Modelling the microbial spoilage and quality decay of pre-packed dandelion leaves as a function of temperature

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ABSTRACT

The objective of the study was to develop and validate predictive models for the effect of storage temperature on the microbial spoilage and quality decay of pre-packed dandelion greens, a product recently introduced in the market. Dandelion leaves packed under modified atmosphere and air, were stored at isothermal conditions (2–15 °C). Quality assessment was based on microbial growth (total viable count, *Pseudomonas* spp., lactobacilli, yeasts-moulds, *Enterobacteriaceae* spp.), texture, enzymatic activity, vitamin C concentration and sensory evaluation. Models were developed and validated at dynamic conditions (T_{eff} = 7.8 °C). The effect of temperature on the different quality indices was expressed by activation energy values (E_a = 37.0–100.4 kJ/mol for air packed and 36.7–98.2 kJ/mol for MAP samples). The shelf life was calculated based on microbial growth (limit for *Pseudomonas* spp. = 8.5 logcfu/g), texture (limit BS = 240 N) and sensory scoring (limit S = 6/9). The developed predictive models can be applied reliably in the dynamic temperature conditions of the real chill chain.

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1. Introduction

Consumers' demand for fresh, healthy and convenient food has increased significantly over the last years. This trend has driven increasing sales of pre-packed leafy salads. New product development and the broadening of the range of salad varieties have supported this growth. Whilst coping with consumer demands for innovative, healthy and low priced products, food producers must balance this with the need to ensure food safety and an appropriate shelf life for their products. A challenge for food scientists is to explore the potential of extending the shelf life and develop models for shelf life estimation.

Fresh cut vegetables are usually pre-packed for convenience and to retain freshness. Lettuce based mixed salads are the most common fresh cut vegetables. However, carrot, tomato, broccoli, cauliflower and cabbage are also available in pre-packed fresh cut form. The shelf life of fresh cut leafy vegetables is about a week (Krasaekoopt and Bhandari, 2011). Shelf life of fresh cut salads is the outcome of combined microbiological-dependent and independent changes during storage, which may represent a vector of quality

and spoilage indicators. A complex indigenous spoilage flora comprises *Pseudomonas* spp., lactic acid bacteria, *Enterobacteriaceae* and yeasts-moulds. Other possible causes of quality degradation include browning and enzymatic softening, which may also partially be attributed to enzymes released by microorganisms. In general, most of the available data and safety parameters of fresh cut salads refer to the effect of storage conditions in these attributes. A limited number of models that describe the effect of processing and storage conditions on the quality and the microbial risk in vegetables, such as 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) have been reported. Most of the available mathematical models have been developed only under isothermal conditions. Such models that could be applied reliable at the variable conditions of the real cold chain could be vital for new product development and shelf life optimization in the fresh produce sector.

Modified Atmosphere Packaging (MAP) has been applied over the last decades to retain high quality of vegetables and extend their shelf life. The positive effect of MAP on the shelf life of fresh cut vegetables may be attributed to the reduction of product respiration, as well as of the overall rate metabolic and biochemical activities (Francis et al., 1999).

Dandelion (*Taraxacum officinale* Wiggers) has long been used as

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medicinal herb, mainly due to its inflammatory and hypoglycemic activities (Schütz et al., 2006). Previously, it was considered a noxious weed, but now, cultivated dandelion has been developed from the wild species. Fresh dandelion greens have a mild, slightly bitter flavour and they were very recently introduced in the fresh produce market. It is reported that dandelion leaves are an excellent source of provitamin A, vitamin C and calcium (Gupta and Rana, 2003). The majority of reports found in the literature are focused in a particular species, *T. officinalis*, and describe antioxidant properties (Hu and Kitts, 2003; Hudec et al., 2007), nutritional value (Escudero et al., 2003), phenolic profile (being flavonoid glycosides and hidroxycinammic acids, mainly chicoric acid) (Shi et al., 2008; Gatto et al., 2011; Sánchez-Mata et al., 2012) and fatty acids (Dias et al., 2014). Little information is available regarding the quality and shelf life of dandelion leaves during different packaging and temperature conditions.

The objective of the study was to develop and validate predictive models for the effect of storage temperature on the microbial spoilage and quality decay of pre-packed dandelion greens.

2. Materials and methods

Dandelion (*Taraxacum officinale* Wiggers) salad leaves were kindly provided by a leading company in the Greek Market for fresh-cut vegetable processing. Fresh-cut, washed and packed in modified atmosphere (MAP: 22% CO_2–78% N_2)) or air dandelion leaves were cold transported to the laboratory immediately after production. Dandelion leaves were sealed in commercial packages of oriented polypropylene (OPP COEX) non perforated ANTIFOG film with 30 μm thickness and an O_2 permeability of 1800 cm^3/m^2 24 h atm (MAP packed) and 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 (aerobically packed). Samples of 400 g each, were stored at controlled isothermal conditions of 2, 5, 10 and 15 °C in high-precision (±0.2 °C) low-temperature incubators (Sanyo MIR 153, Sanyo Electric, Ora-Gun, Gunma, Japan) and shelf life study was carried out. Temperature in the incubators was constantly monitored with electronic, programmable miniature dataloggers (COX TRACER®, Belmont, NC). Samples were taken in appropriate time intervals to allow for efficient kinetic analysis of quality deterioration. All determinations were made in triplicate samples.

In order to validate the applicability of the models from the isothermal experiments to real conditions, a variable temperature experiment was carried out. A time-temperature scenario was applied, that consisted of several repeated 12 h cycles of three isothermal steps, 2 h at 12 °C, 5 h at 5 °C and 5 h at 8 °C, in temperature programmable control cabinets (Sanyo MIR 153, Sanyo Electric, Ora-Gun, Gunma, Japan). The effective temperature (T_{eff}), the constant temperature that results in the same quality value as the variable temperature distribution over the same time period, was calculated based on the Arrhenius model and integrating as described by Giannakourou and Taoukis (2003) and the equivalent value was 7.8 °C. Samples were taken in appropriate time intervals and the rates of the quality deterioration measured during the nonisothermal experiment were compared to the values calculated for T_{eff} by the models based on the isothermal experiments.

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). Samples (0.1 mL) of 10-fold serial dilutions of sample homogenates

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 count was 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. Yeasts and moulds were enumerated on Rose Bengal Chloramphenicol Agar (RBC, Merck, Darmstadt, Germany) incubated for 168 h at 25 °C. For *Lactobacilli* and *Enterobacteriaceae* enumeration the pour-plate method was used. 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. For *Enterobacteriaceae* spp. enumeration Violet Red Bile Dextrose Agar (VRBD, Merck, Darmstadt, Germany) was used, which was incubated at 25 °C for 48 h.

Two replicates of at least three appropriate dilutions were enumerated. The microbial growth was modelled using the Baranyi Growth Model (Baranyi and Roberts, 1995). For curve fitting the in-house program DMfit of IFR (Institute of Food Research, Reading, UK) was used, kindly provided by Dr J. Baranyi. Kinetic parameters such as the rate (k) of the microbial growth were estimated.

2.2. Enzymatic activity

Polyphenol oxidase (PPO) extraction was performed according to Fang et al. (2007) using 5 g of fresh tissue. Sample was blended and homogenized with 10 mL of 0.2 M sodium phosphate buffer (pH 6.5) containing 4% (w/v) polyvinyl poly-pyrrolidone (PVPP) and 1% (v/v) Triton X 100. The homogenate was centrifuged at 6000 rpm for 15 min at 4 °C. PPO activity was assayed using an aliquot of 150 μL of the crude PPO extracts, diluted in 3 mL of 0.05 M sodium buffer (pH 6.5) containing 0.07 M grade catechol. The increase of absorbance in the final solution was measured at 420 nm for 5 min. Enzyme activity (U/g) was estimated by a calibration curve using standard polyphenol oxidase solutions (Worthington, Biochemical Corporation, NJ, USA).

L-Phenylalanine ammonia-lyase (PAL) extraction was performed according to Zhan et al. (2012) with slight modifications using 5 g of fresh tissue. Sample was blended and homogenized with 10 mL of 0.2 M sodium phosphate buffer (pH 8.5) containing 4% (w/v) PVP and 1% (v/v) Triton X 100. After centrifugation at 10,000 rpm for 25 min at 4 °C, the supernatants were filtered through gauze. Subsequently, the enzyme was mixed with 1.9 mL of 0.05 M sodium buffer (pH 8.45) and 50 mM L-phenylanine. The mix was incubated at 40 °C for 1 h. The absorbance in the final solution was measured at 290 nm.

Quinone extraction was performed according to Degl'Innocenti et al. (2007) with slight modifications, using 5 g of fresh tissue. Sample was blended and homogenized with 10 mL of methanol. After centrifugation at 10,000 rpm for 25 min at 4 °C, the supernatants were filtered through gauze. The increase of absorbance in the final solution was measured at 437 nm.

2.3. 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 on vegetable tissue, homogenized, using a pestle and mortar. 5 g of sample homogenates mechanically stirred in 10 mL of a 4.5% (w/v) solution of metaphosphoric acid for 15 min. The total 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);