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CHROMOGENIC INDICATORS FOR TEMPERATURE CONTROL IN THE FOOD COLD CHAIN

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Abstract

Temperature has critical impact on food quality and safety within food supply chain, therefore, food should be kept at the defined storage temperature range. Final consumer should be assured when buying food about actual temperature and thermal history of the selected food product and this is why it should be indicated on the packed or prepacked item.

The chromogenic temperature indicator for cold food chain was prepared from suitable active material packed in the properly structured holder. When temperature rises above the defined storage temperature, the active material changes colour and physical state (solid/liquid). Simultaneously, special packaging structure enables irreversible recording of the time exposed to the elevated temperature. The active material was made of thermochromic composite, consisting of dye, developer and solvent. It changes colour at its melting point, being coloured below and discoloured above it. The temperature is called activation temperature of the composite. Its value was adjusted by appropriate solvent and additives used for preparation of the composite, to reach the desired value. The temperature dependent colour change of the composite was determined by colorimetric measurements. The conditions for best observation of the change by naked eye were also examined. The structure of the active material's holder was analyzed for best displaying of the time spend at high temperature (above the activation temperature).

Functioning of the indicator was examined with growth of pathogens as a function of migration of the active material at temperature above the required storage temperature of the food. It was found out that the described chromogenic temperature indicator for cold food chain shows the thermal history of food storage by colour-, phase- and migration changes of the active composite material and consequently would be reliable as indicator in cold food chain to indicate temperature abuse and would disclose potential growth of psychrophilic microorganisms.

***Key words**: Chromogenic temperature indicator, Food cold chain, Food quality and safety, Temperature control, Thermal history.*

1. Introduction

The major attention of a modern consumer is generally focused on food quality and safety due to awareness of hazards that may affect human health [1, 2]. The shelf life of food products is influenced by both intrinsic (e.g. pH, water activity) and extrinsic parameters (e.g. storage temperature, humidity level), as well as the quality of raw material and sanitary conditions applied during manufacturing. Therefore, temperature control could be one of the critical points for maintaining the safety, durability and quality of food in cold food chain. Final consumer should be informed about actual temperature and thermal history of a food product and this should be indicated on the packed or pre-packed item.

The aim of our work was to prepare simple indicator capable of detecting thermal history and relevant temperature of the product throughout the food continuum (gate to plate). The approach takes advantage of visually recognizable change and does not need any power supply. The proposed solution could eventually become a part of intelligent packaging for monitoring thermal conditions of goods in cold food chain.

The proposed indicator is based on phase-change chromogenic material, suitable structure of holders and appropriate protection. The functional material is

organic thermochromic composite which is based on a solution of leuco dye and color developer in an appropriate solvent. It basic property is color change induced by changes in heat (energy). During melting the thermochromic composite is transformed to its colorless state but returns to the initial colored solid state upon cooling [3, 4]. Color conversion occurs at activation temperature which is controlled first of all by melting point of the solvent. Color developer and leuco dye form colored complex, when composite is in the solid state. Above the melting point reaction between color developer and solvent causes dissociation of colored complex, which leads to discoloration of the composite. The chemical properties of the three components impart the reversible (color change is temporary and multiple) or irreversible composites (color change is unique and permanent) [5].

Good holder for functional material should provide appropriate quantity of the functional material and enable adequate spreading of it after liquefaction. Physically, the phenomenon of wicking is the spontaneous flow of a liquid in a porous substrate driven by capillary forces, which are caused by wetting. This type of flow is governed by the properties of the liquid, liquid-medium surface interactions, and geometric configurations of the pore structure in the medium [6]. Capillary force is influenced by the radius of capillary channel and the contact angle between liquid and capillary channel as well as rheological properties of the liquid [7, 8]. The extent of wicking depends on the substrate and on the functional material. While the rheological properties of a selected phase-change chromogenic material depend on temperature, the combination of all the effects enable us to prepare simple indicator of thermal history and actual temperature. Practical use and importance of such an indicator was shown on a laboratory scale experiment.

The existing commercial time temperature indicators usually consists from phase changing material with dye, where irreversibility of indicator is shown with colored wicking length of material in porous substrate above defined temperature. In this type of indicators color is always the same [9, 10, 11, 12]. Our indicator can also indicate irreversibility with wicking length but has added value due to color change ability of functional material after desired temperature was exceeded.

One of the fundamental cold chain quality preferences is maintenance of temperature within the set boundaries, which is often a critical point of biological risk. A significant concern is caused by psychrophilic microorganisms that are capable of growth at refrigeration temperature and are thus primarily responsible for limiting the stability of many dairy products [13]. Hence the dairy products are among the foods most often involved in listeriosis outbreaks. It is of high importance to primary prevent and limit the growth of *Listeria monocytogenes* [14].

In this work we tried to demonstrate how the temperature abuse could be easily monitored by the usage of designed indicator. A leuco-dye based functional material on a suitable substrate gives us simple warning signals amending the time spend at inappropriate temperature and indicates if the current temperature is within proper limits. The thermal history is followed by the degree of wicking of functional material across the substrate whereas the acceptability of the actual temperature is revealed by coloration of the functional material. Functioning of the indicator was correlated with growth of pathogens depending on the time of wicking length of the prepared indicator at temperature above the required storage temperature of the food (milk).

2. Materials and Methods

Components decanol (DE) (Sigma-Aldrich), crystal violet lactone (CVL) (Sigma-Aldrich), benzyl 4-hydroxybenzoate (B4HB) (Sigma-Aldrich) were used to prepare thermochromic composite as described before [15, 16, 17]. They are denoted here by molar ratio of CVL : B4HB : DE. The composite (0,5 : 6 : 100 + RLD) was made by adding 0,1 mL of red liquid dye (RDL) (Candlechem company, Inc.) into 17,407 g of composite (0,5 : 6 : 100) during mixing. The composite (0,5 : 6 : 100 + P25) was prepared by adding of 5wt% nano TiO_2 (P25) (Evonik Industries) to the composite (0,5 : 6 : 100).

Thermal properties of the composites were analyzed with differential scanning calorimetry (DSC). This method was used to monitor melting point and crystallization temperature of composites.

The dynamic color of composites was evaluated within the CIELAB color space. Each color is represented by three rectangular coordinates: L* sets for the lightness value from 0 (absolute black) to 100 (ideal white); a* determines the color on the red-green axis; b* determines the color on the yellow-blue axis and C* represents the chroma (purity) which may represent the proportion of colorant. The color difference is evaluated by Cartesian distance between two points in the color space. The largest color difference takes place between fully colored and totally discolored states of thermochromic composite. We denote it as total color contrast, TCC, and is one of the most important parameter for the application, thereby, it is calculated from equation:

$$TCC = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

$$\Delta L^* = L^*_1 - L^*_0, \quad \Delta a^* = a^*_1 - a^*_0, \quad \Delta b^* = b^*_1 - b^*_0,$$

Where (L^*_0, a^*_0, b^*_0) refers to the colored state (blue composite) and (L^*_1, a^*_1, b^*_1) to the discolored state of the same composite [18]. Color changes were also

examined by the naked eye. Discoloration and coloration are not proceed at the same temperature, because discoloration appears at higher temperatures, coloration at lower temperatures. As a result color hysteresis is formed. This phenomenon is observed as a function of the individual color values (L*, a*, b*, C*) as a function of temperature during heating and cooling [19].

For TCC evaluation all thermochromic composites were deposited on paper packed in sandwich by self-adhesive films. Temperature depended color values of composite (0,5 : 6 : 100) and (0,5 : 6 : 100 + RLD) were measured as described elsewhere [19].

The pH, air permeability and roughness of the Whatman chromatography papers (Macherey-Nagel GmbH & Co. KG) in Table 3 were measured. The investigated paper was cut in small pieces and mixed in distilled water. After 2 hr of stirring, sample was filtrated and pH of clear water solution was measured using a pH meter. The roughness and air permeability values of the papers were measured with the Bendtsen method [20]. The surface is rougher, if the value of the measured air flow is higher.

Wicking of thermochromic composite was also studied. The proposed chromogenic indicator consists of 0.8 mm thin polymer carrier with a conical recess on the one end of carrier where 0.020 g of liquid composite was deposited. After solidification of composite in the refrigerator, chromatography paper was placed on it and the entire device was covered with a self-adhesive film to protect it against external physical factors. The indicators were placed in refrigerator for 24 hr on aluminium plate (15 x 10 x 1 cm). The plate was used to simulate the thermal capacity of a packed good. When taken out of the refrigerator to room temperature, wicking length of liquidized functional material was studied. The effect was followed on different chromatography papers. The temperature of indicator on aluminium plate and wicking length of composite were measured as a function of time.

In order to demonstrate the practical use of indicator an interruption of cold chain was simulated on laboratory scale. Due to significant concern that is caused by psychrophilic foodborne pathogens in food industry, a *Listeria monocytogenes* strain was used to indicate the importance of maintaining the required temperature. The number of microorganism in artificial inoculated medium was monitored by standard cultivation methods and number of colony forming units (CFU/mL) was calculated. Pure culture of *L.monocytogenes* reference strain was frozen preserved at -20 ^{0}C for long term preservation and maintained on trypton soy agar (TSA) at 4 ^{0}C for routine use. After the inoculation of 4 mL trypton soya broth (Oxoid) with 0,6% yeast extract (Oxoid) (TSB-YE) 10 µL of overnight culture was transferred into 100 mL of fresh TSB-YE medium and 5 mL of inoculum equally distributed into glass test tubes. To show the influence of the initial storage conditions three independent parallels were left at room temperature for two hours with other three independent parallels for positive control. The negative control and test tubes for monitoring the influence of interruption of cold chain were immediately stored at < 4 ^{0}C. The number of colony forming units was determined by drop plate method on TSA-YE immediately after the inoculation (t_0), 2 hr after inoculation (t_1), 4 hr after incubation at < 4 ^{0}C (t_2), 1,5 hr after interruption of cold chain (t_3) and 24 hr after inoculation (t_4). By the prolonged storage of samples we also tried to show that the increase in temperature could have subsequently influenced the number of microorganisms.

3. Results and Discussion

The main purpose of the study was to formulate thermohromic composite with activation temperature below 8 ^{0}C which is also one of the limiting factors of microbial growth and reproduction. Achieving the appropriate activation temperature is primarily based on the choice of solvent. As a solvent we used decanol with melting point 6,3 ^{0}C (Table 1). The melting point of the entire composite depends on all additives to this solvent: different ratios of CVL, B4HB, RLD and P25. The melting and crystallization points of the prepared composites were determined by DSC measurements (Table 1) on heating and cooling respectively. Composite (0,5 : 6 : 100) has the lowest melting and crystallization temperatures. Above 3.4 ^{0}C this composite is colorless liquid and transforms to blue-colored solid when cooled below -3.5 ^{0}C (Table 1).

Table 1. DSC data of melting point (T_m) and crystallization temperature (T_c) of composites and pure DE. The samples are described by ratios of the dye : developer : solvent components. For details see Chapter 2.

Sample	T_m (^{0}C)	T_c (^{0}C)
DE	6.3	-0.1
CVL:B4HB:DE = 0,5:6:100	3.4	-3.5
CVL:B4HB:DE = 0,5:6:100+RLD	5.4	-0.0
CVL:B4HB:DE = 0,5:6:100+P25	4.1	-3.4
CVL:B4HB:DE = 1:6:100	3.7	-3.5
CVL:B4HB:DE = 0,5:12:100	3.6	-2.0
CVL:B4HB:DE = 0,5:20:100	3.5	-1.5

Thermochromic composites (0,5 : 6 : 100), (1 : 6 : 100) and (0,5 : 6 : 100 + P25) show reversible color change. They are blue in solid state and colorless in liquid state. At composites (0,5 : 12 : 100) and (0,5 : 20 : 100) some blue color remains in liquid state, thus no complete discoloration takes place.

The prepared samples with thermochromic composite (0,5 : 12 : 100) and (0,5 : 20 : 100) were colored at low temperature and colorless at high temperature, thereby, the TCC value is the highest (Table 2).

Composite (0,5 : 6 : 100 + RLD) is red at room temperature, where the reaction between B4HB and DE causes discoloration of the thermochromic composite. RLD did not enter in these interactions, thus it remains unaffected. This composite has to be cooled below temperature -0.0 ^{0}C to form solidified material with dark blue/violet color; it remains colored upon heating until 5.4 ^{0}C but above composite becomes red liquid color due to RDL and discolored composite (Figure 1).

At the melting point of the composite (0,5 : 6 : 100) (3.4 ^{0}C) almost full discoloration occurs. Upon cooling blue color is forming immediately after the crystallization temperature (-3.5 ^{0}C) is achieved until -8.9 ^{0}C, where color hysteresis is completed (Figure 1). L* values of the composite (0,5 : 6 : 100) are higher than of the (0,5 : 6 : 100 + RLD). Composite (0,5 : 6 : 100) is getting greener upon heating (a* = -7,44) whereas consequently more redder by heating of composite (0,5 : 6 : 100 + RLD) (a* = 65,69) due to RLD. The reason why initial b* values (b* = -56,42) are lower than 0 for composite (0,5 : 6 : 100) compared to (0,5 : 6 : 100 + RLD) is because RLD is not present in (0,5 : 6 : 100). When composite (0,5 : 6 : 100 + RLD) is heated, it is more yellow (b* = 23,96) than blue (Figure 1).

We observed that color of thermochromic composites held in refrigerator at -20 ^{0}C changes with time. Duration of stored composites with no additives in refrigerator at -20 ^{0}C is affecting the blue colored state. They are getting lighter (higher L*); therefore, TCC values are lowering (Table 2).

Table 2. TCC values of composites with time stored in refrigerator at -20 ^{0}C.

Composite	Day 1	Day 3	Day 6	Day 10
0,5 : 6 : 100	56,0	46,1	39,7	38,7
0,5 : 6 : 100 + RLD	52,0	47,9	46,8	44,3
0,5 : 6 : 100 + P25	89,6	55,5	53,5	49,0
1 : 6 : 100	78,6	54,5	61,5	46,0
0,5 : 12 : 100	97,3	60,0	58,7	54,5
0,5 : 20 : 100	95,0	64,1	50,3	49,3

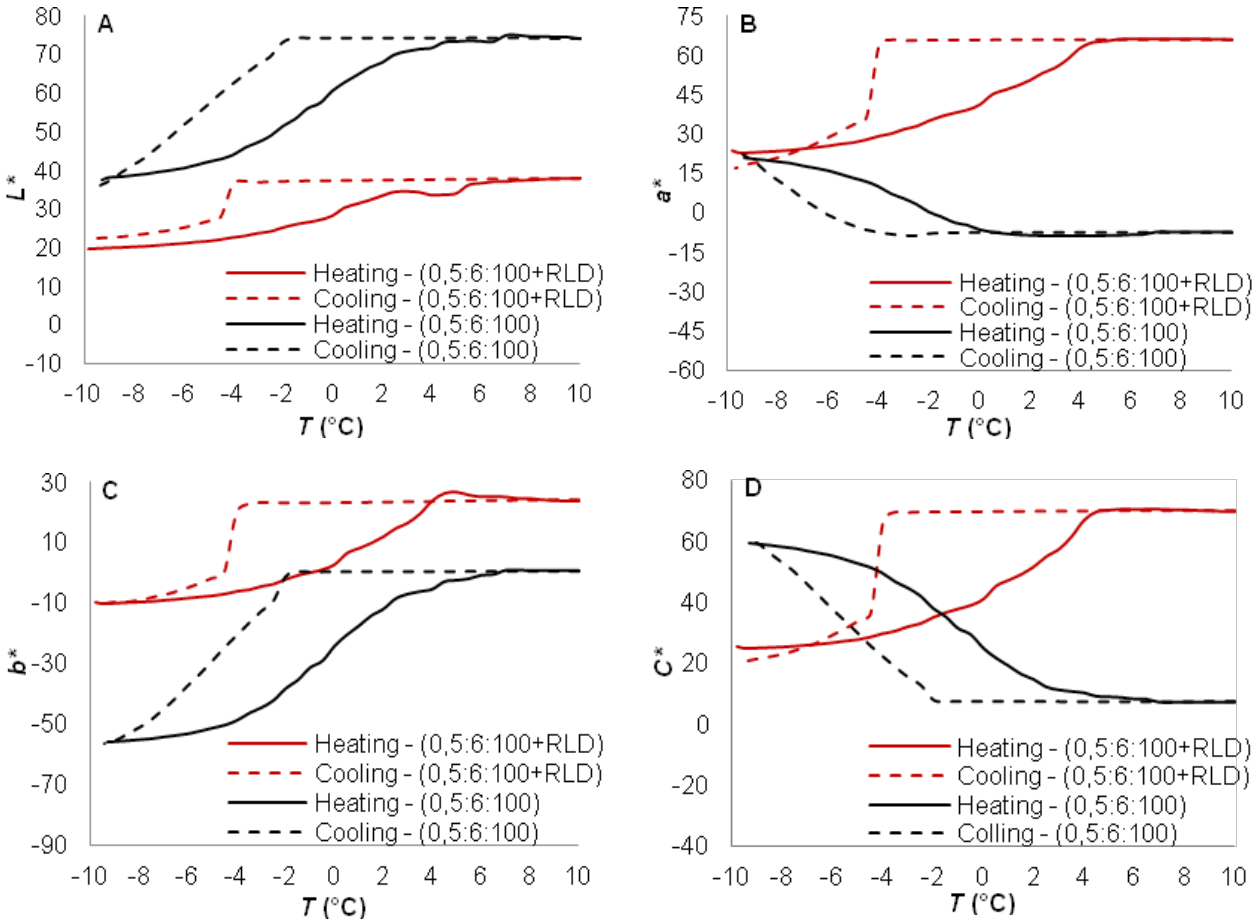

Figure 1. Temperature depended color values L* (A), a* (B), b* (C), and C* (D)

To study the phenomenon of wicking a thermochromic composite (0,5 : 6 : 100) was used with different Whatman filter papers (Table 3). Whatman cellulose filters are made of high-quality cotton fibers, which have been treated to maintain a minimum content of alpha-cellulose at 95 %. Paper 589/3 has the highest efficiency for the collection of small particles, whereas paper 589/1 is paper without ash and allows high flow rates. Pore size and grammage (Table 3) are obtained from product data sheet of Macherey-Nagel GmbH & Co. KG. The most commonly used filter paper for routine analysis is Grade 1. Paper Grade 5 has provide the highest degree of fine particle filtration in the qualitative range.

Table 3. Physical parameters of different filter papers.

Paper		589/3	589/1	Grade 1	Grade 5	674	615 ff
Pore size (µm)		2-5	12-25	11	2,5	2-4	4-12
Grammage (g/m^2)		84	80	88	98	85	70
pH		6,40	6,40	6,30	5,70	7,3	7,3
Air Permeability (mL/min)	A side	1264	2994	2030	654	1250	2700
	B side	1280	2990	2042	726	1225	2740
Roughness (mL/min)	A side	1400	1382	1698	1878	1150	1275
	B side	1328	1432	1214	1678	1350	1125

Wicking length of thermochromic composite (0,5 : 6 : 100) on filter papers was monitored as a function of temperature and time (Figure 2). The indicators were exposed to room temperature in horizontal position on the aluminium plate. The temperature quickly increases, leading to liquefaction of composite and simultaneous spreading across the filter paper. The aluminium plate serves to simulate the thermal capacity of packaging. Before the experiment, indicators were cooled at -20 ^{0}C for 24 hr. When exposed to room temperature, its surface temperature reached room temperature in 50 minutes (Figure 2). We assume that the indicator follows the temperature of the aluminium plate. The wicking length of the functional material depends on the filter paper (Figure 2). Most of the effect occurs within first 20 min, where the temperature of the active material reaches the room temperature. After that the process slows down and spreading of the material stops. If size of pores and air permeability values of the selected paper are small, wicking lengths are shorter (example: 589/3). On the opposite, if size of pores and air permeability values are high, wicking lengths are longer (example: 589/1). Furthermore, the longest wicking length (52 mm) was after 100 min. at room temperature achieved with paper 615 ff, which has small pores and good air permeability (Figure 2, Table 3). We also noticed that lengths are longer due to low roughness. The grammage is lowest in paper 615 ff. pH does not affect wicking length, however, additional studies should be made on relationship between paper pH and the color of thermochromic composites.

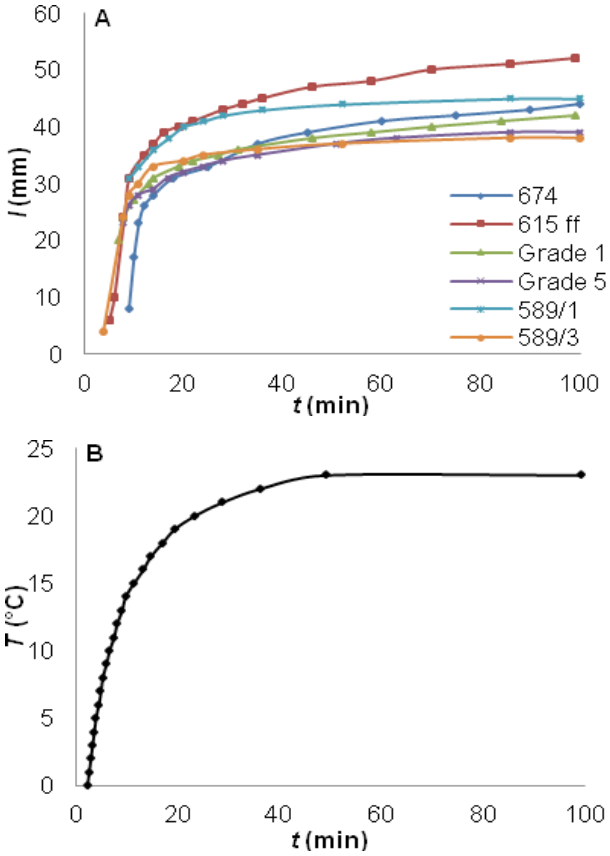

Figure 2. Wicking lengths of composites (0,5:6:100) (A) on different filter papers as function of time. The samples were placed on cooled aluminium plate (B) in horizontal position at room temperature

Functionality of composite (0,5 : 6 : 100) was also checked for double temperature abuse. After 4 hr at 4 ^{0}C, the indicator with paper 674 was left at room temperature for 20 min. and wicking length of 39 mm was achieved. The same indicator was again cooled at temperature -20 ^{0}C for 1 hr. After 70 min. exposure at room temperature, wicking length of composite (0,5 : 6 : 100) on the paper 674 extended for 11 mm and on the paper 615 ff for 9 mm (Figure 3). When temperature abuse of indicator with paper 615 ff was performed twice, wicking length (52 mm) was the same as in single time temperature abuse of 90 min. Multiple interruptions in cold chain did not affect the performance of functional chromogenic material as did not affect the maximum wicking length at defined time of temperature abuse.

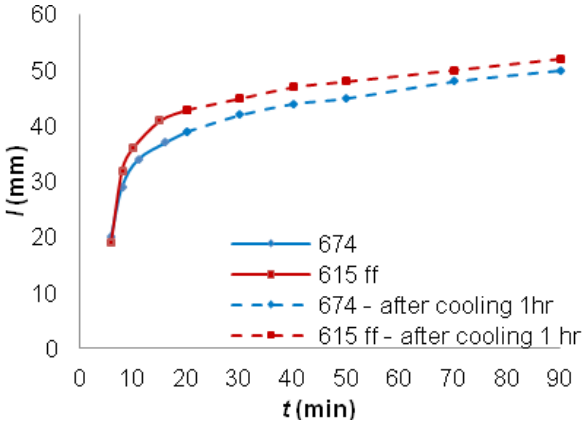

Figure 3. Wicking of thermochromic composite (0,5 : 6 : 100) as function of time at double temperature abuse

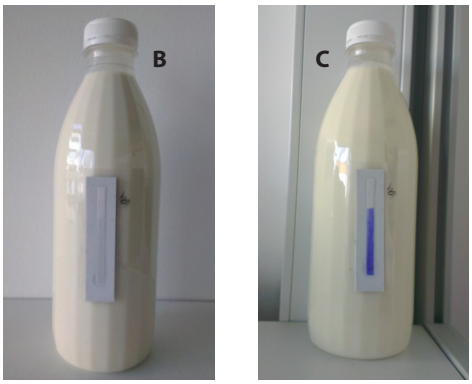

Figure 5. The chromogenic temperature indicator on packaging of milk: activated at room temperature (B) and activated after putting it again at refrigeration temperature (C)

Consumer can see temperature abuse of milk in cold chain by red-colored wicking length at room temperature. Despite of cooling it first below -0.0 ^{0}C, the dark blue/violet wicking of the functional material can be seen again at up to 5.4 ^{0}C (Figure 1, Figure 4).

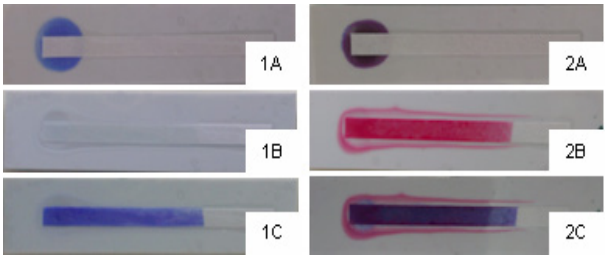

Figure 4. The chromogenic temperature indicator with composite (0,5 : 6 : 100) (1) and (0,5 : 6 : 100 + RLD) (2): non-activated at refrigeration temperature (1A, 2A), activated at room temperature (1B, 2B) and activated after putting it again at refrigeration temperature (1C, 2C)

If the functional material of the indicator does not contain RLD, consumer could check temperature abuse of the product by cooling it to the refrigeration temperature. By adding RLD to the functional material, consumer can immediately see interrupted cold food chain with red wicking observed at room temperature. Reversible color change (violet to red) informs consumers about the appropriate storage temperature and irreversible wicking indicates thermal history, which is here evidence of temperature abuse of product in cold food chain (Figure 5).

Temperature indicators on the basis of thermochromic inks, which are made by printing technology consists from encapsulated material to maintain functional properties [21]. In our case thermochromic composite is not encapsulated and functioning of thermochromic composite is not compromised. This provides advantage to apply the phase change material which adds the irreversible property to the basically reversible thermochromic composite. Both effects can be applied.

Functioning of indicators with composites (0,5 : 6 : 100) and (0,5 : 6 : 100 + RLD) on filter papers 674 and 615 ff was also evaluated in vertical position on packaging of milk. Milk inside packaging has achieved temperature 13 ^{0}C after 90 min. at room temperature (Figure 6). Wicking length of temperature indicator with composite (0,5 : 6 : 100) after 30 min. was equal using both filter papers (674 and 615 ff) but with composite (0,5 : 6 : 100 + RLD) wicking length after 30 minutes are different (Figure 6).

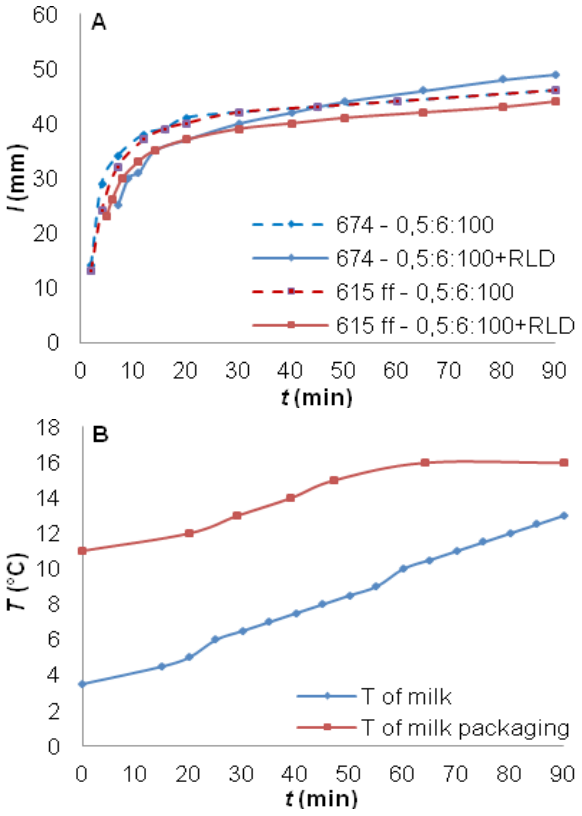

Figure 6. Wicking of thermochromic composites as function of time on cooled milk packaging in vertical position (A) and time depended temperature (T) of milk measured inside and outside the packaging (B)

When composite (0,5 : 6 : 100 + RLD) and paper 674 are used, adhesion and cohesion is better since wicking length is longer. Wicking lengths of thermochromic composite (0,5 : 6 : 100) compared to the horizontal position are shorter in vertical.

Temperature is one of the environmental factors that can significantly influence the growth and reproduction of microorganism. Therefore it could also have a great impact on food safety and quality during storage time and for that reason the duration of exposure to inadequate temperature, should be controlled strictly [22]. Due to its survival under the wide range of environmental influences, *Listeria monocytogenes* is capable to overcome some of the food preservation and safety barriers [23]. Although, this food pathogen is known for its ability to grow at refrigeration temperatures (below 4 ^{0}C to -0.4 ^{0}C), a growth rate of *L. monocytogenes* is considerably increased at higher storage temperatures. That greatly increases the risk that if present, the number of cells could reach the approximate infective dose, which is estimated to be 10 to 100 million colony forming units (CFU) in healthy host [24].

After milking, milk should be cooled down to 4 ^{0}C within 2 hr, to ensure that the present flora does not grow to unacceptable levels during the shelf life of the final food product [25, 26]. The time of sampling points was set according to the fact above mentioned and the average time of milk transportation from farm to distributors. Three parallels of inoculated medium were set as positive control and incubated at room temperature (25 ^{0}C). From the number of colony forming units it could be seen that the number of cells at room temperature in TSB-YE medium has increase linearly (Figure 7.A). To examine the possible influence of conditions directly after inoculation, 3 parallels were exposed to optimal growth temperature of microorganism (37 ^{0}C) and 3 at refrigeration temperature for 2 hr (t_1). After 2 hr samples were cold down to < 4 ^{0}C and incubated for 4 hr till the interruption of cold chain (t_2). Initial conditions of incubation have visible impact on the growth of *Listeria monocytogenes* in TSB-YE, although the number of cells did not reach the same level as in positive control. On the other hand we observed a different dynamic of cell count in inoculated milk medium. There is no big difference in cell number between the positive control and parallels with prior exposure to 37 ^{0}C (Figure 7.B). After 1.5 hr on room temperature the samples were stored back to refrigerator temperature and sampled 24 hr after inoculation of samples (t_4). For negative control a constantly incubated medium at refrigeration temperature (< 4 ^{0}C) was used. Maintaining constant refrigeration temperature of inoculated medium has resulted in stagnation of cell number in both growths medium (Figure 7).

To sum up the initial incubation conditions have an important impact on the number of cells in medium, these so the straight conditions should be ensured from the beginning of the food cold chain. Due to the greater impact of temperature on the number of cells in TSA-YE medium, we could assume that the different composition of milk has somehow contributed to viability of cells. Although, a visible change in number of cells before and 1,5 hr after the interruption of cold chain could not be observed, the temperature abuse could have some further influence on the growth of microorganism. Nevertheless it should be interesting to set further sampling points.

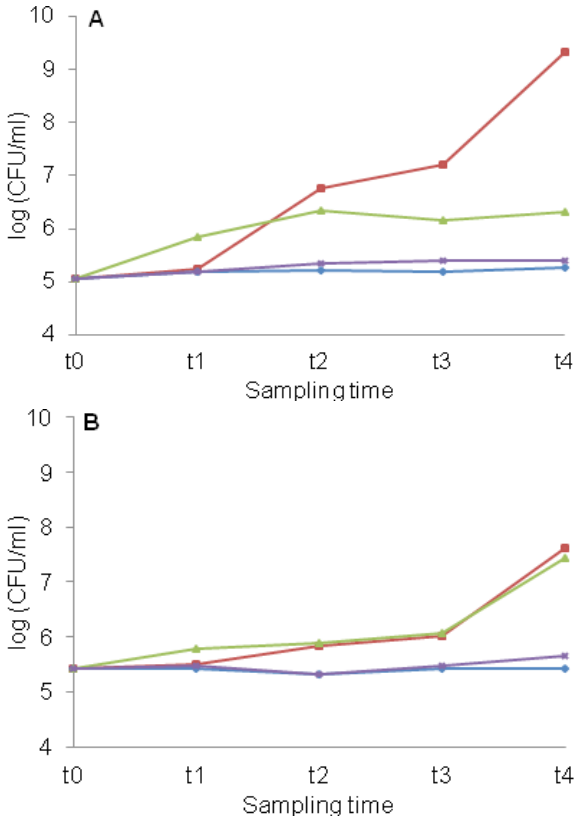

Figure 7. Growth of Listeria monocytogenes constantly at room temperature (—■—); constantly below 4 ^{0}C (—◆—); 2 hr after inoculation at 37 ^{0}C (—▲—); 2 hr after inoculation at 4 ^{0}C (—●—), in TSB-YE (A) and in milk (B) at defined sampling time points

4. Conclusions

- In study chromogenic temperature indicator, melting of thermochromic composite takes place above the desired storage temperature of prepacked or packed item; it transforms the functional material to liquid state (accompanied worth color change) which enable the wicking process to start. Upon cooling under storage temperature the wicking area remains and gives clear evidence of temperature abuse. The time spend at too high temperature (temperature abuse) was determined by wicking length of functional material as a function of time and temperature of the exposure above the storage temperature.

- It was demonstrated that the described chromogenic temperature indicator for cold food chain shows the thermal history of food storage by color-, phase- and wicking length of the active material. Therefore, it would be reliable as temperature indicator in cold food chain where it will indicate temperature abuse up to 1,5 hr. Moreover, it would disclose potential growth of psychrophilic microorganisms and indicate drift of quality parameters of particular food item.

- The studied chromogenic temperature indicators are promising candidates for intelligent packaging because temperature is one of the important physical parameters which influences on the quality and safety food products in cold food chain. This approach is demonstrated to be efficient and informative for educated consumer.

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