Development of a time–temperature indicator (TTI) label by rotary printing technologies

S. Zabala*, J. Castán, C. Martínez

CEMITEC, Polígono Mocholi, Plaza Cein, 4, 31110 Noain, Navarra, Spain

A R T I C L E   I N F O
Article history:
Received 19 December 2013
Received in revised form 30 July 2014
Accepted 5 August 2014
Available online 23 August 2014

Keywords:
Cold chain management
Functional printing
Printed intelligence
Time–temperature indicator
Smart labels

A B S T R A C T
A novel continuous production process is presented for smart labels specifically designed as time–temperature indicators (TTI). Printing and converting technologies are set out on a traditional rotary press that works at high web speed with a great repeatability of the printed product. TTI labels are based on a functional water-based ink that impregnates some areas of an absorbent paper whose edges are waterproofed by a UV graphic ink. A rotary screen printing process is used for the deposition of the inks and a plastic layer is applied by lamination on both sides of the substrate to ensure optimum wetness of the functional ink. The formulation of the ink includes a chromogenic growth medium with a starter culture; the formulation of the ink includes a chromogenic growth medium with a starter culture, hence changing the pH indicator colour. A model based on the Gompertz equation has been developed to simulate the colour change of the labels for different starter culture loads and temperatures, with an accurate match between predictions and experimental data. This model can be a useful tool in the design of new features of the tags that can be a straightforward scale-up to a pre-industrial level. The rotary printing process allows massive production of low cost smart tags.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Consumer behaviour in fresh goods market is heading towards new improved requirements on food traceability and quality. Consequently, companies require systems to guarantee their product healthiness and quality (Nopwinyuwonga, Tranichb, & Suppakula, 2010). Maintaining an adequate temperature during postproduction stages of perishable products is essential to guarantee their quality. This factor gains relevance in fresh goods and pharmaceutical products which suffer from thermal degradation, i.e. cases where cold chain control is a key feature within the quality management.

In order to achieve a safe accessibility to fresh products, an effective cold chain management is required (Calanchea et al., 2013; Koutsoumanis, Taoukis, & Nychas, 2005; Kreyenschmidt, Hübner, et al., 2010; Zhang, Liu, Mu, Moga, & Zhang, 2009). The importance of the different aspects in the postproduction stages is evident in the European legislation CE 178/2002 which describes the need to guarantee the proper management and food handling to ensure safe consumption. Nowadays there are some different systems which allow the management and control of the cold chain. One of them, the TTI smart labels, are time–temperature indicators, cheap and easy to integrate in packaging. These TTIs can be used massively as the understanding of its results is straightforward.

The operation of a TTI label is based on a mechanical, chemical, electrochemical, enzymatic or microbial change which is expressed as a mechanical deformation, colour development or colour evolution. This visual response shows the accumulated information about the storage temperature of the product. There are two kinds of thermal history indicators, partial or total, according to their response mechanism. Partial indicators do not show response unless the pre-set temperature is exceeded, and therefore warning against exposure to a temperature which causes a change in product quality, and consequently a likely health threat. On the other hand, the total ones show a continuous response depending on the temperature along the product lifecycle. The latter represent higher interest for commercial and research purposes.

Different strategies are used for the TTI functionality. Diffusional TTIs are based on the velocity of diffusion of an indicator along a wick of high quality blotting paper. Commercial examples are Monitor Mark from 3M and products as Timestrip. In the literature other diffusional systems for TTIs are found (Galagan & Su, 2008; Wanihsuksombat, Hongtrakul, & Suppakul, 2010). Enzymatic TTI tags have been commercialized by the company Vitsub and different enzymes have been studied at a laboratory scale, like...
amylase (Yan et al., 2008) or peroxidase (Rani & Abraham, 2006). Polymer-based TTIs use polymerization reactions which depend on temperature, as Fresh-Check labels. The solution that is offered by the label OnVu from Freshpoint is based on a material that can be activated by a UV light source and changes its colour quicker as temperature increases (Kreyenschmidt, Henning, et al., 2010; Mai et col., 2010, 2011).

Finally, bacteriological-based TTI labels exploit the growth of selected acid-producing bacteria, which generate a pH reduction and consequently a change in the acid-alkaline indicator colour (Rue et al., 2012). In this case, the labels are freeze-stored and they are activated when they thaw out. The French company Cryolog S.A. has developed microbial TTI labels using a gelling agent to get a thick growth medium. An interesting advantage of microbial strategy is that the label colour progresses at every temperature (it is a total TTI), but faster when the heat transfer rate is higher. It has been demonstrated that these devices are safe when they are used on chilled food packaging (EFSA, 2013).

The TTI label proposed in this paper uses a bacteriological strategy, whose key features are fully compatible with a production process that is completely carried out by rotary printing techniques, converting technologies and using harmless materials compatible with food packaging. In this work, gelling agents are not included in the formulation of the growth medium, and that is an advantage because most of them must be deposited while the mixture is still hot. Instead of using gelling agents, it has been used a thickener to obtain a viscous ink with the growth medium that can be screen printed. The product can be customized using this technology, due to its flexibility as shapes and sizes of the functional areas can be specifically designed. A key design feature of a customized tag is the access to colour settings or changes for specific time and/or temperature transitions. That can be achieved by using a model developed after studying the effect of some components of the formulation of the functional ink on the colour evolution of the tags for different temperatures.

The suggested innovation in this paper is then related to the manufacturing process and the way the product is integrated in a label and its printing process. This is included in the application of the Spanish patent P201231653 (Manufacturing method for printing a flat reservoir to confine functional liquids). The use of roll-to-roll technologies eases continuous processes of high productivity. Moreover, the use of low cost materials and massive production processes helps in giving response to the high-demanding food industry, even to low market-value products. These customizable labels can be easily integrated in food packaging and there are no major issues in being also incorporated into consumer products recycling chain.

2. Materials and methods

2.1. Materials

The printing substrate is an absorbent paper made of pure cellulose (73 g/m², Filtris Anoia, S.A.). Protective plastics are polypropylene (PP) self-adhesive films with 50 μm thickness, white on the back side of the label and clear gloss on the front side. Both substrate and plastics are supplied as rolled materials with 3–6” inch reel cores. Two kinds of graphic inks are used: a UV-curing varnish for the barrier and a coloured water-based (WB) ink for the register layer.

The registered functional ink is water-based, and its standard formulation includes the following components: Glucose 1-hidrate 3% w/w, powdered milk 1% w/w, yeast extract 0.2% w/w, peptone 0.2% w/w and Chlorophenol Red 0.01% w/w. A food industry maturation starter is used as bacteriological source, composed by a mixture of Lactobacillus sakei, Staphylococcus carnosus and Staphylococcus xylosus (TEXEL SA-306, Danisco). The initial amount of starter is on the range of 0.5–2.0% w/w. Different quantities between 0.75 and 2.0% w/w of hydroxypropylcellulose (HPC, Klucel-H, Aqualon) are used depending on the viscosity objective. The manufacturing process of the ink includes the steam sterilization and mixing of all the components, except the maturation starter, using mechanical intense stirring for about 3 h. This is followed by a step where the pH is adjusted with a concentrated NaOH solution. Finally, the starter is dispersed in a small water volume and poured into the mixture. This can be assumed as the starting-time point, and the initial colour is violet.

Ink viscosity has been measured with a rotary viscometer (Viscostar Plus L, Myr, Spain). Some accessories have been used: a low volume adaptor, TL-type impellers and a jacketed vessel with a recirculating bath in order to keep constant temperature.

2.2. Printing press machine

At the laboratory scale the labels are prepared by using a manual screen printer with flat polyester screens, using small scale equivalent tools to those of the pilot plant.

At the pre-industrial scale, the pilot plant is a roll-to-roll plug & play printing machine (Lambda model from Edale, UK) that uses 330 mm max width rolled substrates (Fig. 1) and runs at web speed of up to 50 m/min. It incorporates a web guider system (DRS2202, ELGUISER) and a web contact cleaner (YIELDMAX). In this work an Edale 18” flexographic unit has been used with polymeric plates and a screen printing unit (Drukkop RSI II 16° 1/8”, Stork) has been used with stainless rotary screens (Gallus, Switzerland). The lamination process is of internal development, which uses an expandable mandrill (Re Taurus) and the gravure printing unit (RK PrintCoat Instruments, UK) as the main modules in order to carry out the lamination process. Metallic or rubber rollers can be used and the pressure of lamination is controlled, reaching up to 6 bar.

The WB-ink is dried out in an infrared module (7 IR lamps, XERIC WEB, USA) and the ink for the barrier is cured by a 160 W/cm UV-lamp (VCP-38-1, GEW, UK).

2.3. Colour measurements

Colour measurements of the labels have been made using a colorimeter (Spectroeye, X-Rite, Switzerland) set to D65 illumination source and a 10° angle, working in CIELAB colour system. The measurement result is assumed as the average value of three consecutive measurements. Fig. 2a shows the evolution with time of the reflectance curves of a TTI active area. At the beginning, the device shows an intense violet colour and the curve has a significant valley reaching 580 nm and a small peak at 450 nm. As the label changes its colour, the valley becomes less steep and the peak smoothens; the label loses its reddish tonality turning into yellow. The qualitative evolution is: violet-purple-orange-amber-yellow, as it can be seen on the photographs in Fig. 2. Conversion of data reflectance spectrum into CIELAB parameters is a way to simplify the colour monitoring (Fig. 2b). L parameter is related to the colour tone and increases with colour label evolution, with around 20-units difference. The parameter a quantifies the green component of the colour (negative values) and the red component (positive values). Initial value for this parameter in the label is around 20, due to the big amount of red colour present in it. This value decreases with the colour evolution of the label to slightly negative values. Finally, b parameter has been found to be the best one to show the evolution of the label colour change considering it suffers the biggest changes from -25 (big amount of blue) for the initial violet
label up to +25 for a yellow label. This latter parameter has a substantial increasing trend while the colour becomes yellowish, which is the case-study target.

2.4. Data analysis

A series of 16 different labels were prepared in the lab to study the effect on discolouration of two variables: storage temperature and initial starter load. Four storage temperatures were selected for the kinetics study: the typical temperature of refrigerated fresh products (278 K), an intermediate temperature (286 K), the considered ambient temperature (293 K) and a temperature corresponding to an abuse of the storage conditions (303 K). It was checked that freezing temperatures practically inactivate the starter. After a previous screening survey, four initial starter loads were selected: 0.46, 0.72, 0.97 and 1.16% w/w.

**Fig. 1.** R2R printer: unwinder (1), web guider (2), web cleaner (3), corona surface treatment (4), screen printing unit (5), convection drier (6), flexography unit (7), UV-lamp (8) and IR module (9).

**Fig. 2.** Colour evolution of the tags with time: Spectral curve evolution (a) and L, a, b CIELAB parameters (b). They start in violet and turn into yellow along time.
Colour evolution is a way to analyse the TTI’s behaviour due to its relation with the bacteria growth kinetics. This kinetics has been studied applying a model based on Gompertz equation, which can be described as follows:

\[ b(t) = \Delta b \cdot \exp\left(-B \cdot \exp\left(-s \cdot k_T \cdot t\right)\right) + b_0 \]  

where:

- \( b(t) \) is the value of the \( b \) CIELAB parameter along the lifetime of the label, \( b_0 \) is the starting value;
- \( B \) is a time-independent constant;
- \( s \) is the initial number of starter % w/w.;
- \( k_T \) is the reaction rate for \( T \) temperature.

The following objective function \( (F) \) has been minimized to estimate \( k_T \) and \( B \):

\[ F = \left[ \sum_{i=1}^{NS} \sum_{j=1}^{NT} \sum_{k=1}^{N} \frac{1}{N} \frac{(b_{\text{mod}}(i,j,k) - b_{\text{exp}}(i,j,k))^2}{b_{\text{exp}}(i,j,k)^2}\right] \]  

where:

- \( b_{\text{exp}}(i,j,k) \) and \( b_{\text{mod}}(i,j,k) \) are the \( b \)-parameter values of the colour obtained experimentally and using the model, respectively, for each starter load \( i \), reaction temperature \( j \) and monitoring point \( k \) for a time. \( N \) is the number of experimental data for each label, \( NT \) is the number of studied temperatures and \( NS \) the number of starter loads.

With the estimated reaction rates and using the Arrhenius equation from below, the activation energy \( (E_a) \) is then calculated.

\[ k_T = A \cdot \exp\left(\frac{-E_a}{R \cdot T}\right) \]  

where:

- \( k_T \) is the reaction rate (h\(^{-1}\)), \( A \) is the pre-exponential factor (h\(^{-1}\)), \( E_a \) is the system activation energy (kJ/mol), \( R \) is the ideal gas constant (8.314 J mol\(^{-1}\) K\(^{-1}\)) and \( T \) is the absolute temperature (K).

### 3. Results and discussion

The proposed TTI is a flat and flexible label that is manufactured in a continuous roll-to-roll process, with a final presentation of a sticker-like product. Fig. 3 outlines the construction of the layers. Both sides of a cellulose substrate (1) are printed with a UV curable ink (2) which penetrates the substrate, a waterproof barrier which defines the limits of the functional ink area. Subsequently, the back side of the substrate is laminated with a white plastic film (3), building up a porous central confinement area where the functional material (4) is printed and ultimately changes its colour. Finally, the device is sealed with a clear lamination layer (5). This new TTI manufacturing process based on printing technologies offers the flexibility to modify label designs in shapes and sizes.

The UV curable varnish used as barrier is printed on both sides of the substrate, in such a way that it must flux from the surface to the centre of the paper on each side before being cured. That process implies that the viscosity of the varnish at printing temperature must be adequate to let it flux across the cellulose. Common screen printing varnishes are more viscous than the one used for these layers, which was characterized with 1817 cP (293 K). The quantity of the varnish that is deposited on both sides is adjusted in relation with the absorption capacity and the thickness of the cellulose substrate, with the aim of obtaining a good lateral sealing of the confinement area. In this work, a 170 μm thick pure cellulose substrate with a klemm absorption of 75/70 mm has been used. The optimal screen stencil for the combination of this varnish and this substrate was a 64 mesh/inch and a 100 μm mesh width.

The functional ink contains a growth medium with a pH indicator and a microorganism inoculum (maturation starter). Lactic acid bacteria are selected, then their metabolism increases the acidity of the medium where they are and that causes a colour change in the pH indicator. The colour change of the label can be observed progressively from violet to yellow. The metabolism of the microbiota is quicker as temperature is higher and, consequently, also the discolouration. In a first step we have studied the kinetics...
of that discolouration process in the laboratory. After that, a pre-industrial manufacturing process has been developed to resemble industrial production using printing technologies.

3.1. Kinetics model

The evolution of the colour of TTI labels has been studied under isothermal conditions at temperatures between 278 K (the reference temperature to store refrigerated products) and 303 K (a huge abuse on cold chain conditions). The label colour has been measured at different times recording the \( b \) parameter at each one of them. Fig. 4 depicts an example of the monitoring process carried out on four labels prepared with different amount of starter at a constant temperature of 286 K. Discolouration is a progressive phenomenon and the detection of the colours depends also on the receptor. Therefore, it has been defined an endpoint level, just below \( b = 20 \), where the final colour of the label is clearly detected to a naked eye (dark band in Fig. 4).

The curves have a steeper slope during the first part of the lifecycle of the labels followed by a final smoother section. This shape can be understood as a microbial growth limitation in space and nutrients, as well as toxic metabolite production, which makes that specific growth rate of microbes, is not constant over time (Labuza & Fu, 1995). According to this kind of curves, it is convenient the application of a kinetics model based on the Gompertz function (Eq. (1)). Analysing experimental data, it can be simplified and define \( b_0 \) and \( \Delta b \) as 21.0 and 44.7, respectively. Both the initial value of the colour and the amplitude during lifetime are the same for all labels.

The objective function (Eq. (2)) has been minimized to estimate \( k_T \) and \( B \), and it has been obtained a value of \( B \)-parameter of 2.8013. The estimated values for \( k \), are in the range of 0.0040 h\(^{-1}\) for 278 K and 1.1131 h\(^{-1}\) for 303 K. As it was expected, they are highly temperature-dependent. Fig. 5 shows the comparison between experimental points (dots) and predicted data (dashed lines). In the 293 K graph, the results for different starter loads are shown and the model matches them. The initial small differences between both groups of data are related to the experimental variations. The effect of temperature on the colour evolution is shown on the graph for 0.97% starter load. A good correlation between experimental and predicted data is found, even with different temperature values. The worst model results are those for the label at 303 K when the colour reaches the final \( b \)-values. But in those cases where \( b \) is higher than 20, the labels become completely yellow, and the model predictions are irrelevant for the practical working of the TTI label.

For this initial starter load of 0.97%, the endpoint time is around 4 days under fridge temperature conditions, nearly 6 h at ambient temperature and only 3 h at the hottest situation. These times could be interesting for fresh products with a high spoilage rate that causes a short life expectancy in the market chain. A lower load of starter is advised for products with a higher safe consuming time.

In the case of a starter load of 0.46%, the endpoint under fridge temperature conditions increases up to almost a week, and the times for 293 K and 303 K are 12 and 6 h, respectively.

The Arrhenius plot for the effect of temperature on the TTI discolouration is shown on Fig. 6 \((R^2 = 0.930)\). The system \( E_a \) is calculated, according to Eq. (3), as the slope of the graphical

**Fig. 4.** Colour evolution in labels with different amount of initial starter (% w/w) at 286 K. Dark band shows final colour to the naked eye noticing.

**Fig. 5.** Colour evolution of the labels: experimentally obtained (dots) and model estimated (discontinuous line) for: 293 K of constant temperature and different amounts of initial starter; and 0.97% of initial starter and different temperatures.
representation of \( \ln (k_T) \) vs. \( 1/T \) and the result is 91.92 kJ/mol. This \( E_a \) value approaches those from other TTI labels in the market, which are used for the control of perishable food. For instance, Onvu label \( E_a \) is in the range 92.79–105.75 kJ/mol (Kreyenschmidt, Henning, et al., 2010), and the TTI from Vitsab is a system with an \( E_a \) of 68.7–102.1 kJ/mol (Taoukis, Koutsoumanis, & Nychas, 1999). Other non-commercial TTI proposals have similar values to those from Yan et al. (Yan et al., 2008) with \( E_a = 102–114 \) kJ/mol or Valkousi, Billaderis, & Koutsoumanis (2008, 2009) with \( E_a = 103.2–127.3 \) kJ/mol.

These results suggest that the proposed label concept is usable on a variety of perishable food products. For instance, fish products, which spoilage reaction has an activation energy of 73.6 ± 8.4 kJ/mol (Giannakourou, Koutsoumanis, Nychas, & Taoukis, 2005).

3.2. Scale up to an industrial process

Production process includes industrial common printing and converting machinery that is available in the labelling sector. Fig. 7 outlines the process that is carried out in a continuous way, similar to a standard mass production environment. At our pilot plant, the production is performed step by step, winding and unwinding between stages.

Since it is a product in which several layers are printed, it is necessary to include a previous step to print the registering marks in order to have a correct registration between layers. This layer does not represent technical difficulties, but it allows the remaining layers to overlap properly. A water-based ink for flexographic printing is used and it is dried out in the infrared (IR) module.

The first stage of the characteristic printing process comprises the barrier layer, which is used for limiting the active zone by sealing its edges. For that purpose, a UV varnish is screen printed on both sides of the cellulose substrate (Fig. 7, 2-4 items), forming the anti-dehydration barrier. The following step is the lamination of the back side of the label, using a self-adhesive white-plastic-rolled film (Fig. 7, 5-6 items). At this time the reel is ready for printing the functional layer. Once this has been done, the functional layer can be printed, or the foil can be stored for future work.

The next stage is the functional printing, which includes three processes to be carried out consecutively and immediately: sterilization of the substrate by UV radiation, deposition of the active material and finally its protection with a clear plastic film. The functional layer is also manufactured by screen printing, which can be laid on in large amounts of material. The high deposition screen-printing stencil has 64 mesh/inch ratio and 265 μm mesh width, and a deposition of 115 g/m² (\( \sigma = 10.7 \)) of the functional material is achieved. Due to the functional ink wetness requirement, it must be immediately covered with the clear gloss polypropilene film, to avoid the active area coming into contact with any of the press rollers (Fig. 7, 7-10 items). After this protection step, the material is then wound and frozen.

Laminating conditions must be optimized in order to obtain a good adhesion between the PP film and the substrate (especially on the surface of the UV-varnish printed areas) as well as avoiding the functional ink spillage. This objective can be achieved using at least one metallic roll of the two lamination rolls and a pressure of 1 to 3 bar.

Besides, the viscosity of the functional ink is also a parameter that affects this lamination process, and it should be higher than 11,000 cP at the lamination temperature to avoid spillage.

Adequate control of functional ink viscosity is also important due to the fact that it is a parameter with a significant effect on the deposited quantity of ink and, consequently, on the colour of the tag. The effective viscosity of the ink is controlled by the formulation, mainly by the amount of thicker (1.25–1.60% w/w HPC) used, and by the printing temperature. Fig. 8a shows the exponential effect of temperature on viscosity: viscosity increases as temperature decreases. This effect is more important for higher HPC concentrations, especially at temperatures lower than 20 °C, where curve slopes are maximized.
It was studied the viscosity effect on the initial colour of the label at constant temperature by changing the rotary screen printing parameters (squeegee angle and pressure on substrate). Fig. 8b shows the trend for some applied combinations of angle (20°, 25°) and squeegee pressure (6, 10). All cases present a drop on the values of $b$-colour parameter, which means the initial violet colour gets deeper by increasing the viscosity. Microbial growth velocity was not affected by changes in ink viscosity (in the range of this work) as long as formulation parameters are maintained. Therefore, it is important to control the printing process temperature in order to achieve repetitive results. A relatively small temperature reduction during the printing process may cause changes on ink viscosity, having a negative effect on the colour depth. However, in-situ adjustments of the printing variables (angle and pressure of the squeegee) can be used to achieve the correct ink deposition.

Both the UV-varnish and the functional ink worked properly on the laboratory flat screen printer and on the roll-to-roll screen unit of the pre-industrial machine. The roll-to-roll machine is prepared to reach automatically 100 μm register between layers, and the result is accurate enough for this label product. Tags prepared in the laboratory and tags printed in the pilot plant have similar responses in their colour evolution, as it is shown in Fig. 9 for the case of 293 K and 0.5% w/w of initial starter. The two groups of results, the laboratory ones and the pilot plant ones, match correctly the prediction of the kinetics model. With such a good agreement between the data, the model can be used as a good designing tool for new ranges of TTI labels with a straightforward industrialization.

4. Conclusions

On this paper, a new manufacturing procedure based on roll-to-roll printing techniques has been proposed that allows a functional ink to be confined in certain areas of a porous paper, keeping the wetness conditions inside them. Printed microbial time—temperature indicators have been successfully manufactured using this manufacturing process. These smart labels may have a straight access to the market due, in a way, to the use of traditional printing technologies, such as screen printing, whose main features are repetitiveness, robustness and low costs. TTI labels can be printed in a roll-to-roll press as far as it includes at least a screen printing module, a UV lamp and a lamination unit. Obviously, the product could also be fabricated in a flat printing line.

A kinetics model that predicts the colour of the TTI label along time for temperatures above freezing has been proposed. This model is based on the Gompertz equation and a good correlation between experimental results and estimations has been obtained. The predictions of the model can be a useful tool for designing a bespoke TTI label where colour changes with time and temperature can be customized. The activation energy of these TTI labels is 91.92 kJ/mol. The maximum endpoint times of these tags are 6 days at 278 K and 12 h at 293 K. These figures can be considered as adequate for some perishable-food products monitoring purposes. However, using a different combination of bacteria species in the initial starter could help in expanding this endpoint times.

The printing process proposed in this work can be used for TTI production of a wide range of shapes, sizes and colour changes with time and temperature. These smart labels can be easily integrated in food packaging, and can be a great tool for cold chain management.

Acknowledgements

The work has been funded by the Goverment of Navarre. The authors express their thanks and appreciation for this support.
References


