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Shelf-life prediction models for ready-to-eat fresh cut salads: Testing in real cold chain

Theofania Tsironi, Efimia Dermesonlouoglou, Marianna Giannoglou, Eleni Gogou, George Katsaros, Petros Taoukis *

National Technical University of Athens, School of Chemical Engineering, Laboratory of Food Chemistry and Technology, Greece

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ABSTRACT

The aim of the study was to develop and test the applicability of predictive models for shelf-life estimation of ready-to-eat (RTE) fresh cut salads in realistic distribution temperature conditions in the food supply chain. A systematic kinetic study of quality loss of RTE mixed salad (lollo rosso lettuce-40%, lollo verde lettuce-45%, rocket-15%) packed under modified atmospheres (3% O_2, 10% CO_2, 87% N_2) was conducted. Microbial population (total viable count, *Pseudomonas* spp., lactic acid bacteria), vitamin C, colour and texture were the measured quality parameters. Kinetic models for these indices were developed to determine the quality loss and calculate product remaining shelf-life (SL_R). Storage experiments were conducted at isothermal (2.5–15 °C) and non-isothermal temperature conditions (T_{eff} = 7.8 °C defined as the constant temperature that results in the same quality value as the variable temperature distribution) for validation purposes. *Pseudomonas* dominated spoilage, followed by browning and chemical changes. The end of shelf-life correlated with a *Pseudomonas* spp. level of 8 log(cfu/g), and 20% loss of the initial vitamin C content. The effect of temperature on these quality parameters was expressed by the Arrhenius equation; activation energy (E_a) value was 69.1 and 122.6 kJ/mol for *Pseudomonas* spp. growth and vitamin C loss rates, respectively. Shelf-life prediction models were also validated in real cold chain conditions (including the stages of transport to and storage at retail distribution center, transport to and display at 7 retail stores, transport to and storage in domestic refrigerators). The quality level and SL_R estimated after 2–3 days of domestic storage (time of consumption) ranged between 1 and 8 days at 4 °C and was predicted within satisfactory statistical error by the kinetic models. T_{eff} in the cold chain ranged between 3.7 and 8.3 °C. Using the validated models, SL_R of RTE fresh cut salad can be estimated at any point of the cold chain if the temperature history is known. Shelf-life models of validated applicability can serve as an effective tool for shelf-life assessment and the development of new products in the fresh produce food sector.

1. Introduction

Ready-to-eat (RTE) fresh cut vegetables are usually pre-packed for convenience and retaining freshness. Different lettuce varieties, such as romaine lettuce, iceberg lettuce, lollo rosso lettuce, oak leaf lettuce and escarole, endive, radicchio, rocket, spinach, are available in pre-packed fresh cut form often used in mixed salads. Pre-packed fresh cut mixed salads are highly perishable with a storage life of about 7–10 days under refrigeration at temperatures ≤5 °C (Brocklehurst et al., 1987; Krasaekoopt and Bhandari, 2011).

The shelf-life of pre-packed salads is determined by microbial and chemical changes. The complex indigenous spoilage flora of pre-packed salads comprises *Pseudomonas*, lactic acid bacteria, *Enterobacteriaceae*, yeasts and moulds. Other causes of quality degradation include browning and enzymatic softening, which may also partially be attributed to enzymes released by microorganisms. A limited number of models that describe the effect of processing and storage conditions on the quality and the microbial risk in RTE fresh cut vegetables has been reported. Such models have been referred to spinach (Puerta-Gomez et al., 2013), broccoli (Kebede et al. 2015), carrots (Barry-Ryan and O'Beirne, 1998) and lettuce (Zhan et al., 2012; Zilelidou et al., 2015).

The trend towards more convenient food preparation and consumption has driven sales of pre-packed leafy salads and deli salads during the last years. A challenge for food scientists is to explore the potential of preserving the quality and extending shelf-life (Jacxsens et al., 2015; Tudela et al., 2013). Another challenge is to develop mathematical equations to predict the quality deterioration under different storage conditions and estimate shelf-life (Dermesonluoglu et al., 2016). Most of the available mathematical models have been developed only under isothermal conditions. They must be validated when used under non-isothermal conditions of the cold chain. Therefore, the objective of the study was to develop predictive models for shelf-life estimation of RTE fresh cut mixed salads and test their applicability under non-isothermal

* Corresponding author at: 5, Iroon Polytechniou, Zografou 15780, Athens, Greece.
E-mail address: taoukis@chemeng.ntua.gr (P. Taoukis).

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conditions and at temperature conditions observed in the food supply chain.

2. Materials and methods

2.1. Raw materials and experimental design for the kinetic analysis of quality deterioration

A commercially available in the Greek market RTE mixed fresh cut salad (160 g/package) of leaf lettuce (*Lactuca sativa* L. Var *crispa*) including, lollo rosso (40%), lollo verde (45%) cultivars, and rocket (*Eruca sativa* L.) (15%) was used in this study. Salads were packaged in laser micro-perforated commercial packages (oriented polypropylene OPP COEX perforated ANTIFOG film bags with 30 μm thickness and an O_2 permeability of 9000 cm^3/m^2 24 h atm) of modified atmosphere (3% O_2; 10% CO_2; 87% N_2). Samples were stored at controlled isothermal conditions of 2.5, 5, 10 and 15 °C (30 samples per temperature) in high-precision (±0.2 °C) low-temperature incubators (Sanyo MIR 153, Sanyo Electric, Ora-Gun, Gunma, Japan). Temperature was constantly monitored and confirmed with electronic, programmable miniature dataloggers in the incubators as well as inside one product package (COX TRACER®, Belmont, NC). Samples were taken based on a rough estimate of expected temperature-dependence of quality deterioration rate (2.5 °C: 0, 4, 8, 11, 14, 17, 21 days; 5 °C: 0, 4, 8, 11, 14, 17 days; 10 °C: 0, 1, 4, 5, 6, 8, 11, 13 days; 15 °C: 0, 1, 2, 4, 5 days; Dynamic: 0, 1, 4, 5, 6, 8, 13 days). Quality deterioration assessment was performed in triplicate samples.

2.2. Quality deterioration assessment

2.2.1. Microbiological analysis

For microbiological enumeration, a representative sample (10 g) was transferred to a sterile stomacher bag with 90 mL sterilized Ringer solution (Merck, Darmstadt, Germany) and was homogenized for 60 s with a Stomacher (BagMixer ® interscience, France). Ten-fold serial dilutions of sample homogenates (0.1 mL) were spread on the surface of the appropriate media in petri dishes for enumeration of different spoilage bacteria (Koutsoumanis et al., 2002). Total aerobic viable counts (TVC) were enumerated on Plate Count Agar (PCA, Merck, Darmstadt, Germany) after incubation at 25 °C for 72 h. *Pseudomonas* spp. were enumerated on Cetrimide Agar (CFC, Merck, Darmstadt, Germany) after incubation at 25 °C for 48 h. Lactic acid bacteria (LAB) were enumerated on De Man-Rogosa-Sharpe Agar (MRS, Merck, Darmstadt, Germany) followed by incubation at 25 °C for 96 h.

Two replicates of at least three appropriate dilutions were enumerated. Microbial growth was modelled using the Baranyi Growth Model (Eqs. 1–3) (Baranyi and Roberts, 1994),

$$y(t) = y_o + kA(t) - \ln\left[1 + \frac{e^{kA(t)} - 1}{e^{(y_{max} - y_o)}}\right] \quad (1)$$

$$A(t) = t + \frac{1}{k}\ln\left(\frac{e^{(-kt)} + q_o}{1 + q_o}\right) \quad (2)$$

$$\lambda = \frac{\ln\left(1 + \frac{1}{q_o}\right)}{k} \quad (3)$$

where $y(t)$ is cell concentration at time t, y_o is the initial cell concentration, k is the microbial growth rate, y_{max} is the maximum cell concentration, q_o is a parameter expressing the physiological state of cells when $t = t_o$ and λ = lag phase.

For curve fitting the program DMFit was used (available at http://www.combase.cc/index.php/en/). The kinetic parameters microbial growth rate (k, $days^{-1}$), λ (d), microbial loads N_0 (log cfu/g) and N_{max} (log cfu/g) were estimated at all tested temperature conditions.

2.2.2. Vitamin C content

Vitamin C (L-ascorbic acid) was determined using a high performance liquid chromatography method (HPLC). All analyses were carried out in duplicate homogenized samples, using pestle and mortar. Five grams of sample (of which: 40% *w*/w lollo rosso lettuce, 45% w/w lollo verde lettuce and 15% w/w rocket) homogenates were mechanically stirred in 10 mL of a 4.5% (w/v) solution of metaphosphoric acid for 15 min. Final volume was measured and an aliquot was filtered through a 0.45 μm PVDF filter prior to injection into the chromatographic column. The instrumentation details were: HP Series 1100 (iso pump, vacuum degasser, a Rheodyne 20 μL injection loop and a VWD Detector, controlled by HPChemStation software); Hypersil ODS column (250·4.6 mm) of particle size 5 μm; mobile phase: HPLC grade water with metaphosphoric acid to pH 2.2; detection at 245 nm; calibrated by external standard method (Giannakourou and Taoukis, 2003).

The average retention of vitamin C (C_{vitC}) is expressed relatively to an initial, average value of day 0 of the experiment ($C_{0,vitC}$), where C represents the concentration of ascorbic acid in 100 g of fresh weight (*f.w.*). In all cases, vitamin C loss was found to be adequately described by an apparent first order reaction (Eq. 4).

$$\frac{C_{vitC}}{C_{0,vitC}} * 100 = e^{-k_{vitC}t} \quad (4)$$

where, k_{vitC} is the apparent reaction rate of vitamin C loss, estimated by the slope of the linearized plot of $\ln(C_{vitC}/C_{0,vitC})$ vs t.

2.2.3. Colour measurement

Colour was measured using a chroma meter (Minolta CR-200 Chromameter, Chuo-Ku, Japan) at predetermined time intervals. Colour measurement was based on the CIELab scale and the three value parameters; L, a and b were determined. Colour measurements were performed individually for the mixed salad ingredients (lollo rosso lettuce, lollo verde lettuce and rocket) in triplicate. Different leaf salads presented different colour as well as different colour deterioration mechanism and rate during storage.

The *L-value* change with storage time was mathematically described by a linear increase described by Eq. 5.

$$L = L_0 + k_{colour}t \quad (5)$$

where, L is the *L-value* after storage time t (days) and L_o the initial value at time zero, k_{colour} the apparent rate of colour deterioration ($days^{-1}$).

2.2.4. Texture analysis

Textural degradation of each one of the mixed salad ingredients (lollo rosso lettuce, lollo verde lettuce and rocket) was measured against storage time and temperature. The required force to burst the leaves (force in g) of each of the salad ingredients was used as the representative texture index. Experiments were performed using a Texture analyser (Texture Analyser TA.XT2i, Stable Micro Systems Ltd., Godalming, UK). The film support rig HDP/FSR (containing perspex film support platform, aluminum circular top plate, thumb screws, 5 mm spherical probe) was used to measure burst strength of salad leaves. Prior to test performing, samples were placed over a hole in a raised Perspex platform. A top plate prevented sample from slipping during testing. The test was then carried out as the arm of the texture analyser that brought a 5 mm stainless steel ball probe was going down into the aperture. Maximum force to rupture the product was recorded and was referred as burst strength (g). Method details used were: Method = Measure force in compression/Return to start; Test speed = 1.0 mm/s; Pre-test speed = 2.0 mm/s & Post-test speed = 10.0 mm/s; Target mode = Distance; Distance = 10 mm; Trigger Type = 5 g; Data Ac. Rate = 500pps; Calibration Force (1000 g) & Calibration Height (50, 10, 4 cm) before analysis. A linear equation was