Food Packaging and Shelf Life 40 (2023) 101172

Contents lists available at ScienceDirect

Food Packaging and Shelf Life

journal homepage: www.elsevier.com/locate/fpsl

Assessment of the secondary shelf life of Bolognese sauce based on domestic use simulation

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ARTICLE INFO

Keywords:
Household food waste
Period after opening
Microbial acceptability
Sensory acceptability
Sustainability

ABSTRACT

Household food waste contributes significantly to global food waste throughout the food supply chain, resulting in a substantial waste of resources. The inability of consumers to utilize food before it spoils generally leads to its disposal, thus representing one of the causes of food waste. The secondary shelf life (SSL) is the period a product should be consumed within once open, specified in the label, which sometimes is extremely short and may lead to a significant amount of discarded food. This study focuses on evaluating the SSL of Bolognese sauce through domestic use simulation and monitoring its microbiological and sensory quality, to explore the potential extension of the SSL, allowing consumers more time to consume the product. Results show that, even under harsh usage conditions, Bolognese sauce can have a SSL longer than 13 days, 2–3 times longer than the current SSL. The results highlight the influence of consumer behavior in determining the SSL, suggesting that improved food management could further reduce household food waste.

1. Introduction

One third of the global food production intended for human consumption is lost every year, from the agricultural production to consumers' homes. It is estimated that the extent of food that was still suitable for consumption before its loss is about 1.3 billion tons per year (FAO, 2013).

The production of food that is wasted causes unnecessary environmental impacts, arising from the consumption of resources and energy and from the release of pollutants and greenhouse gases (GHG) in the atmosphere. FAO estimated that the total carbon footprint of food wastage is between 3.5 and 4.1 Gtons of CO_2 equivalents per year. It means that, if food waste was a country, it would rank third, after China and USA, for GHG emissions (FAO, 2013; FAO, 2014).

The environmental impact depends on the type of food product that is wasted, e.g., plant products require less energy input than products of animal origin (Licciardello & Piergiovanni, 2020). To get an idea, the waste of meat products represents about the 2% by weight of the total food waste but impacts for the 16% of the greenhouse emission of food waste (Venkat, 2011). In addition, the food waste occurring at the consumption phase requires the highest amount of energy, and has the highest carbon footprint, compared to a loss occurring in the early or middle stages of the product life. In this context, the reduction of wastes at the consumption phase would have a great effect on their environmental impact mitigation.

Household food waste constitutes a significant proportion of global food waste throughout the entire food supply chain, especially in high-income countries, accounting for approximately 61% of the total food waste (FAO, 2011; UNEP, 2021). The amount of household food waste in industrialized countries is higher than in developing countries mainly due to economic reasons, since in the firsts people can afford to waste food, while in developing countries it is considered unacceptable (FAO, 2011).

According to Waste and Resources Action Programme (WRAP, 2013) the majority (around 60%) of the household food waste is defined 'avoidable', i.e. edible food and drink that could have been consumed but become waste for various reasons (European Commission, 2018). Therefore, focusing on waste prevention at the household level may be a highly effective strategy for reducing food waste and promoting the establishment of sustainable food systems (Falasconi et al., 2019; Giordano, Alboni, Cicatiello, & Falasconi, 2019).

The generation of food waste at the consumption level in

Abbreviations: PSL, primary shelf life; SSL, secondary shelf life.

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https://doi.org/10.1016/j.fpsl.2023.101172
Received 9 March 2023; Received in revised form 30 July 2023; Accepted 10 September 2023
Available online 27 September 2023

industrialized countries is influenced by a complex set of factors, mainly driven by consumers' individual behaviors both in-store and at home. These behaviours are given by psychological, socio-cultural, economic, and demographic factors (Falasconi et al., 2019; Vittuari et al., 2023). These factors affecting consumer behavior are defined as 'drivers' of consumer food waste at the household level (Vittuari et al., 2023). Among the factors influencing household food waste, frequency of grocery shopping, dining-out behavior, storage strategy (e.g., unseen stored food), extra cooking, skills in using leftover food, grocery expenditure, cultural identity, and household composition are some of the most significant influencers (Ananda, Karunasena, Mitsis, Kansal, & Pearson, 2021; Giordano et al., 2019).

Additionally, the interpretation of date labels plays a main role in determining domestic food waste generation (WRAP, 2011). The most commonly cited reasons for discarding food by consumers include "spoiled food", "not used in time", "bought too much", "cooked, prepared, and served too much", along with other reasons related to personal preferences and accidents (Falasconi et al., 2019; Giordano et al., 2019; WRAP, 2013). These reasons for disposal may vary depending on the category of food being considered (WRAP, 2013).

In the light of these various reasons, a recent review provides an analysis of the 'levers' for designing interventions aimed at reducing consumer food waste, based on specific drivers (Vittuari et al., 2023). From the perspective of consumers, interventions should focus on improving food managements skills, such as list writing, meal preparation, and storage methods (Ananda et al., 2021; Vittuari et al., 2023). Additionally, the authors proposed communication strategies that emphasize the environmental impact of food waste and promote the results derived from good practices at home, in order to raise consumers' awareness (Vittuari et al., 2023). Other levers are related to regulations defined beyond the consumer level, such as the different types of expiration dates, including the extension of package date labels (Vittuari et al., 2023).

The present study specifically focuses on the latter lever, namely the label information concerning the period for consumption after opening. Traditionally, food technologists have been concerned with extending the primary shelf life (PSL) of products. However, considering that most of food waste occurs at home, scientific studies should also focus on extending the product life after opening, known as the secondary shelf life (SSL), to prevent food waste without compromising food safety (WRAP, 2015).

The secondary shelf life is the period that a product should be consumed within once opened, and it is specified on the label with indication such as 'best if used within *x days after opening*' (European commission, 2018; WRAP, 2015). This indication is crucial for food products which undergo a deterioration after opening, while it is recommended to not indicate the SSL for foods where opening only influence quality deterioration, and not food safety (WRAP, 2015). The indication of SSL supersedes other durability coding such as 'use-by' and 'best before' dates and it is often very short (e.g. 24 – 48 h for some food categories), resulting in strict timespans for food consumption after opening and potentially leading to high amount of wasted food (WRAP, 2015).

In the Regulation (EU) No 1169/2011, the 'best before', the 'use by' date, and any special storage or use conditions are included within the mandatory food information, while the indication about SSL is not mandatory. Article 25 of the Regulation states that "to enable appropriate storage or use of the food after opening the package, the storage conditions and/or time limit for consumption shall be indicated, where appropriate" (European Parliament & Council of the European Union, 2011). Therefore, the indication concerning the SSL is at the discretion of the food business operator (FBO), or the food company itself. It was reported that, for many products, the information provided is set historically or as an industry standard (WRAP, 2015), rather than based on an experimental approach.

According to the European Commission's Directorate-General for Health and Food Safety (European commission, 2018), avoidable food waste can be reduced when the indication concerning the SSL is based on safety and quality studies. The Waste and Resources Action Programme (WRAP, 2015) states that is possible to extend the shelf life of a wide range of products, without any change to packaging or product itself. Even a small increase (e.g., one day) in SSL can extend the timespan for the consumer to use the product and potentially reduce the waste of foods with short shelf lives (e.g., between 3 and 12 days). This is particularly true after package opening, since the SSL is the shortest among the date markings, and because it supersedes other expiration dates of the product. In this context, our study proposes an experimental verification of the SSL information provided on the label of some food products, to assess its accuracy and to explore its possible extension, without changing the product formulation or the packaging.

However, studying the SSL faces challenges since it is characterized by indefinite conditions, unlike the primary shelf life (PSL). Food exposure to microorganisms may vary depending on the opening conditions of the product, such as the contamination of the environment, surfaces and kitchen utensils, as well as the time and number of openings of the same package. These sources of variability compromise the stability of the product. Indeed, besides the recontamination, the package opening involves variation in intrinsic, extrinsic, and implicit factors, which can influence microbial growth and therefore determine a more rapid deterioration (EFSA, 2021).

Given the importance of the information regarding storage conditions and shelf life of food after opening, the EFSA has developed a decision tree to support FBOs in determining the time limit for consumption after opening and information to consumers (EFSA, 2021). The decision tree guides the FBO through five questions, to decide whether the secondary shelf life should be the same as or shorter than the primary shelf life (either 'best before' or 'use by' date). Additionally, EFSA recommends indicating the SSL for those products where safety is the main concern after pack opening. Despite EFSA's guidance helps understand the mechanism involved in food deterioration after opening, it does not provide methods for the scientific determination of the SSL.

A proposed method for assessing the SSL found in the literature is a deterministic approach (Nicoli & Calligaris, 2018), in which the real domestic conditions and consumers' use are simulated. We previously implemented such a deterministic approach for the assessment of SSL of Pesto alla Genovese (Nicosia, Fava, Pulvirenti, Antonelli, & Licciardello, 2021) and UHT milk (Nicosia, Fava, Pulvirenti, & Licciardello, 2022), based on microbiological, sensory, and physicochemical analyses, that allowed us to establish the real duration after opening of the products.

The purpose of this study was to objectively re-assess the SSL of "Ragù alla Bolognese" (Bolognese sauce), a traditional dressing for pasta widely used worldwide, based on the simulation of domestic use and storage and on the monitoring of microbiological, sensory and physicochemical attributes. The study aims to explore the possibility of extending the labeled indication of SSL, without modifying the product characteristics or packaging.

2. Material and methods

2.1. Bolognese sauce

In the first part of the study, we collected samples of all the different brands of shelf-stable Bolognese sauces available in the main supermarkets in Reggio Emilia (Italy). The range of brands found is representative of the Italian retail chains. A total of sixteen commercial brands were identified, and they were all collected for evaluation of their intrinsic parameters, including water activity (a_w) and pH, as well as indications of SSL mentioned in their labels. The measurement of water activity (a_w) was performed through the dew point water activity meter AquaLab 4TE (Meter Group, Inc., Pullman, WA, USA), and the pH was measured following the method described in Section 2.5.

Based on the results of the intrinsic parameter analysis, one among the sixteen brands was chosen for further testing, as explained in Section 3.1. The selected brand of Bolognese sauce was packaged in a 400-g glass jar with a metal cap. The composition was: chopped tomatoes 28%, water, tomato concentrate 6.5%, beef 9.5%, pork 9.5%, onions, carrots, celery, sunflower seed oil, cornstarch, salt, yeast extract, sugar, bay leaves 0.06%, sage extract, black pepper. The indication reported in the label is to consume within 5 days from opening, under refrigerated storage.

2.2. Protocol of domestic use

The assessment of the SSL is subjected to high variability, mainly due to the domestic conditions of use. Since the variability of the consumers' behavior can impact the sensory and microbiological stability of the product, SSL studies require a simulation of domestic use of the products.

50 jars of the same production batch of Bolognese sauce were distributed in 10 households (coded A-J), where a simulation of domestic use was performed. The participants involved were selected based on their availability and their willingness to participate on a voluntary basis. The participants were working at the laboratory of Food Sciences and Technologies at the Department of Life Sciences, University of Modena and Reggio Emilia. The test was carried out in two steps, in five households at a time, due to the large number of samples and analyses to be performed.

Prior to the test, participants were provided with instructions on how to perform the domestic use simulation protocol, with a schedule containing information on the opening days and sampling days, and properly coded Bolognese jars. The jars were opened simultaneously in the five domestic environments (day 0) and left uncapped for 15 min. In the meanwhile, 3 spoonful (about 70 g) were removed. The opening procedure was performed on five jars in each home environment. Subsequently, jars were closed and maintained at room temperature for 40 min, before being placed in domestic fridges. To simulate repeated use and to exacerbate consumer use conditions, this protocol was replicated after 2 and 4 days from the first opening. Temperatures of the ten domestic refrigerators (named from A to J) were recorded at 1-h intervals for the first four days of storage, to get an idea of possible temperature fluctuations. The measurements were carried out using mini TH temperature dataloggers (XS Instruments, Carpi, Italy).

After 5, 8, 13, 16, and 19 days of domestic storage after first opening, one jar from each household was delivered to the laboratory, while maintaining refrigeration temperature using a cooler bag. Therefore, on each sampling day, 10 jars of Bolognese sauces, each coming from a different household, were subjected to microbiological, sensory, and chemical-physical analyses.

2.3. Microbiological analysis

Microbial quality assessment was performed at 5, 8, 13, 16, and 19 days after the first opening. Although packaged Bolognese sauce is sterile, due to the thermal treatment to which it is subjected, the package opening allows the recontamination of the product. Thus, microbiological analyses were carried out to assess the degree of contamination of Bolognese sauce during 19 days of refrigerated storage after the first opening and to verify compliance with food safety requirements.

Ten grams of each Bolognese sauce were diluted with 90 g of sterile physiological solution (0.9% NaCl) in sterile stomacher bags and homogenized in a laboratory Stomacher 400 blender (Seward Limited, Worthing, UK) at high speed for 120 s. Ten-fold serial dilutions were prepared in sterile physiological solution. The pour plate method was used for the microbial count. The following culture media and incubation parameters were used. The Plate Count Agar (PCA, Tryptic Glucose Yeast Agar, Biolife, Milan, Italy) was used for the quantification of the aerobic mesophilic count, after incubation at 30 °C for 48 h. Rose Bengal Chloramphenicol (RBC) Agar (Biolife, Milan, Italy) was selected for the enumeration of yeasts and molds after incubation at 25 °C for 4–5 days.

All experiments were performed in duplicate. The results of the microbiological analyses are expressed as $\log_{10}$ CFU/g of Bolognese sauce.

As reported in our previous study (Nicosia et al., 2022), a microbial threshold of 6 $\log_{10}$ CFU/g for the aerobic mesophilic count was chosen to define the end of the SSL of Bolognese sauce. Indeed, this limit represents the maximum acceptable microbial load in food, while the value of 7 $\log_{10}$ CFU/g indicates spoiled foods (Wijtzes, van't Riet, Huis in't Veld, & Zwietering, 1998).

2.4. Sensory analysis

Sensory analysis is an essential tool for assessing the acceptability of food, and has proven to be crucial, especially in SSL studies (Nicosia et al., 2022). Indeed, sensory evaluation throughout the period after opening is complementary to hygienic quality.

Sensory sessions were performed at 5, 8, 13, 16, and 19 days after the first opening of jars. The sensory evaluation, in particular, aimed to assess the degree of acceptance of the samples throughout the period after opening, from a visual, olfactory, and gustatory point of view. Based on the results of microbiological analyses, the sensory evaluation was either complete or based only on a visual and olfactory evaluation. A sensory panel of 10 trained members was selected among the staff of the Department of Life Science of Unimore and was trained before the analysis. During the training session the panelists were provided with a reference sample (from a just-opened jar) to familiarize with the appearance, taste, and odor of the product. During each sensory evaluation, judges were asked to compare the reference sample with samples withdrawn from jars opened and stored according to the simulation protocols.

Ten grams of each Bolognese sauce were placed into plastic spoons and left at room temperature for 15 min before the analysis. The order of the samples was randomized for each assessor and for each test day. In addition, the participants were provided with unsalted crackers and still water to be used during the tasting. Each assessor was asked to evaluate six samples of Bolognese sauce, the first of which was a reference (just-opened) sample. The remaining five samples were from home environments (A to E, and F to J) and were divided into two separate sessions due to the large number of samples to be evaluated.

The judges were asked to rate the global acceptability of the Bolognese sauces on a scale from 0 to 10 (extremely dislike to extremely like). Based on our previous work (Nicosia et al., 2022), two sensory thresholds were established and samples were evaluated as not acceptable for consumption when: *i*) the average acceptability was less than 6, or when *ii*) 40% or more of the judges rated the sample acceptability lower than 6.

2.5. pH determination

The pH of Bolognese sauces was monitored throughout the period after the first opening, performing the measurements at 5, 8, 13, 16, and 19 days after opening.

The product was homogenized with physiological solution and the pH was measured under stirring using a CyberScan pH 310 (Thermo Fisher Scientific Inc., Waltham, MA, USA), previously calibrated with buffer solutions at pH 7.0 and 4.0 (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). The pH measurements were conducted in triplicate.

2.6. Statistical analysis

Statistical analysis was performed by one-way and two-way analysis of variance (ANOVA). Tukey's HSD post hoc test was performed to assess significant differences amongst groups ($p < 0.05$). In addition, the relationships among variables were studied by applying Pearson correlation test. The analyses were performed using SPSS version 26 (IBM

Corp., New York, NY, USA) and RStudio (version 2022.12.0 +353; RStudio, Boston, MA). Unless stated otherwise, the experiments were carried out in duplicate. The results are expressed as means ± standard deviations (SD).

3. Results and discussion

To objectively define the SSL, microbiological and sensory parameters were compared with the established threshold values to determine the acceptability of the products. In this study, the assessment of the SSL follows a risk-based approach. For this reason, only the most deteriorated samples or those with the worst ratings will be reported in the results.

3.1. Brand comparison

Sixteen different brands of Bolognese sauce were found in the market, and pH and a_w values were analyzed. In addition, the information about SSL found on the product label was reported (Table 1).

The chosen brand of Bolognese sauce (Brand 1 in Table 1) was selected based on its wide availability in retail shops and on its intrinsic properties (i.e., pH and a_w, both above the average), which theoretically make it more sensitive to spoilage compared to other brands.

Although some ingredients, such as tomato, increase the acidity of Bolognese sauce, the pH is often higher than 4.5 and in addition, the a_w higher than 0.94 can support the growth of several microorganisms (Coorey et al., 2018).

3.2. Storage temperatures

Fig. 1 shows the temperature profiles of the ten domestic refrigerators (households A - J), recorded for the first four days of storage after the first opening of the Bolognese sauces. The recorded temperatures are highly variable, ranging from a minimum value of 3.1 °C (day 3, refrigerator C) to a maximum of 15.4 °C (day 4, refrigerator F). The complete set of temperature values can be found in Table S1, among the Supplementary material.

As already reported in other SSL studies (Nicosia et al., 2021; Nicosia et al., 2022), in most cases the recorded temperatures were higher than recommended by producers for refrigerated storage (0–6 °C). Despite three refrigerators (C, E, and J) were in the range of recommended temperatures, seven fridges out of ten had very high mean temperatures,

Table 1
Intrinsic parameters and labeled secondary shelf life of sixteen commercial Bolognese sauces.

Brand of Bolognese Sauce	pH	a_w	Labeled SSL
1	**4.92^{bc}**	**0.9851^{bc}**	***5 days***
2	4.32^{g}	0.9802^{g}	*3–4 days*
3	4.75^{d}	0.9847^{bcd}	*3–4 days*
4	4.61^{e}	0.9877^{a}	*2 days*
5	5.20^{a}	0.9840^{bcdef}	*3–4 days*
6	4.44^{f}	0.9841^{bcdef}	*4 days*
7	4.95^{bc}	0.9861^{ab}	*3 days*
8	4.40^{fg}	0.9845^{bcde}	*3–4 days*
9	4.46^{f}	0.9823^{defg}	*3–4 days*
10	4.86^{bcd}	0.9830^{cdef}	*Short time*
11	4.97^{b}	0.9832^{cdef}	*3 days*
12	4.42^{fg}	0.9823^{efg}	*4 days*
13	4.88^{bc}	0.9838^{bcdef}	*Short time*
14	4.35^{fg}	0.9818^{fg}	*4 days*
15	4.37^{fg}	0.9800^{g}	*4 days*
16	4.84^{cd}	0.9828^{cdef}	*2–3 days*
average ± SD	4.67 ± 0.28	0.9835 ± 0.002	3–4 days*

* the mean was calculated considering the average of two days, where SSL was reported as a range. The indications "short time" were excluded from the mean calculation. Different superscript letters in the same column indicate significant differences among mean values ($p < 0.05$).

reaching peaks of 13–14 °C in refrigerators A and F.

The temperatures measured in this study can give a representation of realistic conditions that may be found in domestic environments. Based on the recorded temperatures, we can conclude that the Bolognese sauce samples were stored under both optimal and stress conditions.

3.3. Microbiological analysis

The standard industrial production of Bolognese sauce consists of cooking for 2 h at 90–100 °C, followed by hot filling at 70 °C, and sterilization at 121 °C (Prandi et al., 2019). This intense thermal treatment leads to the inactivation of both the vegetative and the spore forms, resulting in a sterile product. Nevertheless, the recontamination of sterilized Bolognese sauce occurs after the package opening at home. For this reason, microbiological spoilage is considered as the most important parameter determining the end of the SSL for sterilized foods (Nicoli & Calligaris, 2018).

Results of microbiological analyses are reported in Table 2, that shows the aerobic mesophilic count and yeasts and molds count of the Bolognese sauces, during 19 days of domestic storage after opening.

As already observed in previous studies on the SSL of sterile products, the contamination of the samples throughout the time after opening is subjected to a certain variability (Nicosia et al., 2021; Nicosia et al., 2022). Indeed, within each domestic environments, the opening protocol is performed simultaneously on five Bolognese jars (see Section 2.2). Each jar is used for each sampling day, therefore the contamination of the jars may be different depending on casual factors, such as the different air flow entering the specific jar, through which the samples can be contaminated.

The Bolognese samples showed a high stability throughout the period after opening. All the samples were highly acceptable until the 13th day after opening, with a maximum growth of the aerobic mesophilic bacteria of 3.2 $\log_{10}$ CFU/g, observed on day 5 in the sample from domestic environment I. On the 16th day after package opening, one among ten samples (sample D) slightly exceeded the microbial threshold with a count of 6.2 $\log_{10}$ CFU/g, indicating the unacceptability of the product. It is worth reminding that the above-mentioned samples were selected based on significant contamination, while other samples (such as those from houses A, B, C, E, and H) showed very low or no contamination for the entire 19-day period.

As regards yeasts and molds, contamination was only recorded in two samples (I and J), while in the other eight samples, no growth was detected throughout the time span examined. Considering sample I, which had the highest yeast and mold contamination, the maximum value observed was 3.7 $\log_{10}$ CFU/g on the 19th day after opening.

Interestingly, samples from household A never showed detectable growth for any of the microbial groups and at any day of sampling, even though refrigerator A was the one with the highest average temperature of 10.3 °C. Indeed, no significant correlation was observed between the microbial growth and the storage temperature of samples from household A.

Pearson's correlation analysis highlighted a correlation ($p < 0.05$) between storage temperature and aerobic mesophilic bacteria only for the samples from home environments I and F. Furthermore, in the samples from household I, a correlation ($p < 0.001$) was observed between the storage temperature and yeast and molds count.

Overall, the Bolognese sauces showed a high stability from a microbiological point of view. Based on a worst-case approach, the analyzed samples are fit for consumption up to 13 days after first opening, even under stressful storage and use conditions.

3.4. Sensory analysis

Sensory evaluation was crucial for determining the consumer acceptance of the samples. As previously stated (see Section 2.4), the overall acceptability rating of the ten samples from households A to J

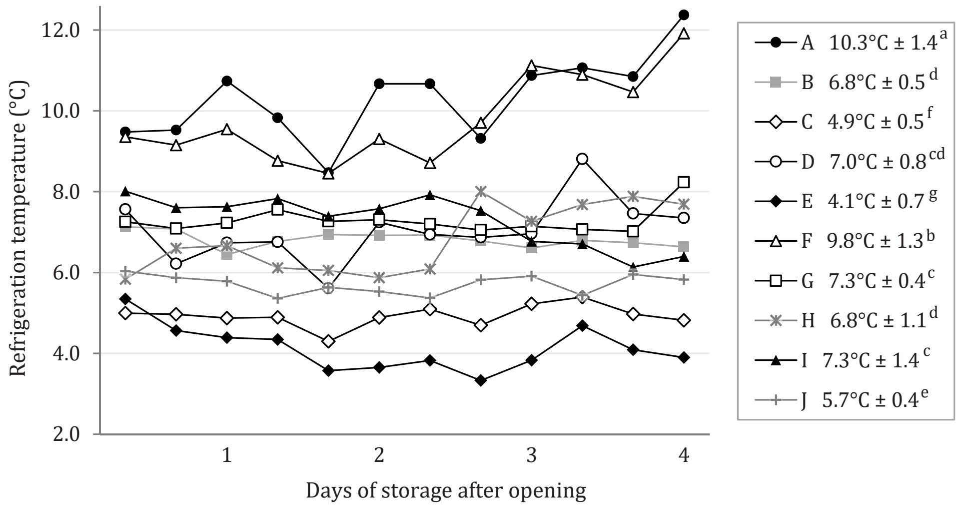

Fig. 1. Temperatures (°C) of the ten domestic refrigerators, recorded during the first four days of storage after opening of the Bolognese sauces. Measurements were taken at 1-hour intervals (n = 96). For the sake of simplicity, the data shown in the figure represent the average values over an 8-hour period, while the legend reports the overall average temperature of each refrigerator. Different superscript letters in the legend indicate significant differences among mean values ($p < 0.05$).

Table 2
Microbial growth of (a) aerobic mesophilic count and (b) yeasts and molds observed in Bolognese sauce samples stored in ten domestic environments (A to J) for 19 days after the first opening. Values are expressed as $\log_{10}$ CFU/g of Bolognese sauce.

(a) Aerobic mesophilic bacteria ($\log_{10}$ CFU/g)										
Days after opening	Domestic environment									
	A	B	C	D	E	F	G	H	I	J
5	n.d.	0.87 ± 1.23	n.d.	n.d.^{b}	n.d.	2.77 ± 0.07	1.84 ± 0.09	1.87 ± 0.12	**3.16 ± 0.07^{a}**	n.d.
8	n.d.	n.d.	n.d.	n.d.^{b}	n.d.	1.79 ± 0.44	1.09 ± 1.54	n.d.	**2.07 ± 0.16^{b}**	n.d.
13	n.d.	n.d.	n.d.	n.d.^{b}	n.d.	1.84 ± 0.34	**1.93 ± 0.21**	0.85 ± 1.20	n.d.^{c}	1.35 ± 1.91
16	n.d.	n.d.	n.d.	**6.15 ± 0.21^{a}**	n.d.	2.04 ± 0.19	1.84 ± 0.09	n.d.	2.04 ± 0.19^{b}	n.d.
19	n.d.	n.d.	n.d.	n.d.^{b}	n.d.	1.54 ± 0.34	1.91 ± 0.19	n.d.	**3.58 ± 0.1^{a}**	2.92 ± 0.01
(b) Yeasts and molds ($\log_{10}$ CFU/g)										
Days after opening	Domestic environment									
	A	B	C	D	E	F	G	H	I	J
5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.^{c}	n.d.^{b}
8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**2.07 ± 0.16^{b}**	0.5 ± 0.71^{b}
13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.^{c}	n.d.^{b}
16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**1.92 ± 0.11^{b}**	n.d.^{b}
19	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**3.68 ± 0.44^{a}**	2.89 ± 0.0^{a}

Data are shown as mean ± standard deviation. Different superscript letters in the same column indicate significant differences ($p < 0.05$) among mean values of the different days of sampling. If letters are not provided, no significant difference was observed.
n.d.: microbial growth not detected.
bold: highest value of that sampling day.

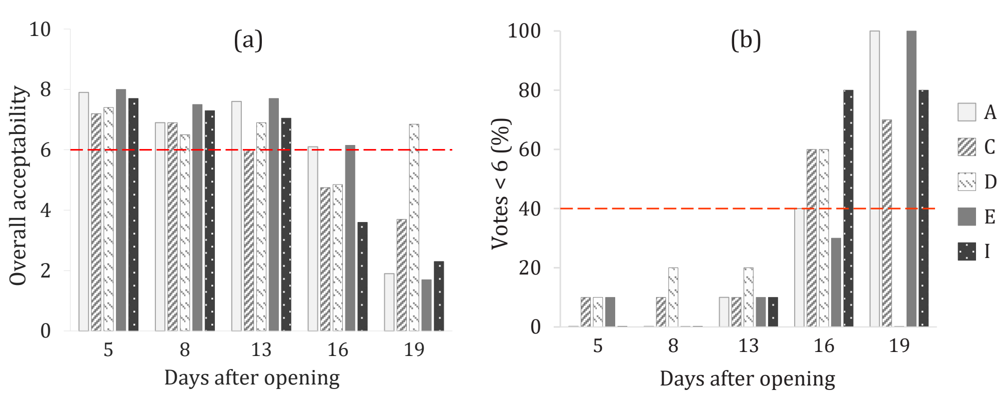

Fig. 2. (a) Average acceptability values and (b) percentage of ratings lower than six for Bolognese sauce samples, during 19 days of domestic storage after opening.

was compared with two sensory thresholds.

For the sake of simplicity, only the samples with the worst evaluations are reported in Fig. 2. The graph shows the acceptability factors, namely: the average acceptability rating of the Bolognese samples (Fig. 2a) and the percentage of panelist that rated the samples below 6 (Fig. 2b).

All the Bolognese sauce samples scored an average acceptability greater than or equal to 6, up to the 13th day of storage after opening. Similarly, in the first 13 days after package opening the percentage of judges who found the samples unacceptable was well below the established limit, reaching a maximum of 20%. Thus, considering both sensory thresholds, the samples were acceptable up to 13 days after opening. On the 16th day of storage, several samples exceeded the limits: samples C, D, and I had average acceptability values between 3.5 and 5. Moreover, the second threshold was overcome by the same samples and sample A, with 40–80% of the assessors giving negative judgement. Statistical analysis highlighted a negative correlation ($p < 0.05$) between sensory evaluation and aerobic mesophilic count in sample D, characterized by high contamination and low sensory evaluation on day 16 after opening.

In general, the sensory acceptability of the Bolognese sauces decreased over the time after opening, indeed the two parameters were found to be negatively correlated ($p < 0.001$) by the Pearson's test. Nevertheless, the samples of domestic environments F and G did not follow this trend; in fact, their overall sensory acceptability remained constant over time. The two samples were also characterized by a low and nearly stable microbial load during the 19 days of analysis.

3.5. pH measurements

The initial pH of the Bolognese sauce used in this study was 4.92 ± 0.06. In line with this value, Sapers, Phillips, & DiVito (1982) assigned tomato-meat sauce to the low acid foods category (pH > 4.6) and reported a pH in the range of 4.6 – 5.0 for this product. In another study, the initial pH of a tomato and pork sauce cooked in *sous vide* was similar to the value found in our study, namely 4.82 and 5.16, depending on the packaging method used (Díaz, Garrido, & Bañón, 2010).

In the present study, we observed a slight but not-linear decrease in pH values during the 19 days after opening the package. Table 3 shows the average pH value considering the Bolognese samples from the ten home environments, while the complete pH data can be explored in the Supplementary file (Table S2).

The observed pH changes were not influenced by microbiological contamination, both aerobic mesophilic bacteria and yeasts and molds. Indeed, the variables pH and microbial growth were not correlated according to Pearson's test. When tomato products are contaminated by molds, the pH tends to increase over time (Mundt, 1978). The only mold-contaminated samples in our study were samples I and J, which reached maximum contamination on day 19. However, the pH was not affected by mold growth, as their pH on day 19 was not significantly different from the other samples.

Changes in pH over time were found to be significant ($p < 0.05$) by one-way ANOVA; in addition, Pearson's analysis highlighted a negative correlation between pH values and time (Pearson's coefficient – 0.61, $p < 0.001$). However, it is worth noting that the observed changes were within a narrow range, from a mean of 4.92 at day 0, to a minimum of 4.69 recorded at day 16.

Table 3
pH of Bolognese sauce throughout a period of 19 days after package opening. Each value is the average of the samples opened and stored in ten different domestic environments.

Days after opening	Mean pH
0	4.92 ± 0.06^a
5	4.85 ± 0.03^b
8	4.77 ± 0.05^c
13	4.82 ± 0.04^b
16	4.69 ± 0.04^d
19	4.74 ± 0.06^c

Different superscript letters indicate significant differences ($p < 0.05$).

In line with our findings, slight pH changes over time were observed in a tomato and pork sauce cooked in *sous vide* and stored at 2 °C for 90 days (Díaz et al., 2010). Furthermore, in our study on the SSL of pesto sauce (Nicosia et al., 2021) we observed a pH variation over time, similar to that observed in Bolognese sauce. Therefore, slight fluctuations in pH might be considered normal during refrigerated storage after package opening. Furthermore, the pH trend did not affect the microbiological stability of the Bolognese sauce.

3.6. Reassessment of the secondary shelf life

The degradation phenomena responsible for the end of the secondary shelf life are different from those causing the end of the primary shelf life (Nicoli & Calligaris, 2018). In the case of a complex food system, such as Bolognese sauce, the factors affecting the durability of the product may be various, depending on the formulation and method of preparation. The primary shelf life of Bolognese sauce may be determined by chemical changes, such as oxidation, and the resulting quality deterioration related to changes in sensory and nutritional attributes. Indeed, these are the main factors influencing the PSL in tomato puree and meat products (Munekata et al., 2020), the main ingredients of Bolognese sauce.

Conversely, once the packaging is opened, different degradation phenomena occur which affect the secondary shelf life of food. In the case of sterilized food, the activity of spoilage microorganisms is the principal cause affecting the secondary shelf life (Nicoli & Calligaris, 2018). Furthermore, as mentioned above (see Section 2.4), we have established two sensory thresholds. Based on both microbiological and sensory thresholds, it is possible to objectively assess the duration after opening of the Bolognese sauce and re-evaluate its secondary shelf life.

The opening protocol was performed in ten domestic environments, whereby ten Bolognese jars from the different households were analyzed on each sampling day. The end of the SSL was determined when the first sample out of ten overcame at least one of the established thresholds.

Results from microbiological analysis suggested that the Bolognese samples were perfectly suitable for consumption up to 13 days after the package opening, as the first threshold was exceeded on the following sampling day. Likewise, the sensory test showed that both thresholds were exceeded on day 16, while up to 13 days of storage after opening the samples were fully acceptable to consumers.

Overall, the Bolognese sauce used in this study can achieve a secondary shelf life of at least 13 days, even when opened and stored under stressed conditions.

4. Conclusion

We have described a method for simulating domestic use of foods, which can represent a guiding procedure in the case of secondary shelf life studies. This work combined end-user household use, carried out in ten households, with objective monitoring of safety and quality parameters. Considering the established microbial and sensory thresholds, our findings suggest that the secondary shelf life of Bolognese sauce can be extended up to 13 days, compared to the 5 days currently indicated on the label.

Considering the high variability to which secondary shelf life studies are subject, our results should be confirmed by further studies, possibly with more home environments in which to perform the opening procedure.

It is worth noting that our result was achieved with samples subjected to stress conditions, such as a long period uncapped and at room temperature, repeated opening of jars, and storage at medium to high refrigeration temperatures. In addition, the end of SSL was defined when the first of ten samples exceeded one of the defined thresholds: hence, a risk-based approach was used to assess the SSL of Bolognese sauce.

Consequently, the re-evaluated SSL would be even more precautionary if the end user paid more attention when handling and using of the product. Thus, manufacturers should consider informing consumers about the proper use of the product through appropriate label claims.

Extending the SSL on the label may have positive consequences on reducing household food waste and related resource waste. Our hope is that a longer durability may allow consumers to use the "forgotten" or "unseen" stored food that is still fit for consumption, rather than discarding it. This work ultimately aims to promote the reassessment of the SSL of foods in order to contribute to prevent the generation of food waste and take a step toward greater sustainability of the food supply chain.

Funding

Ivan Mezza was supported by Europass (joint structure connecting the Emilia Romagna Region with the EFSA) through a Research Fellowship titled "Identification and analysis of emerging risks in the agri-food chain of the territory of the Emilia-Romagna region".

CRediT authorship contribution statement

Carola Nicosia: Data curation, Methodology, Formal analysis, Validation, Writing – original draft, Writing – review & editing. **Ivan Mezza**: Data curation, Methodology, Formal analysis, Writing – original draft. **Andrea Pulvirenti**: Funding acquisition, Investigation, Supervision. **Fabio Licciardello**: Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare no actual or potential conflict of interest.

Data availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fpsl.2023.101172.

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