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Effect of temperature in domestic refrigerators on fresh-cut Iceberg salad quality and waste

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Corresponding Author: Professor Lara Manzocco, Ph.D.

Corresponding Author's Institution: Dipartimento di Scienze AgroAlimentari, Ambientali e Animali

First Author: Lara Manzocco, Ph.D.

Order of Authors: Lara Manzocco, Ph.D.; Marilisa Alongi; Corrado Lagazio; Sandro Sillani; Maria Cristina Nicoli

Abstract: The evolution of different quality parameters (firmness, weight loss, colour changes, microbial counts, consumer rejection) of packed fresh-cut Iceberg salad was assessed at 4, 8 and 12 °C to simulate domestic refrigerators running at different conditions. The increase in storage temperature did not affect salad firmness and weight loss but increased colour changes, microbial growth and consumer rejection. A survey among Italian consumers was also carried out and demonstrated that fresh-cut salad was mainly consumed within the first 5 days after purchasing. Consumer rejection data were combined with data relevant to the distribution of salad consumption over the days following product purchase, to estimate salad wasting risk.
When salad was stored at 4 and 8 °C, estimated wasted packages within the expiration date (7 days) were less than 1%. By contrast, 13% of the packages was estimated to be wasted within 7 days of storage at 12 °C. Quantification of wasting risk is a necessary information to identify efficient and sustainable interventions to tackle food waste.

Effect of temperature in domestic refrigerators on fresh-cut Iceberg salad quality and waste

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Ms. Ref. No.: FOODRES-D-17-01926
Title: Effect of temperature in domestic refrigerators on fresh-cut Iceberg salad quality and waste
Food Research International
Lara Manzocco

Anderson Sant'Ana
Editor
Trends in Food Science and Technology

Dear Prof. Sant'Ana,
Thank you for reviewing the paper. All referees' comments were carefully considered and following you will find an itemised list of the changes made to the paper:

Reviewer #1:
The authors thank the referee for his/her interesting suggestion that allowed improving the paper.

Introduction
P2 L27-29. Please, add a suitable reference supporting this sentence.
Reference was added as suggested by the referee.

P2 L29-30. Please, add a suitable reference supporting this sentence.
Reference was added as suggested by the referee.

P2 L44-47. Please, add a couple of references about the temperature usually found in the household refrigerators: 1) It was reported that in South European countries 30% of refrigerated foods were kept above 10°C in household refrigerators (Kennedy et al. 2005. Journal of Food Protection, 68, 1421-1430); 2) 19 thermal histories recorded in Italy highlighted that in 75% of the cases the storage temperature in domestic refrigerators was 8.4°C (Limbo et al., 2010, Meat Science, 84, 129-136).
The text was modified as suggested by the reviewer.

P3 L67-68. Please, add suitable references supporting this sentence.
Reference was added as suggested by the referee.

Materials and methods

P4 L80-83. Please, provide the shape (rectangular?) and the size (…x…cm) of the packages.
Information was added.

P4 L87-89. Please, specify the sampling frequency or sampling times.
Sampling times were specified in the text.

P4 L102. Please substitute "stomacher" with "Stomacher".
The text was corrected.

P6 L131-133. Authors stated that in the study were involved 650 consumers. Were all they salad consumers? How did you check it? Did you ask any question about their salad consumption frequency, before performing the test (it seems that you asked them to fill in the consumption habit questionnaire after doing the salad rejection evaluation)? If, not all of them were consumers, please substitute the term "consumers" with "subjects".

Subjects participating to the study were actually all salad consumers. The procedure for consumers' selection was specified in the text.

P6 L133-134. Please, add the average age.
This information was added.

P6 L135-136. I agree with the fact that consumers should be not told to be involved in a study relevant to domestic food waste. However, I expect that you informed them about the study and asked them to sign an informed consent. Please, add this piece of information.
This information was added in the text.

P6 L140-142. Was the temperature of the portable refrigerated display readable/visible/shown on it? If yes, did you apply any precaution to prevent the subjects reading the information? Please, provide details on this point.
Temperature was not visible to consumers. Details were added in the text.

P6 L144. Each consumer was asked to look at a salad package. Did all the 50 subjects at a sampling time evaluate the same salad sample? In total, how many salad packages were used for the presentation to the 50 subjects?
Each consumer required less than 1 min for rejection evaluation. The 50 consumers visually evaluated the sample within 2 hours. Given the short time of the assessment session, we decided not to substitute salad samples in the refrigerated cabinet during the session. In this way, all consumers evaluated the same sample. This information was mentioned in the text.

P6 L145-146. It is not clear to me what you meant with "Each evaluation session required approximately 2 hours." You meant the time required to complete the evaluation by all the 50 subjects? How long did it take the evaluation by each subject? Please, clarify this topic.
The assessment of acceptability or unacceptability by consumers was very quick. Each consumer looked at the salad and express his/her response. This generally occurred in less than 1 min. Immediately after having given the acceptability response subjects were led to another room for the consumption survey. This is why the overall session for acceptability evaluation was about 2 hours long. More details were added in the text.

P6 L144-148. Authors stated that for each storage time, salad was assessed by 50 consumers and that 6, 9 and 4 times of analysis for salad stored at 4, 8 and 12 °C were required to reach the 100% rejection. Previously, it was stated that in total 650 subjects were involved. It is not clear to me the criteria used to associate the subjects to the tests over the experiment. Please, provide details on this point. Were the subjects different in the different evaluation sessions? Were the 50 consumers the same for all the evaluations of the salad samples stored at the same temperature? Was the subject-sample association randomized? How many samples did evaluate each subject in each session?
The referee correctly identified a misinformation. The experimentation was initially performed only at 8 and 12 °C (650 consumers were involved in this trial, 50X9 times at 8 °C and 50X4 times at 12 °C). The consumers participating to this first set of trials were also involved in the consumption survey. Following, a second experimentation was carried out to evaluate rejection on samples stored at 4 °C. Given the previous experience at 8 and 12 °C, only 6 times were considered (50 X6 times=300). This second group of consumers was not involved in the consumption survey. For this reason, the total number of consumers participating to the consumption survey was 650. By contrast, the total number of participants in the rejection study was 950. This number was corrected in the text. The text was also modified to provide details about numerosity of consumers and times of rejection analysis.

P6 L150-152. After salad rejection evaluation, each consumer was led in another room and asked to fill in a questionnaire. To me, that means that each subject participated just in one evaluation session. Is it correct? If not, a subject who participated in a previous evaluation session would not be required to fill in the questionnaire once more. Please, make clear this point.
Each consumer evaluated only one sample. Only the 650 consumers participating to the first trial (samples stored at 8 and 12 °C) were involved in the salad consumption survey. The 300 consumers involved in the second trial (samples stored at 4 °C) were only used to evaluated salad acceptability. The number of consumers involved in the consumption survey was specified in the text.

P7 L165-166. Please, define "m" and "s".
"m" and "s" were defined as suggested.

P8 L183-184. Please, define "p" and "n".
"p" and "n" were defined as suggested.

Results and discussion

P9 L227-229. "Since changes in fresh-cut salad appearance (Table 1) are generally associated with microbial growth, …". Please, add suitable references supporting this sentence.
Reference was added as suggested by the reviewr.

P11 L258-261. I would suggest to increase the result discussion relating the probability of salad rejection by subjects with the visual changes over storage time resulted from the image analysis data. Please take into account that appearance attributes (e.g. green colour, leaf turgidity) and the perceived level of freshness are the most important attributes for ready to eat salad choice and consumption (Dinnella et al. 2014, Food Research International, 59,108-116; Vidal et al. 2013, Food Quality and Preference, 28, 1-7).
Regression analysis between rejection and colour data was performed and results added in the text. Discussion was also implemented as suggested by the reviewer.

P14 L336. The sentence "This means that 13 packages out of 100 purchased packages are likely to be thrown away by the Italian consumers within expiration date if the product is stored in the refrigerator at 12 °C" is redundant. Please, delete it.
The sentence was deleted.

P14 L342-344. Please, add a suitable reference supporting this sentence.
Reference was added as suggested by the referee.

P 14 L 352-361 and P 15 L368-370
I would suggest to include in the discussion section also a presentation of the limitations of the work. In my opinion, an important limitation is that participants could accept or reject a salad sample based only on the appearance of the product, when it was still packaged. This evaluation condition can be different from the once in a real situation where a consumer at home has to evaluate the level of freshness of the product and decide to consume it or not. It can be hypothesized that, in a real situation, consumers have the possibility to open the package and to touch the salad, for a more complete evaluation of the level of freshness of the product. Moreover, in a real situation, consumers can take into account the expiration date of the product printed on the package as factor influencing the decision of acceptance/reject. In fact, it was observed that

information about storage time significantly affected freshness perception and liking of fresh-cut salad samples (Dinnella et al. 2014, Food Research International, 59,108-116; Vidal et al. 2013, Food Quality and Preference, 28, 1-7). That means that other factors than the visual appearance could contribute to the consumption decision. In my opinion, it is relevant to mention that they could be such others factors not considered in your study able to play a role.
Both discussion and conclusions were implemented by considering the methodology limitations and its possible improvements.

Tables

Table 1. Please substitute "… at 8 and 12 °C" with "… at 4, 8 and 12°C".
The Table was corrected.

I apologize but I do not understand the superscripts. Did you compare the means intra-temperature or inter-temperature? Form the table footnote "for each column and storage temperature means indicated by the same letter are not significantly different" it should be an intra-temperature comparison. However, looking at the superscripts it seems more that it is an inter-temperature comparison. Please, make very clear this point. What is the meaning of the superscript "ac"? Is it for "abc"?
Table 1 was corrected and reorganised to make clear significance of differences among data. In particular, we decided not to show the mean vale of data relevant to the control sample but to insert time 0 data relevant to experiments carried out at the different temperatures.

Reviewer #2:

General comments

We agree with the reviewer that the effect of different storage temperature on salad quality is well documented in the literature. However, as stated in the introduction section, the aim of the study was that of comparing the effect of different storage temperature not only on salad quality but also on household waste generation (which is the study novelty). To this aim, product quality parameters were assessed to provide a description of the samples subjected to evaluation of wasting risk. In other words, the research was not intended to provide suggestions to modify storage conditions in order to increase shelf life. On the contrary, the focus was on the possibility to develop a methodology allowing to get reliable indication about the risk that the product is wasted at domestic level.

Minor issues:

- The questionnaire for consumers addresses their habits of buying certain number of packs and time of rejection of the product. This is insufficient in scientific point of view. The questions should be formulated so that to allow more precise evaluation of visual appearance involving more specific ranking based on perception of yellowing, browning, objectionable defects, occurrence of microbial spoilage, turgor and other criteria known as overall visual quality, for which there are well elaborated scales for assessment of the shelf life of this product.
Survival analysis is widely applied in several scientific domains (e.g. engineering, health sciences, pharmaceutics). In the present research, it was applied to model food acceptability/rejection. This approach is well accepted by the scientific community, with special emphasis in the case of shelf life estimation (Hough, 2010. Sensory Shelf Life Estimation of Food Products. Boca Raton: CRC press, Taylor & Francis Group.; Labuza & Schmidl. 1988, Cereal Foods World, 33, 193-206).

Consumption survey was carried out according to the methods and procedures conventionally applied in the Economics and Marketing studies (Kahn & Cannell, 1957. The dynamics of interviewing; theory, technique, and cases. Oxford, England: John Wiley; Peter, Olson & Grunert, 1999. Consumer behavior and marketing strategy. Mcgraw Hill Higher Education (UK); Gillham, 2005. Research Interviewing: The range of techniques: A practical guide. McGraw-Hill Education (UK)) to collect information about consumer attitude towards salad wasting at domestic level.
According to what stated in the introduction, the paper aim was not to precisely describe salad quality by specific analyses. For this reason, we focus on some general quality indices only to describe salad quality before presenting data relevant to wasting risk.

Page 2 line 31-34 "in the absence of an inactivation step" - not clear to me what is that
The sentence was modified, omitting any mention to inactivation step.

Page 4 L 96 "Aliquots of 10 g of salad"- aliquots is not an appropriate word in this particular case?
The text was modified as suggested.

Page 4 L 96; Page 6 L 131 When a sentence starts with a number, it is to be written in words.
The text was modified as suggested.

Page 9 L 215-217 Although the data are not shown, I find strange that, line 216, "Independently on storage temperature, no significant changes in firmness and weight loss of salad were detected during the entire observation time". Usually with advancement of wilting and occurrence of deteriorations such as exudates and colour changes, the firmness and weight also change.
The sensitivity of salads to alterative phenomena occurring during storage depends on several factors, including species, cultivar, tissue structure, physiological respiration and enzymatic activity. In this context, Iceberg salad is particularly sensitive to colour changes, which are earlier alterative events as compared to changes in texture and turgidity. Other salads (e.g. Valerianella locusta or rocket salad) show opposite trends.

- In my opinion it is better to present Results and Discussion in separate sections
The results and discussion sections were largely improved based on the indications of the other reviewers. Based on their comments, we decided to keep them together.

Reviewer #3:

Highlights: Points 1 seems to be the conclusion and may be better switched with point 4.
The order of highlights was modified as suggested.

Page1(P1) Line19(L19): packages wasted may be better changed to “estimated wasted package" since it is exploited data, not actual results.
The text was modified as suggested.

P2L29-30: Only the convenience? How about concerns on freshness and nutrition?
The text was modified as suggested.

P3L60-64: "its effects" refers to what? Please clarify.
As suggested by the reviewer, the text was modified to clarify this point,

P4L86: How many samples were taken for each temperature?

At the beginning of the experimentation, 15 salad packages from a same production batch were stored at each temperature. At each storage time, two packages were used: one package was used for the sensory assessment, while another one was used for the microbiological analysis, firmness, weight loss and colour evaluation. Depending on the number of storage times tested at each temperature (page 4 L 87-89), the overall number of packages used changed, so that some packages remained unused by the end of the experiments.

P5L108-111: How to determine Pseudomonas spp. on surface after aerobic incubation at 30C for 48h. Please provide more details.
Pseudomonas spp. was not determined on salad leaf "surface". It was assessed by counting colonies on plate "surface". The text was modified to avoid misunderstanding.

P6L134: What is the distribution of age? Were more young people recruited in the study, which may create potential bias for Italian consumer data?
The average age of consumers was added to the text.

P9L217-219: Since visual appearance includes colour change, why did the author separate the word "colour changes" in Line 204?
The text was modified to avoid misunderstanding.

Table 2: For data of 8 °C and 13 days, why did the counts of pseudomonas even exceed total mesophilic counts? The author should provide details for identifying pseudomonas.
Microbiological analyses were performed based on conventional plate counting. As highlighted in the text, Pseudomonas spp. represent the major spoilage microorganisms in fresh-cut salad. For this reason, for each sample, total mesophilic count and Pseudomonas spp. were always in the same magnitude range. In these conditions, Pseudomonas spp counts exceeding total mesophilic ones are quite common and are attributed to intrinsic experimental error of the plate counting methodology.

P13 L312: This part seems to be the repetition of the description in the material and methods and could be deleted or abbreviated
The authors agree with the reviewer. This part was deleted.

Best regards,
Lara Manzocco

Highlights:

Salad waste was estimated based on consumption and rejection probabilities

Less than 1% of salad stored below 8 °C was wasted within 7 days shelf life

When salad was stored at 12 °C its waste reached 13% within 7 days

Storage temperature affects fresh-cut salad quality and wasting risk

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Effect of temperature in domestic refrigerators on fresh-cut Iceberg salad quality and waste

L. Manzocco[1]*, M. Alongi[1], C. Lagazio[2], S. Sillani[1] and M.C. Nicoli[1]

[1]Department of Food, Agriculture, Environment and Animal Sciences, University of Udine, Via Sondrio 2A, 33100, Udine, Italy

[2]Department of Economics, University of Genova, Via Vivaldi 5, 16126 Genova, Italy

*Corresponding author.

E-mail address: lara.manzocco@uniud.it

ABSTRACT

The evolution of different quality parameters (firmness, weight loss, colour changes, microbial counts, consumer rejection) of packed fresh-cut Iceberg salad was assessed at 4, 8 and 12 °C to simulate domestic refrigerators running at different conditions. The increase in storage temperature did not affect salad firmness and weight loss but increased colour changes, microbial growth and consumer rejection. A survey among Italian consumers was also carried out and demonstrated that fresh-cut salad was mainly consumed within the first 5 days after purchasing. Consumer rejection data were combined with data relevant to the distribution of salad consumption over the days following product purchase, to estimate salad wasting risk.

When salad was stored at 4 and 8 °C, estimated wasted packages within the expiration date (7 days) were less than 1%. By contrast, 13% of the packages was estimated to be wasted within 7 days of storage at 12 °C. Quantification of wasting risk is a necessary information to identify efficient and sustainable interventions to tackle food waste.

Keywords: fresh-cut salad; temperature abuse; refrigeration; consumer; waste

1. INTRODUCTION

Fresh-cut salad is nowadays regularly consumed in most developed countries. In addition, its market continues to grow at a sustained pace in countries undergoing the industrialisation process (Soliva-Fortuny and Martin-Belloso, 2003). The reason for this global success lies not only in freshness and nutritional value of the product but also in its convenience (Rocha and Morais, 2007). Minimal processing is known to make fresh-cut salad particularly prone to biochemical reactions and microbiological spoilage, leading to changes in colour and appearance that compromise product acceptability (Martin-Diana, Rico, Barry-Ryan, Frias, Mulcahy & Henehan, 2005; Gonzales-Aguilar, Ayala-Zavala, De La Rosa, & Ivarez-Parrilla, 2010). To delay quality depletion and guarantee product shelf life, the control of temperature is crucial. The cold chain is compulsorily maintained during production, distribution and retail (DM No 3746/2014; EC No 1234/2007; EU No 1169/2011). In addition, the product should be stored under refrigerated conditions (below 5 °C) during domestic storage until use. However, literature data indicate that recommended temperature for refrigerated foods is barely maintained at household level (Marklinder & Eriksson, 2015). A survey carried out in France indicated that the average temperature in the refrigerators was 6.6 °C with a minimum value of 0.9 °C and a maximum value of 11.4 °C (Laguerre, Derens, & Palagos, 2002). Bakalis, Giannakourou, & Taoukis (2003) not only found large temperature differences among the compartments of 110 refrigerators but also observed that 8% of them were running at 10-12 °C. In addition, Kennedy et al. (2005) reported that 59 out of 100 domestic refrigerators tested in their study had an average temperature higher than 5 °C and 6 of them were kept above 10 °C. According to Limbo (2010), 19 thermal histories recorded in Italy highlighted that in 75% of the cases the temperature of food stored in domestic refrigerators was higher than 8 °C. According to James, Evans, & James (2008), domestic storage of chilled foods appears to be the weakest link in the entire chill-chain.

Based on these considerations, domestic storage temperatures higher than recommended, being responsible for a fastest quality decay of fresh-cut salad, could also be associated to a higher

wasting risk. It has been estimated that lowering home refrigerated temperature from 7 to 4 °C could annually save 32,000 t of leafy salad waste in UK (Brown, Hipps, Easteal, Parry, & Evans, 2014). However, this estimate was based on the general assumption of waste savings proportionality with shelf life extension at different storage temperature, since specific data were not available. This is quite surprising, considering that consumers are the largest contributors to global food discard and that food wasted at domestic level ranges between 15 and 30% with fruit and vegetables accounting for one third of the entire waste (Williams, Wikstrom, Otterbring, Lofgren, & Beretta, 2012; Gunders, 2012; Lebersorger & Schneider, 2011; Scott Kantor, Lipton, Manchester, & Oliveira, 1997). Despite these evidences, to our knowledge, the effect of domestic storage temperature on food waste has never been directly quantified. As indicated by Brown et al. (2014), truly comparative data about the potential effect of domestic storage temperature on waste saving would require product quality and consumption behaviour be carefully monitored during storage at different temperatures.

In the light of these considerations, the present research was addressed to compare the effect of different storage temperatures in domestic refrigerators on fresh-cut salad quality and waste generation. To this aim, Iceberg salad was chosen as a typical example of fresh-cut salad due to its susceptibility to storage temperature and wide diffusion at global level (Casati and Baldi 2012). Commercial Iceberg salad pouches were stored at 4, 8 and 12 °C. At increasing time during storage, salad was analysed for quality indices (firmness, weight loss, colour, microbial counts) and consumer rejection by survival analysis. A survey about habits of salad consumption of Italian consumers was also carried out to obtain data relevant to the frequency of salad consumption during its storage in domestic refrigerators. Waste of salad during domestic storage at different temperatures was then estimated by multiplying consumer rejection and consumption data. Differences in salad quality and waste were discussed as a function of storage temperature.

2. MATERIALS AND METHODS

2.1. Sample preparation

Rectangular packages of transparent bi-axially oriented polypropylene pouches (BOPP, 0.035 mm) measuring 30 x 25 cm and containing 200 g Iceberg salad (*Lactuca sativa* var. *Capitata* L.), sealed under modified atmosphere (8% CO_2, 8% O_2, 84% N_2), were provided by a local producer on the production day between February and May 2015. Salad variety and package size were chosen since the most commonly available on the Italian market. The expiration date was set by the producer after 7 days from the production. Salad packages were stored in dark conditions at 4 ± 1 °C, 8 ± 1 °C or 12 ± 1 °C (fifteen packages for each storage temperature). At increasing time during storage samples were removed from the refrigerator and submitted to the analyses. In particular, analyses were carried out on samples stored for: 4, 7, 10 and 14 days at 4 °C; 2, 3, 5, 6, 7, 8, 9, 10 and 13 days at 8 °C; 1, 2, 5 and 7 days at 12 °C.

2.2. Salad characterization

2.2.1. Weight loss

Weight loss was determined by weighting the content of the package before and after the storage period. Weight loss was expressed as g kg^{-1}.

2.2.2. Firmness

Salad firmness was examined using a ten-blade Kramer shear cell, attached to Instron 4301 (Instron Ltd, High Wycombe, UK). Ten grams of salad were placed into the Kramer cell and compressed 50 mm at a 2.5 mm s^{-1} speed. Maximum force was recorded by using the software Automated Materials Testing System (Version 5, Series IX, Instron Ltd.). Force-distance curves were recorded and firmness was taken as the maximum force required to compress salad (kN). For each sample, eight measures were performed at each storage time.

2.2.3. Microbiological analyses

Ten grams of fresh-cut salad was aseptically removed from the package, placed in a Stomacher bag with 90 mL of maximum recovery diluent (Oxoid, Italy) and homogenised for 1 min at normal

speed and temperature in a Stomacher (International PBI, Milan, Italy). Serial dilutions (1:10) were made in sterile maximum recovery diluent and 0.1 or 1.0 mL were spread on agar plates for aerobic microorganisms or mixed with agar base for anaerobic microorganisms, respectively. The media and conditions were the following: Plate Count Agar (Oxoid, Italy) was used for enumeration of aerobic mesophilic bacteria and incubation was carried out at 30 °C for 48 h; Pseudomonas Agar Base supplemented with Pseudomonas Cetrimide Fusidine Cephaloridine Supplement (Oxoid, Italy) was used for *Pseudomonas* spp., which were determined after aerobic incubation at 30 °C for 48 h. The salad extract was gently mixed with violet red bile glucose (Oxoid, Italy) and incubated at 37 °C for 24 h to enumerate enteric bacteria. Pour plating in ColiID (BioMérieux, France) with a covering layer of the same medium incubated at 37 °C for 24 h was used for enumeration of total and faecal coliforms.

2.2.4. Picture acquisition and image analyses

Images of fresh-cut salad were acquired by using an image acquisition cabinet (Immagini and Computer, Bareggio, Italy) equipped with a digital camera (EOS 550D, Canon, Milano, Italy). The digital camera was placed on an adjustable stand positioned 60 cm above a black cardboard base where the sample was placed. Light was provided by four 100 W frosted photographic floodlights, in a position allowing minimum shadow and glare. Other camera settings were: shutter time 1/250 s, F-Number F/2,8 and focal length 60 mm. Images were saved in jpeg format resulting in pictures of 5,184 x 3,456 pixels, 72 x 72 dpi.

Image analyses were performed using Image-Pro Plus (ver. 6.3, media Cybernetics, Inc., Bethesda, Md., U.S.A.). Attention was focused on quantification of the percentage of brown and green pixels in the images. RGB (Red Green Blue) values corresponding to the brown areas of fresh-cut salad were R (77-111), G (47-85), B (15-35) while those corresponding to the green ones were R (50-130), G (80-140), B (10-70). Browning and greenness indices were calculated as the percentage ratios between the sum of brown or green pixels and the sum of all pixels of the pictures.

2.3. Consumer data collection

Nine hundred-fifty consumers of fresh-cut salad were selected by asking students and workers from the University of Udine (Italy) if they generally consume fresh-cut salad. Only subjects providing a positive answer participated to the study. They were between the ages of 18 and 63 years with average age of 25 ± 8 years, and approximately balanced between males (47%) and females (53%). Participants were not told to be involved in a study relevant to domestic food waste but were informed that acquired data would have been used for research purposes and asked to sign an informed consent.

2.3.1. Fresh-cut salad rejection

At increasing time during storage, salad packages were shown to consumers in a portable refrigerated cabinet. The latter guaranteed temperature maintenance of the sample during the assessment without allowing consumers to visualise the temperature display which was covered by a piece of cardboard. Each consumer was asked to look at a salad package and answer to the following question: "If this salad was in your refrigerator, would you consume it, or would you throw it away?". For each storage time, one salad package was visually assessed by 50 consumers. Each consumer required about 1 min for acceptability evaluation. Completing the evaluation by all the 50 consumers required approximately 2 hours. Analyses were performed on samples stored for increasing time until 100% rejection was approached. Reaching this percentage required 6, 9 and 4 times of analysis for salad stored at 4, 8 and 12 °C, respectively.

2.3.2. Fresh-cut salad consumption

After salad rejection evaluation, consumers were led in another room and asked to provide information about fresh-cut salad consumption habits by filling a questionnaire (Fig. 1). Six hundred-fifty consumers were involved in this survey. In particular, consumers were invited to indicate the number of the usually purchased salad packages and the number of purchased packages they usually consume at each day during domestic refrigerated storage up to 10 days. For instance, consumer 1 could declare that he/she usually buys one salad package, which he/she assumes to

consume on the purchase day. By contrast, consumer 2 could declare that he/she usually buys 4 salad packages and consumes one package per day, starting from purchase day, up to the third day after purchase.

2.4. Data study area

2.4.1. Elaboration of data relevant to fresh-cut salad rejection

The probability that the consumer rejects fresh-cut salad at a given time during refrigerated domestic storage due to unacceptable characteristics was estimated by elaborating rejection data via survival analysis. Based on its wide application (Hough, 2010), the Weibull function (1) was used to describe the evolution of the probability of salad rejection $P(R_t)$ during storage. $P(R_t)$ is thus the probability of the food to be rejected by consumers at time t (1), where μ and σ are the intercept and the scale parameters, respectively.

$$P(R_t) = 1 - e^{-e^{\left(\frac{\ln(t)-\mu}{\sigma}\right)}} \qquad (1)$$

The likelihood function was used to estimate the unknown parameters and the rejection probability percentage was computed by multiplying $P(R_t)$ by 100. The rejection probability percentage was used to estimate the shelf life of fresh-cut salad. The latter was computed as the storage time corresponding to 25 % rejection probability (Ares, Gimenez, & Gambaro, 2008a; Ares, Martinez, Lareo, & Lema, 2008b).

2.4.2. Elaboration of data relevant to fresh-cut salad consumption

Fresh-cut salad consumption data were elaborated to estimate the probability that the consumer decides to consume fresh-cut salad at a given time during its refrigerated domestic storage. In particular, consumption data for each consumer at each storage time (Fig. 1) were normalized by computing the ratio between the number of packages consumed at each day after purchase and the total number of purchased packages. The average of normalized consumption data at each day after purchase was then calculated.

The Negative Binomial model (2), which is particularly effective for the analysis of discrete data (Byers, Allore, Gill, & Peduzzi, 2003), was fitted to the average consumption distribution, to describe the consumption probability of fresh-cut salad during storage time. $P(C_t)$ is thus the probability that the consumer decides to consume the food at time t (2), where n and p are the size and the probe parameters, respectively.

$$P(C_t) = \left(\frac{(t+n-1)!}{(n-1)!t!}\right)(1-p)^n p^t \qquad (2)$$

Minimum chi-square method was used to fit model-based probabilities to observed frequencies and the consumption probability percentage was computed by multiplying $P(C_t)$ by 100.

2.4.3. Wasting risk model

A probabilistic approach was adopted to estimate fresh-cut salad domestic food waste. The basic assumption of the wasting risk model was that turning salad into a domestic waste at a given storage time t would require the joint occurrence of two events at t: i) the consumer should decide to consume the salad (salad consumption probability); ii) the consumer should reject the salad (salad rejection probability). The consumption decision is expected to depend on consumer behaviour and social characteristics as well as on his/her awareness about product stability. By contrast, product rejection depends on the interaction between consumer expectations and product properties, intended as the result of the technological interventions applied to guarantee product safety and an adequate shelf life. Based on these considerations, the events of consumption decision and product rejection were considered as independent. The probability of the food to become a waste $P(W_t)$ (3) at the storage time t was thus expressed in mathematical terms as the product of $P(C_t)$ (2) and $P(R_t)$ (1):

$$P(W_t) = P(C_t) \cdot P(R_t) \qquad (3)$$

Substituting equations 1 and 2 in equation 3, the wasting risk model results as follows:

$$P(W_t) = \left[\left(\frac{(t+n-1)!}{(n-1)!t!}\right)(1-p)^n p^t\right] \cdot \left[1 - e^{-e^{\left(\frac{\ln(t)-\mu}{\sigma}\right)}}\right] \qquad (4)$$

The total amount of wasted food, expressed as a percentage, until time *t* was calculated by summing up $P(W_t)$ values over the desired time interval.

2.5. Computational details

Results are averages of three measurements at least and are reported as means ± SD (standard deviation). Analyses of variance (ANOVA) was performed with significance level set to $P < 0.05$. The Tukey procedure was used to test differences between means. All the computations were carried out using R, ver 3.2.3 (R Core Team, 2015).

3. RESULTS AND DISCUSSION

3.1. Effect of storage temperature on the evolution of quality parameters of fresh-cut salad

The evolution of different quality parameters of fresh-cut salad was assessed during storage at 4, 8 and 12 °C. These temperatures were selected to simulate domestic refrigerators running at different conditions. Independently on storage temperature, no significant changes in firmness and weight loss of salad were detected during the entire observation time (data not shown). By contrast, remarkable changes in salad visual appearance were detected following colour changes as well as development of wilting and exudates. The latter included green colour fading of leaves, formation of dark and necrotic stains on their surface and browning of cut edges and midribs. To quantify these colour changes, image analysis was used to assess the percentage of green and brown pixels in salad images (Table 1).

As expected, storage promoted a decrease in the green index, which can be attributed to chlorophyll degradation upon the metabolic stress induced by cut operations, and an increase in the brown index due to phenol oxidation (Ferrante, Incrocci, Maggini, Serra, & Tognoni, 2004; Agüero, Yommi, Camelo, & Roura, 2007) (Table 1). The increase in storage temperature resulted in progressively faster colour changes (Table 1). Since changes in fresh-cut salad appearance (Table 1) are generally associated with microbial growth (Paillart et al., 2017), microbial counts of fresh-cut salad during storage were also compared (Table 2). Initial microbial counts (Control) were in the same

magnitude range reported by other authors for this product (King, Magnuson, Török, & Goodman, 1991; Baur, Klaiber, Hammes, & Carle, 2004; Conte, Conversa, Scrocco, Brescia, Laverse, & Elia 2008). Total viable count was mainly represented by *Pseudomonas* spp., which are known as the major spoilage population in salad due to their easy adaptation to refrigeration. A low presence of total coliforms and *Enterobacteriaceae*, as well as faecal coliforms below the detection limit, indicated an adequate hygienic level of the product.

When salad was stored at recommended temperature (4 °C), a 3-log increase in total mesophilic bacteria was observed within 10 days of storage. During the following week, only a minor increase was noticed. Due to the prevalence of *Pseudomonas* spp. in the fresh-cut salad microbiota, a behaviour mimicking that of total mesophilic count was also observed for this microbial population. A 2-log increase was detected for *Enterobacteriaceae* and total coliforms. By contrast, faecal coliforms never exceeded the detection limit (5 cfu g^{-1}). As expected, when salad was stored at abuse temperature (8 and 12 °C), the growth of alterative microflora was progressively faster.

Specific microbiological criteria for minimally processed fruits and vegetables have been adopted in some European countries. For instance, Spain, France and Germany recommended 7 log cfu g^{-1} as a maximum limit for total viable count (Francis, Thomas, & O´Beirne, 1999). In our case, this value was reached at 10 day-storage at 4 °C. This is consistent with literature data, suggesting an expiration date of about 10 days for this category of fresh-cut salads (Tsiros & Heilman, 2005). It is noteworthy that the producer of the salad considered in this study actually attributed the product a shelf life of 7 days. It is likely that the company choice of the expiration date is mainly the result of considerations regarding the changes in product appearance (Table 1), which may be easily perceived by consumers, rather than in hygienic parameters (Table 2). In agreement with the fastest growth of microbial count (Table 2), the 7 log cfu g^{-1} limit was reached in times progressively shorter when temperature was increased to 8 or 12 °C.

3.2. Effect of storage temperature on the evolution of consumer rejection of fresh-cut salad

Fresh-cut salad samples stored for increasing time at 4, 8 and 12 °C were presented to consumers, asking them to express an acceptability/rejection judgment. Salad rejection progressively increased as its green index decreased ($r > 0.80$, $p > 0.5$), in agreement with the evidence that green colour of salad leaves is among the most important attributes for fresh-cut salad choice and consumption (Dinnella et al. 2014). Rejection data (Figure 2) were analysed by survival analysis to estimate the probability that salad is rejected by consumers at time *t* during storage ($P(R_t)$, equation 1). Estimates of the experimental parameters μ and σ of the salad rejection function are reported in Table 3.

Figure 2 shows the evolution of the percentage of consumers rejecting salad during storage under recommended (4 °C) or abuse (8 and 12 °C) temperatures up to 10 days.

The percentage of consumers rejecting the product during storage increased according to the typical shape of the Weibull function. As known, the percentage of consumers rejecting the product during storage (Figure 2) can be used to estimate shelf life. The latter is generally taken as the storage time corresponding to 25% consumer rejection (Ares *et al*., 2008a; Ares *et al*., 2008b). This level of consumer rejection was achieved in considerably different times depending on storage temperature. For instance, only 3 days at 12 °C were required to reach the 25% consumer rejection limit whilst this value was achieved in 10 days when salad was stored at 8 °C. By contrast, the consumer rejection remained well below the 25% value up to 10 days of storage at 4 °C. In addition, it can be noted that, within the expiration date declared by the producer (7 days), the percentage of packages rejected by the consumers ranged from 4% to 80% depending on storage temperature. In other words, this means that when salad is stored at 4°C, it is likely that only 4% of the consumers that would occasionally decide to consume the product stored for up to 7 days, would find it unacceptable and would probably decide to waste it. By contrast, a 20-times higher percentage of rejection was estimated when the salad was stored at 12 °C.

Results shown in Figure 2 emphasise the role of storage temperature in affecting consumer rejection, and reasonably the consumer tendency to waste the product. For instance, if salad was

always consumed on the purchase day (*t=0*), there would be no salad waste since, on that day, product rejection would be equal to zero (Figure 2) and all consumers would decide to consume the salad without wasting it. By contrast, if most consumers decided to eat the salad after several days of storage, the probability of rejection would increase, accounting for a higher salad wasting. In addition, if this were the case, an intense effect of storage temperature on salad waste would be expected since product rejection is significantly affected by storage temperature (Figure 2). Based on these considerations, rejection data could be further elaborated to compute how much salad is likely to be wasted at the different storage temperatures. To this aim, rejection probability (Figure 2) should be merged with data relevant to the distribution of salad consumption during refrigerated storage at domestic level.

3.3.Distribution of fresh-cut salad consumption by Italian consumers

The distribution of salad consumption during refrigerated storage at domestic level was obtained by performing a consumption survey to describe the consumption habits of Italian salad consumers. Data were analysed to estimate the probability that a consumer decides to consume a salad package at a given time during its refrigerated domestic storage (Figure 3).

At the purchase day, the percentage of consumption was approximately 30%, indicating that about 30% of the purchased salad packages is generally consumed on that day. These data suggest that Italian consumers are aware of product freshness. Based on the short shelf life of fresh-cut salad, they tend to consume it as soon as possible to avoid consumption of salad stored for more days, which could have a lower quality level, running a higher risk of being thrown away. The probability of salad consumption quickly decreased after purchasing and approached zero for storage time longer than 5 days. This indicates that it is unlikely that salad packages would remain in the refrigerator of Italian consumers more than 5 days. The Negative Binomial model (Equation 2) was fitted to consumption probability data (Figure 3). Estimates of the experimental parameters *n* and *p* were respectively 1.438 (SE 0.124) and 0.449 (SE 0.020).

3.4.Effect of storage temperature on consumer waste of fresh-cut salad

In order to estimate salad waste, a wasting risk model was developed (Equation 3). The basic assumption was that a consumer throws the purchased salad in the garbage, on a given storage day, only if he/she is willing to consume salad on that day and, once taken the salad pouch out of the fridge, he/she finds it unacceptable for the meal. It should be noted that this approach allowed to estimate the probability of salad waste without directly asking consumers to describe their wasting behaviour. On the contrary, the methodologies most commonly applied to estimate food waste are based on a direct investigation of consumer wasting behaviour. These methodologies often lead to non-representative data since consumers tend to minimize their wasting behaviour as it has intrinsic moral and ethical implications (Beretta, Stoessel, Baier, & Hellweg, 2013; Lebersorger & Schneider, 2011; Scott Kantor et al., 1997). For instance, about 20% of Italian consumers declare a highly virtuous behaviour that does not fit with actual food waste data (Waste Watcher, 2013). The estimation of the waste probability (Equation 3) by multiplying consumption and rejection functions minimizes these statistical biases. It also allows comparison of waste probability for products submitted to different processing or storage conditions. In the case of salad, Figure 4 compares the effect of storage at 4, 8 or 12 °C on the cumulative probability that the product is wasted by consumers since unsuitable for the meal. It can be noted that the percentage of purchased packages that was expected to be wasted within a given storage time progressively increased during storage, reaching considerably different values, depending on temperature.

In particular, at the recommended temperature (4°C), only a negligible amount of salad packages was expected to be wasted within the expiration date set by the producer (7 days). This confirms that expiration date of this product is consistent not only with its quality evolution but also with the need of minimising the risk for product waste. It is interesting to note that a slight increase in wasted salad was observed when storage time was increased to 8 °C, suggesting a certain tolerance of the product to temperature abuse. This means that quality changes perceived by consumers, although faster occurring at 8 °C (Tables 1 and 2), were still limited and associated to wasting risk

lower than 1%. By contrast, an abrupt increase in salad waste was estimated in the case of storage at 12 °C. In this case, 13% of the purchased packages was estimated to be wasted within the expiration date (7 days).

Temperature was demonstrated to dramatically affect not only quality and hygienic indicators of fresh-cut salad (Tables 1 and 2) but also consumer rejection (Figure 2) and the tendency of consumers to waste the product at domestic level (Figure 4). Proper campaigns should be carried out to inform consumers about the importance of food temperature control at domestic level as well as on the correct use of the different fridge compartments to decrease food waste and contribute to environmental sustainability. In 2011, the Stockholm Consumer Cooperative Society urged food industry, producers, wholesalers and distributors to lower the temperature to 4 °C in the food chain (Stockholm Consumer Cooperative Society, 2011). The main hindrance to the implementation of this indication was found in the increase costs of reducing storage temperature. However, net savings in terms of money and greenhouse gas emissions were estimated based on the expected decrease in waste for some fresh food categories (Eriksson, Strid, & Hansson, 2016). Consumer reaction towards campaigns with the message "reduced storage temperature to 4 °C in your refrigerator" could be significantly affected by information relevant not only to the environmental impact of our domestic behaviour but also about the amount of money which could be annually saved by decreasing domestic food waste.

Although the methodology here proposed allowed to obtain information about product wasting risk at domestic level, its predictive efficacy could be largely increased by collecting data relevant to product acceptability in conditions as near as possible to those experienced in a real domestic setting. In the present study, consumers evaluated salad acceptability based only on the appearance of the packaged product. By contrast, consumers at home have the possibility to open the package as well as to smell and touch the salad, for a more complete evaluation of its acceptability. They can also take into account the expiration date of the product printed on the package as factor influencing the decision of acceptability. To this regard, it has been reported that such information may

significantly affect freshness perception and liking of fresh-cut salad samples (Dinnella et al. 2014,; Vidal et al. 2013).

4. CONCLUSIONS

This study demonstrated that there are great opportunities for reducing domestic food waste by setting the recommended temperature in household refrigerators. Although the work was focussed on the study case of fresh-cut salad, the methodological approach here proposed could be extended to different foods and exploited to study the effect of factors other than storage temperature (i.e. food processing and distribution conditions) on food wasting risk. In addition, the proposed methodology could also be used to estimate food waste as affected by social characteristics and waste attitude of consumers as well as by communication aspects, including expiration date.

The study of the relation between the application of specific technological interventions and the risk that food is wasted by the consumers is still very much in its infancy. This kind of information could greatly benefit food sustainability by allowing the identification of technological interventions able to efficaciously tackle consumer waste. Using food technology in the pursuit of the production of more sustainable products is worth stressing, and further development on this topic seems needed.

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Legislation

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Commission implementing regulation (EU) No 1169/2011 of the European parliament and of the council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

Captions for figures

Figure 1. Questionnaire used to collect consumer data about habits of fresh-cut salad consumption after purchase. Two filling examples are reported.

Figure 2. Percentage of consumers rejecting fresh-cut salad during storage at 4, 8 and 12 °C. Symbols: data. Line: Weibull function estimate.

Figure 3. Consumption probability of fresh-cut salad during domestic storage. Symbols: data. Bars: Negative Binomial estimates.

Figure 4. Cumulative probability of fresh-cut salad to become a waste during domestic storage at 4, 8 and 12 °C.

Figure 1

How do you usually distribute the consumption of the packages purchased during a single shopping?

NUMBER OF PURCHASED PACKAGES	CONSUMPTION DISTRIBUTION (number of daily packages)										
	0	1	2	3	4	5	6	7	8	9	10 (days)
Consumer 1											
	0	1	2	3	4	5	6	7	8	9	10 (days)
1	1										
Consumer 2											
	0	1	2	3	4	5	6	7	8	9	10 (days)
4	1	1	1	1							

Figure 1.

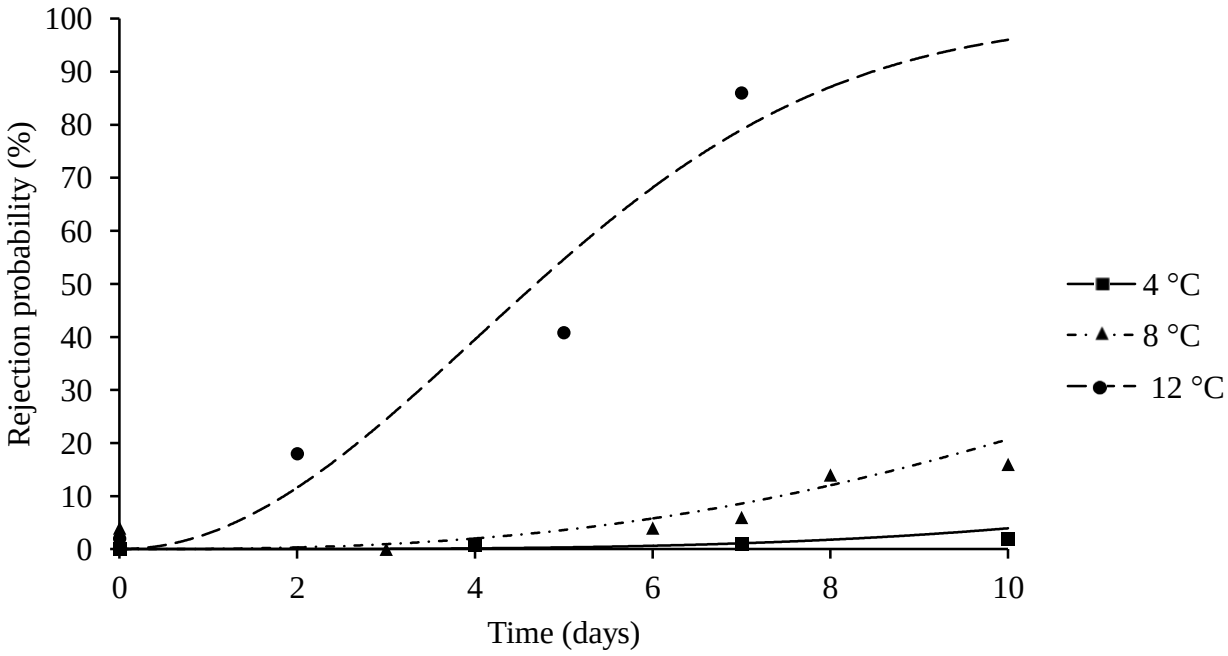

100
90
80
70
60
50
40
30
20
10
0
Rejection probability (%)
0
2
4
6
8
10
Time (days)
4 °C
8 °C
12 °C

Figure 2.

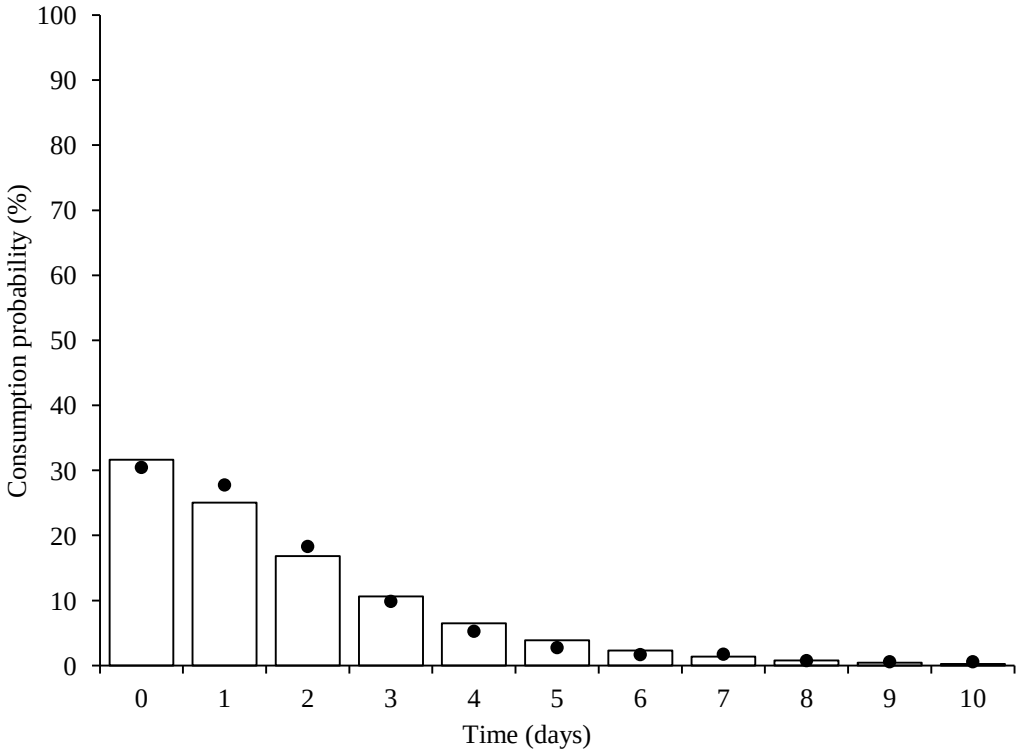

100
90
80
70
60
50
40
30
20
10
0
Consumption probability (%)
0
1
2
3
4
5
6
7
8
9
10
Time (days)

Figure 3.

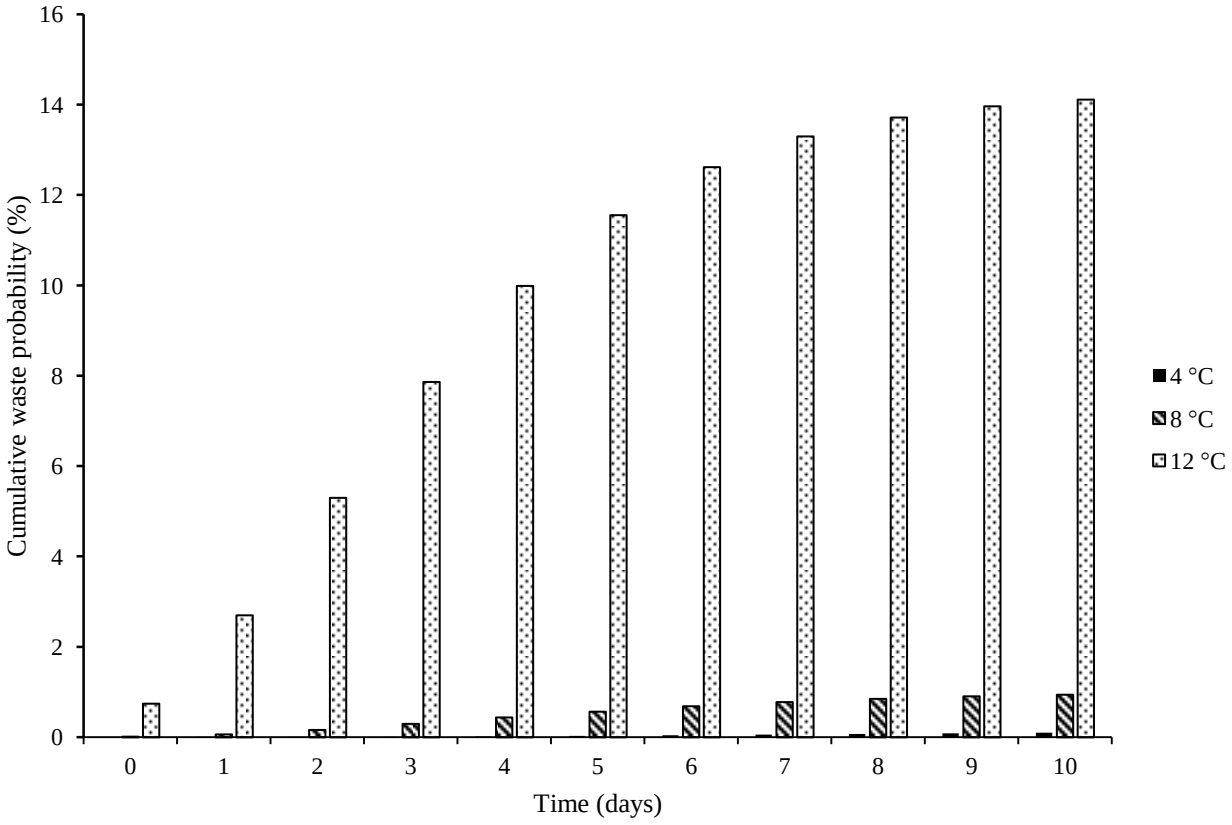

16
14
12
10
8
6
4
2
0
Cumulative waste probability (%)
0
1
2
3
4
5
6
7
8
9
10
Time (days)
4 °C
8 °C
12 °C

Figure 4.

Table1

Table 1. Green and brown indices of fresh-cut salad stored for increasing time at 4, 8 and 12 °C.

Temperature (°C)	Time (days)	Green index (%)	Brown index (%)
4	0	47 ± 6 [a]	0.1 ± 0.1 [b]
	4	38 ± 4 [ab]	0.3 ± 0.1 [b]
	7	36 ± 2 [ab]	0.4 ± 0.2 [b]
	10	33 ± 7 [ab]	0.4 ± 0.5 [b]
	14	30 ± 3 [b]	0.3 ± 0.3 [b]
8	0	45 ± 10 [a]	0.1 ± 0.1 [b]
	2	38 ± 10 [ab]	0.1 ± 0.1 [b]
	5	36 ± 4 [ab]	0.5 ± 0.2 [b]
	6	30 ± 7 [b]	0.3 ± 0.1 [b]
	7	30 ± 4 [b]	0.6 ± 0.5 [ab]
	9	25 ± 2 [b]	0.3 ± 0.3 [b]
	13	28 ± 9 [b]	1.2 ± 0.3 [a]
12	0	47 ± 7 [a]	0.1 ± 0.0 [c]
	1	33 ± 2 [ab]	0.0 ± 0.0 [c]
	2	28 ± 1 [bc]	0.2 ± 0.2 [bc]
	5	20 ± 4 [bc]	0.9 ± 0.3 [b]
	7	14 ± 0 [c]	4.3 ± 0.3 [a]

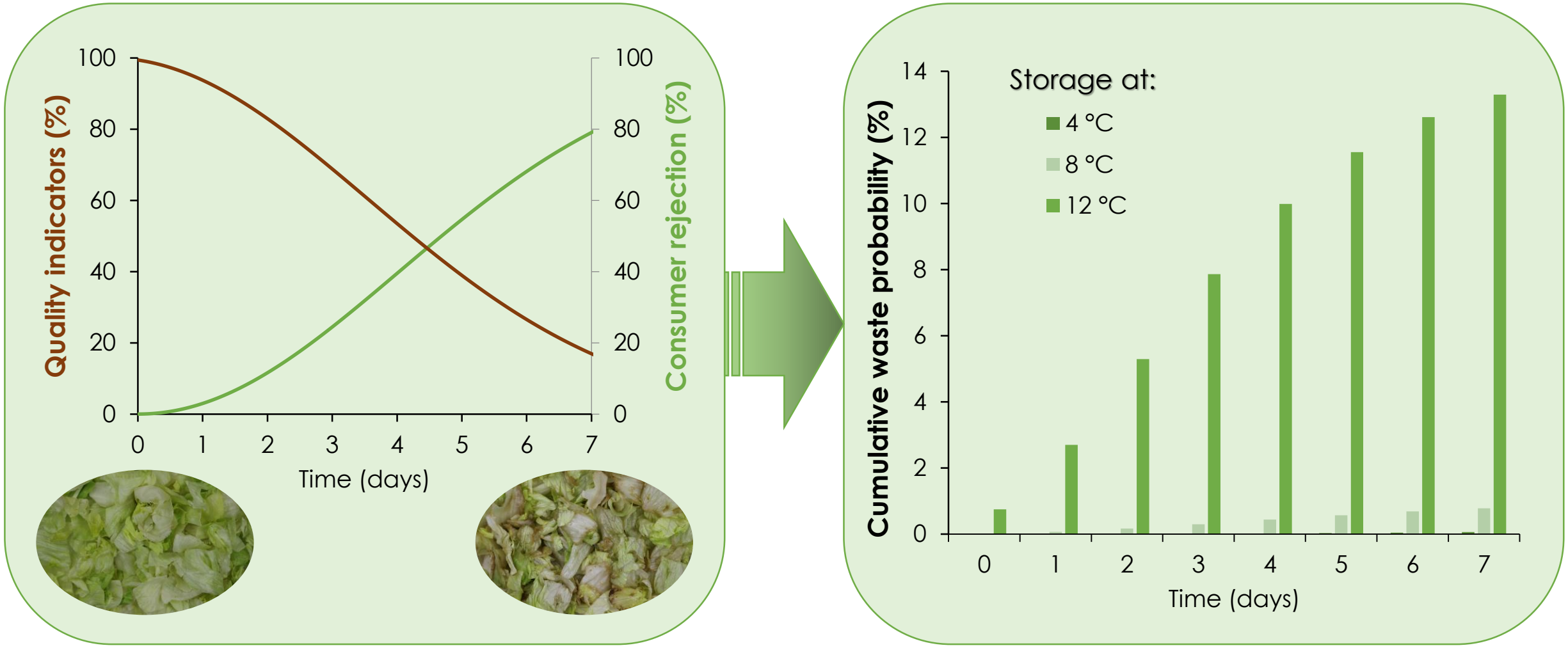
[a] for each column and storage temperature means indicated by the same letter are not significantly different ($p > 0.05$).

Table 2

Table 2. Total mesophilic bacteria, *Pseudomonas* spp., *Enterobacteriaceae*, total coliforms and faecal coliforms in fresh-cut salad stored for increasing time at 4, 8 and 12 °C.

Temperature (°C)	Time (days)	Total mesophilic bacteria (cfu g^{-1})	*Pseudomonas* spp. (cfu g^{-1})	*Enterobacteriaceae* (cfu g^{-1})	Total coliforms (cfu g^{-1})	Faecal coliforms (cfu g^{-1})
Control	0	1.1×10^4	1.2×10^4	1.3×10^2	1.0×10^1	< 5
4	4	2.8×10^6	3.1×10^6	7.2×10^2	1.0×10^2	< 5
	7	7.1×10^6	6.9×10^6	5.1×10^2	<5	< 5
	10	3.2×10^7	2.8×10^7	1.6×10^4	7.6×10^2	< 5
	14	7.8×10^7	5.6×10^7	3.1×10^4	3.4×10^3	< 5
8	2	5.0×10^5	7.2×10^4	6.4×10^3	6.0×10^3	< 5
	5	1.6×10^7	1.9×10^7	2.6×10^4	5.0×10^2	< 5
	8	2.0×10^7	1.3×10^7	7.5×10^4	2.0×10^3	< 5
	13	1.0×10^8	3.0×10^8	7.0×10^4	5.0×10^3	< 5
12	1	3.4×10^5	3.0×10^5	4.2×10^2	< 5	< 5
	2	4×10^6	3.0×10^6	3.4×10^4	7.2×10^3	< 5
	5	1.5×10^7	1.4×10^7	1.5×10^4	6.0×10^3	< 5
	7	6.8×10^7	5.5×10^7	6.7×10^5	3.7×10^5	< 5

Table 3. Estimates of the experimental parameters μ and σ of the rejection function of fresh-cut salad at 8 and 12 °C.

Temperature (°C)	μ (SE)	σ (SE)
4	3.197 (0.044)	0.278 (0.035)
8	2.852 (0.043)	0.376 (0.040)
12	1.726 (0.063)	0.493 (0.071)

*Graphical Abstract