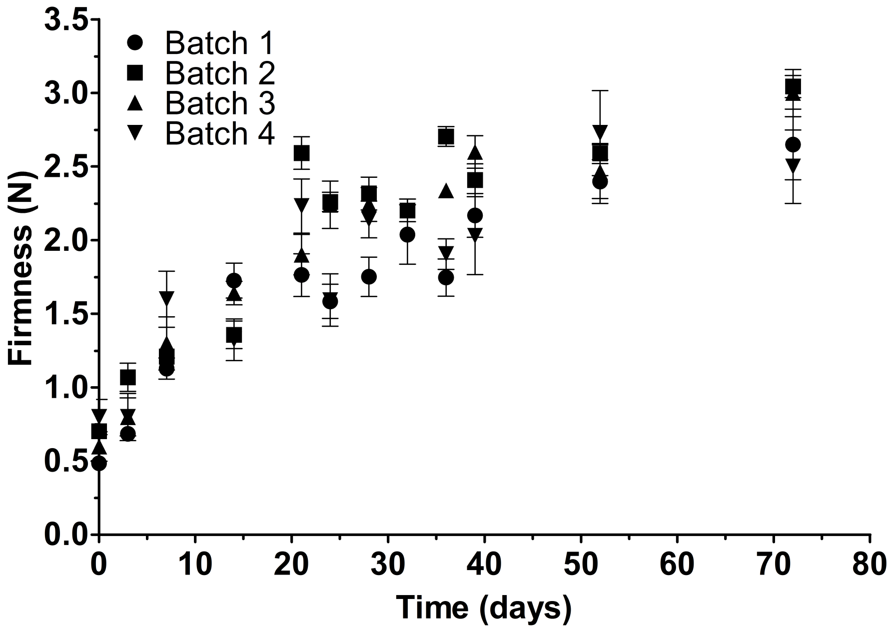

Figure 1: Firmness of sliced bread from different batches stored in the dark at 25°C under laboratory conditions.

Figure 1 shows the firmness increase of the crumb of bread from four different batches during storage at 25°C under laboratory conditions. Just produced samples showed different mean values of firmness accounting for the product batch variability. Although the increase in firmness of the different bread batches proceeded according to similar trends, samples from batches 1 and 4 were generally characterized by lower firmness values than those of batches 2 and 3.

The increase in bread crumb firmness during storage is known to be a complex phenomenon in which multiple mechanisms operate, mainly involving starch retrogradation of gelatinized starch and moisture redistribution (KULP and PONTE, 1981; ATWELL *et al*., 1988; ZOBEL and KULP, 1996; GIL *et al*., 1999; GALIC *et al*., 2009; RONDA *et al*., 2011). Since no changes in moisture were recorded during bread storage at 25°C confirming the moisture barrier properties of the packaging material (data not shown), crumb firming can be mainly attributed to starch recrystallization phenomena. This reversible aggregation involves the progressive re-association of the non-linear amylopectin fraction to form a molecular structure with increased order, which can be assimilated to a crystalline one (ZOBEL and KULP, 1996). Based on these considerations, starch recrystallization in bread can be considered a nucleation controlled phenomena and the changes in firmness can be analyzed by the Avrami equation (COLWELL *et al*., 1969) (equation 1). Firmness data reported in Figure 1 were thus used to predict the value of θ (Fig. 2).

In order to fit the Avrami model to the data, non-linear regression was performed to estimate the model parameters k and n. Table 1 shows that the Avrami exponent n, although close to 1.0 for all batches, actually assumed different values for each batch. According to the Avrami model, the exponent n should be an integer that indicates the crystal growth mechanism. In particular, values close to 1.0 indicate that crystallization occurs by instantaneous nucleation, with nuclei appearing all at once early on the process, and proceeds with formation of rod-like crystals.

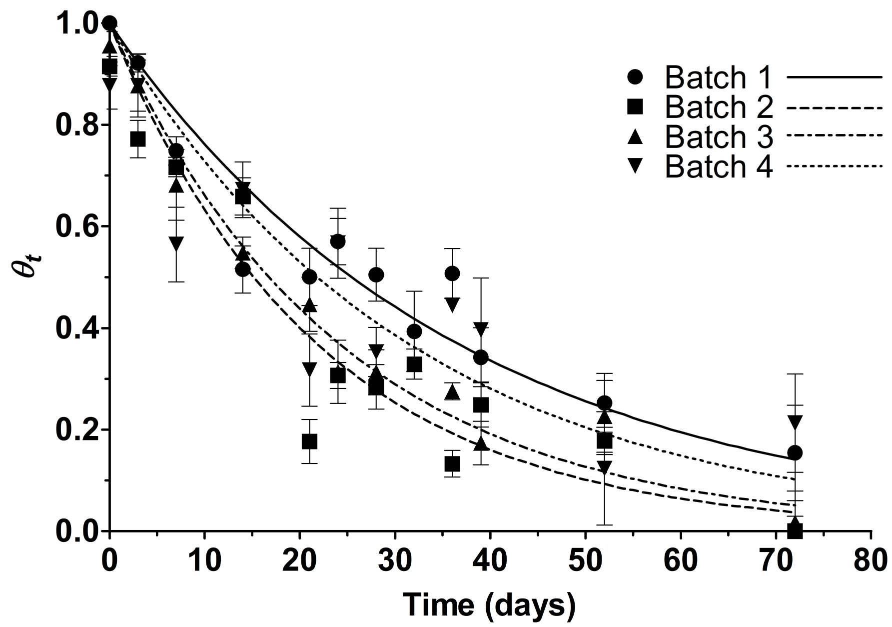

Figure 2: θ_t values (non-firmed fraction) of sliced bread from different batches stored in the dark at 25°C under laboratory conditions. Points: experimental data; lines: predicted values under the restricted Avrami model.

For this reason, the restricted Avrami equation, with n set equal to 1, was fitted to θ_t (Table 1). Reaction rates obtained in this case have the same unit ($days^{-1}$) and can thus be compared. Statistical analysis actually showed that the evolution of sliced bread firmness was significantly affected by the bread batch ($p < 0.05$). It is evident that the production of the bread samples considered in this study is not completely standardized and that samples with higher or lower firming tendency are produced.

Table 1: Parameter estimates of Avrami and restricted Avrami models for sliced bread from different batches stored in the dark at 25°C under laboratory conditions.

	Avrami model		Restricted Avrami model	
	n	*k* ($days^{-n}$)	*n*	*k* ($days^{-1}$)
Batch 1	0.770±0.109	0.060±0.022	1.0	0.027±0.002[b]
Batch 2	0.869±0.167	0.069±0.038	1.0	0.046±0.005[a]
Batch 3	0.871±0.083	0.063±0.017	1.0	0.041±0.002[a]
Batch 4	0.651±0.168	0.102±0.058	1.0	0.032±0.004[ab]

[a,b]: Estimates indicated by the same letter are not significantly different ($p > 0.05$) with the *t* test with Bonferroni adjustment.

The definition of shelf life implies that there should be a quality level discriminating products that are still acceptable for consumption from those no longer acceptable (MANZOCCO, 2012). To estimate the shelf life of sliced bread based on its crumb firmness changes, it is thus necessary to define the acceptability limit. The latter corresponds to the maximum bread firmness the company can tolerate at the end of shelf life. In particular, a company can choose a certain level of θ_t as possible criterion to estimate the limit firmness. For instance, if θ_t at shelf life is chosen to be equal to 0.20, the end of bread shelf life is assumed to be reached when 80% of starch crystallization is achieved. In our samples, this condition would be reached when bread firmness is equal to 2.53 N, as calculated from θ_t defined in equation (1). More restrictive choices can be done by high quality standard companies which may decide that the product is out of shelf life when θ_t is equal to 0.50 (50% starch crystallization), corresponding to a firmness equal to 1.77 N in our samples. Reversely, companies addressing consumers less sensitive to quality aspects could accept a θ_t value equal to 0.10, associated with a 90% starch crystallization. Our bread samples reached this level of staling at a firmness of 2.79 N. Based on these considerations, a reasonable simulation of company choices was performed by considering values of firmness equal to 1.77, 2.53 and 2.79 N as acceptability limits to estimate sliced bread shelf life. Table 2 shows shelf life estimates of sliced bread from different batches in correspondence of these acceptability limits.

Table 2: Shelf life estimates in correspondence of different θ_t values criteria and acceptability limits (firmness) for sliced bread from different batches stored in the dark at 25°C under laboratory conditions. 95% lower and upper confidence limits (LCL and UCL) of shelf life are also reported.

Batch	θ_t	Firmness (N)	Shelf life (days)		
			LCL	Estimate	UCL
1	0.10	2.79	73	85	101
	0.20	2.53	51	59	70
	0.50	1.77	22	25	30
2	0.10	2.79	41	50	65
	0.20	2.53	29	35	45
	0.50	1.77	12	15	19
3	0.10	2.79	50	56	64
	0.20	2.53	35	39	44
	0.50	1.77	15	17	19
4	0.10	2.79	56	72	104
	0.20	2.53	39	51	73
	0.50	1.77	17	22	31

It can be noted that the shelf life estimate was strictly dependent on the choice of the acceptability limit. This result is in agreement with data reported by GUERRA *et al*. (2008), showing that the acceptability limit easily affects the final shelf life by more than 20%. As expected based on the different reaction rates (Table 1), the shelf life of sliced bread resulted significantly affected by the batch. In particular, for a given acceptability limit, the shelf life of sliced bread from batches 2 and 3 resulted comparable and almost half than that of batch 1. Even if batch 4 shelf life estimates were similar to those of batch 1, they were characterized by large confidence levels suggesting the existence of a high variability

within this batch. In other words, each bread batch may show specific firmness variability, begetting shelf life values with considerably different confidence interval. The latter dramatically increase the uncertainty of shelf life.
It is noteworthy that the producer attributes this sliced bread a commercial shelf life equal to 75 days. It can be thus inferred that the company highly tolerated bread firming at shelf life. The latter actually corresponded to an acceptability limit approaching 0.10 θ_t, equal to 2.79 N bread firmness. By selecting such an acceptability limit, the shelf life assessment tests carried out in the laboratory would indicate a shelf life, computed as the average value of the shelf life estimates of the four batches (Table 2), equal to 66±16 days. It is evident that this shelf life estimate intensely suffered by the variability of the firmness kinetics of the four bread batches considered in the experiment. Under these conditions, the application of the conventional laboratory procedure to estimate shelf life would lead to a product dating strictly dependent on the bread batch considered in the experimental test.

3.2. Market shelf life test

To validate whether the laboratory shelf life estimates really account for the shelf life of the product available on the market, bread samples analogous to those considered in the laboratory shelf life test were purchased in local stores having different size, sale volume, logistics and distribution platforms. Collected bread samples not only had different storage time but also had suffered storage conditions that varied depending on the logistics and distribution modalities of the selected store. Bread samples were then analyzed for firmness (Table 3).
It can be noted that, in some cases, samples having the same storage time but taken from different stores exerted considerably different firmness. For instance, samples stored for 43 days presented firmness values between 2.81 and 1.76 N.
Firmness data were then used to compute the evolution of θ_t value (Fig. 3) and modelled according to the restricted Avrami equation (equation 1 with n = 1.0). The relevant rate constant resulted equal to 0.024±0.002. Statistical analysis showed that the rate was not significantly different from that observed for bread slices from batches 1 and 4 stored under laboratory conditions ($p > 0.05$), but significantly lower than those observed for batches 2 and 3.
Table 4 reports the shelf life values of sliced bread taken from the market assuming as acceptability limits firmness values analogous to those selected for shelf life estimate of bread stored under laboratory conditions. Shelf life values similar to those observed for bread slices from batch 1 and 4 (Table 2). The 95% confidence limits appeared similarly wide to those of batch 1, indicating that the shelf life test performed in the lab on samples from batch 1 provided a reasonable estimation of the shelf life of the product stored on the market. By contrast, confidence limits of market shelf life were largely lower than those of batch 4, indicating that the use of this batch to estimate the shelf life of the product on the market would provide an uncertain shelf life value. Finally, performing the shelf life test on samples from batch 2 and 3 would imply an almost 50% underestimation of the actual shelf life of the product on the market.
The discrepancy observed between laboratory and market shelf life could be attributed to a high batch variability as well as changes of environmental conditions during storage and distribution. Results suggest that shelf life estimates obtained in the laboratory should be validated by checking the evolution of product quality on the market. Although time consuming and cost effective, this process could become highly sustainable when integrated in the analysis of historical data concerning the "real" performance of the product on the shelves, including consumer complaints and recall data.

Table 3: Firmness of bread samples collected in different stores and belonging to different production batches as a function of storage time.

Batch	Storage time (days)	Firmness (N)
1	7	0.68±0.03
2	13	1.02±0.09
3	13	1.02±0.16
4	18	1.43±0.13
5	19	1.46±0.16
6	20	1.45±0.23
7	20	1.90±0.03
8	24	1.58±0.10
9	24	1.73±0.02
10	24	2.43±0.20
11	24	2.62±0.09
12	27	0.98±0.10
13	31	1.71±0.14
14	31	1.77±0.13
15	34	1.39±0.04
16	34	2.00±0.09
17	34	2.11±0.09
18	35	1.62±0.27
19	38	1.77±0.17
20	38	2.01±0.20
21	43	1.76±0.14
22	43	1.93±0.15
23	43	2.58±0.10
24	43	2.81±0.18
25	51	1.92±0.09
26	51	2.18±0.15
27	56	2.34±0.18
28	56	2.62±0.02
29	61	2.40±0.22
30	61	2.59±0.15
31	69	2.47±0.11
32	69	3.03±0.06
33	75	2.23±0.19
34	75	2.34±0.10
35	75	2.38±0.23

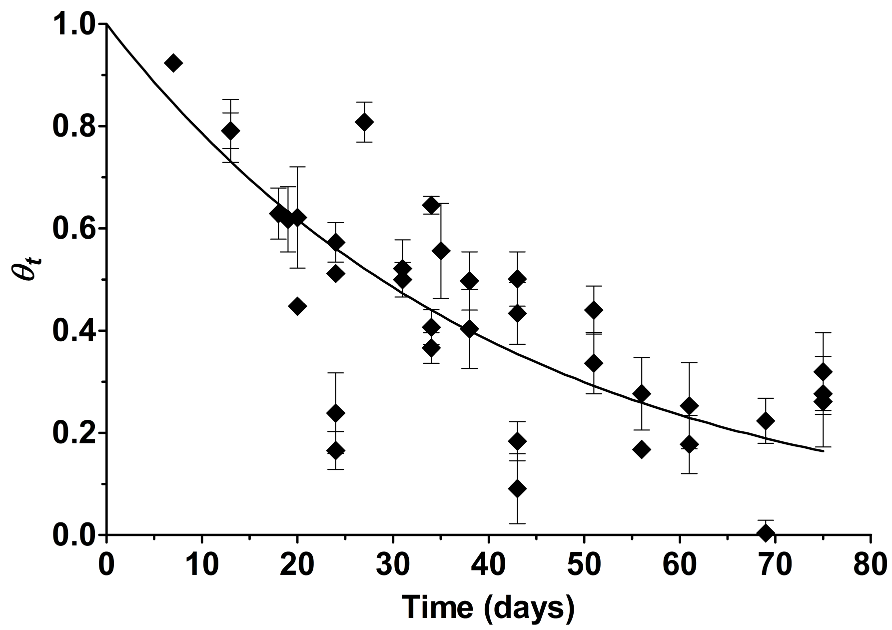

Figure 3: θ_t values (non-firmed fraction) of sliced bread taken on the market. Points: experimental data; lines: predicted values under the restricted Avrami model.

Table 4: Shelf life estimates in correspondence of different θ_t values criteria and acceptability limits (firmness) for sliced bread taken from the market. 95% lower and upper confidence limits (LCL and UCL) of shelf life are also reported.

θ_t	Firmness (N)	Shelf life (days)		
		LCL	Estimate	UCL
0.10	2.79	83	95	112
0.20	2.53	58	67	79
0.50	1.77	25	29	34

4. CONCLUSIONS

The methodology here proposed could represent a powerful tool to validate the reliability of laboratory shelf life dating. Moreover, its application would allow the continuous improvement of shelf life accuracy by progressively adding new data coming from further product batches. On the other hand, the availability of market shelf life data could be exploited to easily identify batch outliers, obtaining indications about possible issues deriving from variability of raw material, formulation or processing conditions.

Although data were relevant to the study case of sliced white bread and additional studies should be accomplished to fully validate the proposed approach, results suggest that caution should be paid to shelf life estimation carried out only under laboratory conditions. It is a matter of fact that further complications should be expected in products stored under chilled and frozen conditions, for which slight temperature changes could dramatically modify the kinetics of quality depletion as well as the prevalent event affecting shelf life.

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