Shelf-life modelling of ready-to-eat coconut

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Summary In this research the possible use of coconut as raw material to produce ready-to-eat fruit was investigated. The development of spoilage micro-organisms in packaged cut coconut stored at 4, 12, 21 and 24 °C was monitored. Spoilage of minimally processed coconut was correlated with high levels of mesophilic bacteria and was strongly dependent on temperature; however the modified atmosphere, created by respiration of the fruit when contained under a film barrier, could be fundamental to the determination of the product's shelf-life. The shelf-life of the product was kinetically modelled in order to check the effects of storage temperature and to assess the microbial indices most relevant for hygiene and quality of the product. Our results suggest that mathematical modelling could allow manufactures to rely on more objective parameters to determine the shelf-life of their products.

Keywords Predictive modelling, ready-to-eat fruit, *Serratia rubidea*, shelf-life, spoilage, storage temperature.

Introduction

Minimally processed refrigerated fruits have become a very important area of potential economic growth in the fresh-cut produce industry (Buta *et al.*, 1999). Such fruits can be categorized as convenience foods, offering valuable features such as freshness, easy practical use and good retention of nutritional qualities. Deterioration of minimally processed fruit is a very complex process apparently resulting from wound-induced biochemical and physiological modifications that mainly affect sensorial properties, appearance and flavour (Nguyen-the & Carlin, 1994). In particular activation of enzymatic systems leads to changes in colour, softening, flavour and loss of nutritional value (Svensson, 1977; Nicoli *et al.*, 1994). Moreover, the destruction of tissue and the subsequent release of nutrients enhances the growth of naturally occurring micro-organisms.

Most of the studies concerning the extension of shelf-life have been done by using ready-to-use vegetables, which are characterized by longer biochemical stability and different spoilage patterns than minimally processed fruits (Francis *et al.*, 1999). In fact, the marketing of fresh-cut fruit has been limited to 5–7 days compared with 15–20 days for preparations from vegetative and root tissues; this is because of the more rapid deterioration (Watada, 1997).

Improvements of shelf-life can be achieved through special care during cleaning and processing along the trade chain, as well as using high quality raw products and careful control of temperature and relative humidity.

Moreover, as minimally processed fruits continue to respire during storage, the depletion of oxygen and the accumulation of carbon dioxide can retard the microbial proliferation.

Coconut (*Cocos nucifera* L.) is one of the ten most useful trees in the world. It is a primary source of food, drink and shelter. In Sanskrit the coconut palm is called 'kalpa vriksha', which is defined as 'the tree which provides all the necessities of life'. In fact, man can use every part of the coconut; the white nut-meat can be eaten raw or shredded and dried and used in most cooking recipes.

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After harvesting fresh coconuts may be stored at temperatures of 0–15 °C in the shell and relative humidity of 75% or less, for 1–2 months. In storage, they are subject to loss in weight with drying up of nut milk.

No work has been done on the possible use of coconut as a raw material to produce ready-to-eat fruit.

The aim of this study was to investigate the value of predictive modelling in the estimation of the shelf-life of packaged fresh coconut.

Materials and methods

Fresh-cut coconut preparation

Deshelled fresh coconuts, obtained from 'Piccola Società Cooperativa, ARVUUM' (Bari, Italy), were manually sliced with sharp knives. The slices were packaged in high barrier plastic bags [Nylon/Polyethylene, 30 μm nylon and 120 μm of polyethylene (Tecnovac, San Paolo D'Argon, Bergamo, Italy)] by means of the S100-Tecnovac equipment. The permeability of films to oxygen, carbon dioxide and vapour was 2.53 cm^3 mm^{-1} 100 cm^{-2} day^{-1} atm^{-1}, 7.11 cm^3 mm^{-1} 100 cm^{-2} day^{-1} atm^{-1} and 0.014 g 100 cm^{-2} day^{-1}, respectively. The samples were packaged in ordinary atmosphere and stored at 4, 12, 21 and 24 °C.

Isolation and enumeration of micro-organisms

The medium and the conditions used were as follows: plate count agar (PCA; Biolife, Milano, Italy) incubated at 30 °C for 48 h for mesophilic bacteria.

Microbiological data are the average of at least four repetitions. The variability coefficients, expressed as the percentage ratio between the standard deviation and the mean value, were $<7\%$.

The cell load data, collected during the storage of the products, were modelled according to the Gompertz equation as modified by Zwietering *et al.* (1990):

$$y = k + A\ \exp\{-\exp[(\mu_{max}e/A)(\lambda - t) + 1]\}$$

where y is the log[CFU g^{-1}], k is the initial cell concentration, A is the maximum bacterial growth attained at the stationary phase, μ_{max} is the maximal growth rate (Δlog[CFU g^{-1}] day^{-1}), λ is the lag time (days) and t is the time.

The experimental data were modelled through the nonlinear regression procedure of the statistic package Statistica per Windows (Statsoft, Tulsa, USA).

Identification of bacteria

For identification three colonies of each different morphological type were selected from the primary cultures and kept on PCA (Biolife) at 4 °C until they were identified. All bacteria strains were grouped on the basis of a staining reaction, catalase test, oxidative-fermentative metabolism of glucose, motility reaction, cell shape and spore formation by heating cultures at 80 °C for 10 min and successive plating on PCA according to Collins *et al.* (1989). The isolates were identified at the species level, using the appropriate API identification system (BioMerieux, Marcy l'Etoile, France).

Determination of physico-chemical and organoleptic parameters during refrigerated storage

Concentration of CO_2 and O_2 were measured by colorimetric kits (Draeger Safety Italia S.p.A. Corsico, Milano, Italia).

A simple organoleptic evaluation was performed by trained people when the samples were opened for analyses. A hedonistic scale of 0–4 (0 = unacceptable, 1 = poor, 2 = fair, 3 = good and 4 = very good) for colour, texture, odour and yellowing of the slices was used.

Colour measurements

Colour analysis was made on sliced coconut. The changes in colour during the storage at 4, 12, 21 and 24 °C were monitored by colorimetric measurements using a tristimulus colorimeter Chromameter-2 Reflectance (Minolta, Osaka, Japan) equipped with a CR-300 measuring head. The instrument was standardized against a white tile before each determination. The colour of sliced coconut was determined as L', a' and b' values; in this work the changes in colour were expressed as yellowing (b_{tx}*/b_{t0}*). Data were the average of at least five repetitions. The variability coefficients, expressed as the percentage ratio between the standard deviation and the mean value, were $< 5\%$. The yellowing indices collected during the storage of the products were fitted with a Gompertz equation modified by Zwietering *et al.* (1990).

Shelf-life modelling

The microbiological shelf-life was calculated as the time necessary to attain a mesophilic bacteria count of 5×10^7 CFU g^{-1} (7.69 log CFU g^{-1}), as recommended by French regulations for fresh-cut vegetables (Ministere de l'Economie des Finances et du Budget, 1988). This parameter was calculated as follows:

$$T_{\text{shelf}} = [A/(e\mu_{\max})] + \lambda - \{[A \ln(-\ln 7.69)/(k + A)]/(e\mu_{\max})\}$$

where k is the initial level of mesophilic bacteria count, A is the maximum bacterial growth attained at the stationary phase, $\mu_{\max}$ is the maximal growth rate and λ is the lag time estimated with the Gompertz function.

Colour modification was defined by a stability time, which was assessed as the point of maximum acceleration of the yellowing reaction (dY^2/dt) estimated by the Gompertz function. In fact, when colour modification attains its maximum acceleration, the fruit undergoes very fast changes with a rapid loss of quality requirements.

A hedonistic grading of 2 was chosen to indicate the end of useful shelf-life, as based on sensory attributes. This was defined when the mean of the attributes fell to a value of 2.

The relationship between acceptability times (At) for bacteria, yellowing reaction and for sensory characteristics and temperature was represented by a linear equation:

$$\text{At} = a + bT$$

where a is theoretical shelf-life at 0 °C, T is temperature (°C) and b (the slope) represents the increase of degradative reaction rates for each 1 °C temperature increase (Singh, 1994). This parameter multiplied by a factor of 10 is known as the Q_{10} value.

Results

Figure 1 shows the growth of mesophilic bacteria at different temperatures of storage. At every

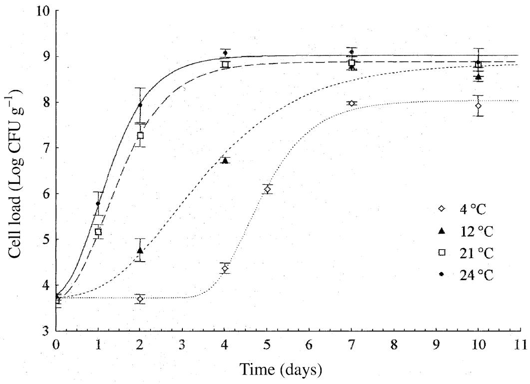

Figure 1 Evolution of mesophilic bacteria cell load during storage at different temperatures in ordinary atmosphere.

temperature, the microbial growth followed a classic pseudoexponential trend, described by the Gompertz function: an increase of storage temperature reduced the lag phase and quickened the growth rate. In Table 1 the estimated Gompertz parameters are reported.

As reported before, the kinetic model allows the evaluation of an acceptability time range, namely the time necessary to reach the mesophilic bacteria count value of 5×10^7 CFU g^{-1}, established by French regulations for ready-to-eat vegetables.

Figure 2 shows the effect of temperature on the yellowing reaction of coconut slices. This index also followed a trend that can be described by the Gompertz equation. A stability time can be assessed at the maximum acceleration of yellowing reaction (dY^2/dt^2) estimated with the previous Gompertz function.

Table 2 summarizes acceptability time (time necessary to reach the total bacterial count legal limit) and stability time (time corresponding to the maximum of the second time derivative of the Gompertz function; i.e. the maximum acceleration of the yellowing reaction) for each storage temperature. In the same table is also reported the values of shelf-life calculated on the basis of sensorial attributes.

The predicted values of the end of shelf-life were when the mesophilic bacteria count reached 5×10^7 CFU g^{-1} and were well related to the beginning of spoilage from a sensory point of view.

On the contrary, the maximum acceleration time of yellowing reaction agreed well with neither microbial nor sensorial shelf-life. In fact, the

Table 1 Parameters of the Gompertz equation for the growth of mesophilic bacteria in ready-to-eat coconut at different storage temperatures

Temperature (°C)	*K**	*A*	μ_{max}	λ	R^2
4	3.72 ± 0.06†	4.31 ± 0.10	1.96 ± 0.15	3.74 ± 0.09	0.997
12	3.71 ± 0.27	5.13 ± 0.55	1.17 ± 0.34	1.21 ± 0.73	0.993
21	3.60 ± 0.22	5.29 ± 0.30	2.41 ± 0.52	0.35 ± 0.29	0.998
24	3.71 ± 0.11	5.32 ± 0.12	2.87 ± 0.92	0.29 ± 0.26	0.994

*Gompertz parameters: *K*, initial cell load (log CFU g^{-1}); *A*, maximum cell load attained in stationary phase; μ_{max}, maximum growth rate (Δlog CFU g^{-1} day^{-1}); λ, lag phase (days); R^2, determination coefficient.
†Standard errors.

discriminant capability of the colorimeter, which is superior to that of the human eye, can demonstrate colour modifications.

The temperature dependence of the degradation kinetics can be easily expressed in terms of Q_{10} (Saguy & Karel, 1980).

In Fig. 3, the temperature dependence of the total bacterial count is compared with sensorial and colour indices; both the latter indices yielded analogous Q_{10} values equal to 3. This finding means that sensory evaluation strongly correlated with colour modification. Mesophilic bacteria

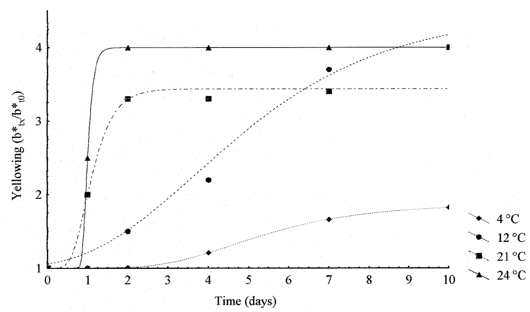

Figure 2 Yellowing evolution of coconut samples during storage in ordinary atmosphere at different temperatures.

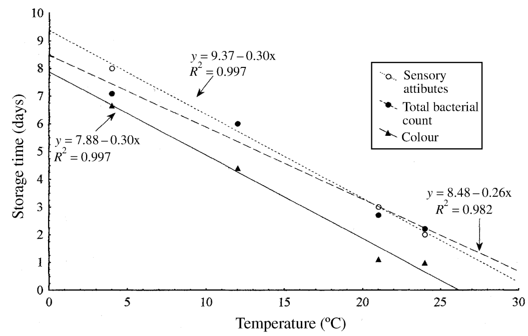

Figure 3 Influence of storage temperature on shelf-life of minimally processed coconut.

Table 2 Prediction value of coconut slice shelf-life time (days) according to storage temperature, expressed as the time required for mesophilic bacteria count to reach a level of 5×10^7 CFU g^{-1}, resulting in a loss of optimum sensory characteristics

Temperature (°C)	Mesophilic bacteria count*	Yellowing†	Sensory attributes‡
4	7.01	6.70	8
12	6.00	4.40	6
21	2.71	1.10	3
24	2.21	0.99	2

*Time (days) taken by mesophilic bacteria count to reach 5×10^7 CFU g^{-1}.
†Time (days) of maximum acceleration of yellowing reaction.
‡Time (days) taken to reach spoilage value as defined by the mean of the sensory attributes.

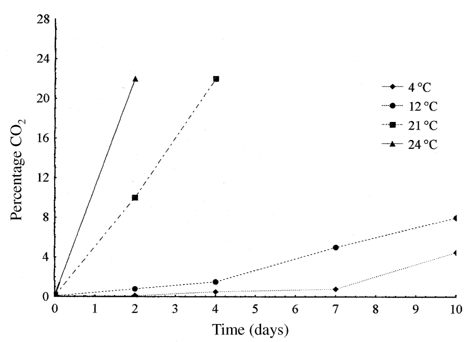

Figure 4 Influence of temperature on CO_2 concentration produced during the storage of coconut samples at different temperatures in ordinary atmosphere.

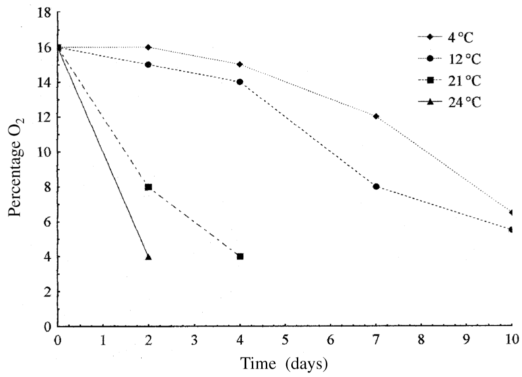

Figure 5 Influence of temperature on O_2 concentration consumed during the storage of coconut samples at different temperatures in ordinary atmosphere.

count seems to be influenced less by temperature, the Q_{10} value is 2.6, which means that the microbiological shelf-life decreased 2.6-fold (from 7 to 4.4 days) when changing from 5 to 15 °C.

Figures 4 and 5 show the evolution of gases inside the package, produced by coconut respiration. It is possible to observe that the modified atmosphere created inside the package developed by an increase in the level of CO_2 and a decrease in the O_2 concentration. This development is increased by the increase of storage temperature.

Discussion

The use of coconut as a raw material for ready-to-eat products has not been proven as no work has been done on the microbiological safety at a pH value of about 6. Moreover, evaluation of modified atmosphere packaging for fresh-cut fruit remains a trial-and-error exercise.

However, the application of predictive models would help to establish some scientific basis to solve questions such as length of commercial shelf-life or food safety.

The shelf-life of packaged cut coconut was strongly dependent on temperature. However, the modified atmosphere created by respiration of the fruit when sealed by a film barrier could be fundamental to the determination of the product's shelf-life. Although both CO_2 and O_2 vary very little in the samples stored at 4 °C for about 7 days, at higher temperatures the respiration of coconut rose, with an increase in CO_2 and decrease in O_2 inside the packages.

The predominant microflora are mesophilic bacteria with an initial count of approximately 10^4 CFU g^{-1}. *Serratia rubidea* (also called *S. merino rubra*) was the most frequent bacterial species isolated from packaged coconut. The *S. rubidea* frequency, calculated as the number of isolates of a species over total isolates of the species, was 0.80; the other species isolated were *Rahnella aquatilis* (0.10) and *Pseudomonas fluorescens* (0.05). The occurrence of the other species was sporadic. *Serratia* species are important in food microbiology, because not only are they involved in food spoilage but they are also opportunistic pathogens that can cause various diseases in humans and animals. Members of *Serratia* are distributed in soil, air and water, and are associated with large numbers of plants and animals. In particular, *S. rubidea* has been isolated in spoiled coconut (Grimont & Grimont, 1984) demonstrating that this micro-organism can grow on this kind of product.

The prevalence of *S. rubidea* in coconut, probably due to its ability to metabolize specific carbon sources, could be regarded as indicative of a potential human health hazard. However, the connection between the development of disease and consumption of food contaminated with *Serratia* is not well established (Rafii, 2000).

The prediction of a product's shelf-life indicates the time that the manufacturer could expect his product to stay in the market. Depending on the temperature of the distribution chain of the product, different commercial shelf-lives could be predicted. The importance of the storage temperature is very apparent and a careful control of temperature, together with the use of high quality raw material, seem to be the principal tools to achieve a high production standard and acceptable shelf-life. Current practice in food marketing uses a 'best before' date for consumption. In the case of coconut, 7 days under refrigeration conditions could be suggested by the producer; however, it has been demonstrated that the temperature in the distribution chain is always closer to 10 than to 5 °C. At this temperature the shelf-life shrinks to 5 days; consequently action should be taken to control the temperature.

This paper represents a contribution to our knowledge about shelf-life of ready-to-eat coconut. Moreover the results suggest that mathematical modelling could allow manufactures to rely on more objective parameters to determine the shelf-life of their products. However, deeper investigations are necessary to assess the safety of fresh coconut slices.

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