Evaluation of the effectiveness of Delvocid in-cooperated Antimicrobial Edible Coating made from the Whey Protein Isolate base to improve the shelf life of Swiss Cheese

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Abstract— *The objective of this research was to evaluate the effectiveness of an antimicrobial edible coating made from whey protein isolate base to improve the shelf life of Swiss cheese throughout 60days of storage, as a substitute for commercial nonedible paraffin coatings. Whey protein isolate which is producing through the cheese production process, have natural lactic acid. So it can inhibit the growth of other bacteria species by lowering the pH. Delvocid which contain 50% of Natamycin was added to inhibit the growth of yeasts & molds. Three types of antimicrobial solutions were prepared by adding different concentration of Delvocvid. Edible coated, paraffin coated and uncoated cheeses were kept at 10^0C, 85% relative humidity for 60 days and all cheeses were compared with each other. Physicochemical properties including hardness, moisture, weight loss, colour variations, pH, fat and salinity were measured of each samples in subsequently 0, 10, 20, 40 and 60 days of storage. Edible coated cheeses exhibited decreased moisture loss, pH change, hardness, colour change and increment of salinity and fat contest was relatively lower than other Cheeses. Sensory evaluation tests were carried by 10 trained panellists using seven point hedonic scales. Highest Delvocid concentration contained edible coated cheeses obtained highest acceptability score on 20, 40 and 60 day of storage and relatively low level of microbial count throughout the storage and thus it was selected as the best Delvocid concentration for coating preparation. This research was reviewed the effectiveness of Delvocid for edible cheese coatings as an antimicrobial agent.*

Keywords— **Antimicrobial edible coatings, whey protein isolate, shelf life, Delvocid**

I. INTRODUCTION

Swiss cheese is a generic name in North America for several related verities of cheese, mainly of North American manufacture which resemble Emmental cheese, a yellow, medium hard cheese that originated in the area around Emmental, in Switzerland. Cheese industry has now evolved into a global business where research has an important role on the increase of shelf life and promotion of cheese products' quality and safety. One of the main problem happens during the storage of cheese is the high moisture loss. It could be lead to increase the hardness of cheese, so it is leading to undesirable organoleptic properties. Also the contamination of cheese by bacteria, molds & yeasts will develop the off flavours hence decreasing the quality of cheese, mainly during agitation when storing without package. Different packaging systems including modified atmosphere packaging & vacuum packaging used to minimize these problems. Normally polyamide, polyethylene & polypropylene are used as packaging materials. Coatings have been used as an individual packaging material which provide additional protection when combine with vacuum packaging or modified atmospheric packaging (Costa et al., 2018). Dairy industries do use paraffin or microcrystalline for waxing cheese. While cheese rinds in many types of cheeses are edible, people wonder if it is safe to eat wax on the cheese. Paraffin & microcrystalline waxes help to keep the cheese fresh but it is not edible. Those waxes need to be removed before eating cheese. Paraffin and microcrystalline waxes are food grade and hence safe for waxing cheese but it is not edible because it is non digestible. It's also nontoxic so, if eat cheese wax unknowingly, it will not do harm. Food grade colours are used to colour wax, so they too are nontoxic and safe for coloring wax. But those paraffin & microcrystalline waxes only inhibit the growth of Yeasts and molds and it is not specific for inhibit the growth of bacteria (Ramos et al., 2012) & also tend to be crack.

Edible coatings can prepare by adding several antimicrobial agents to inhibit the growth of bacteria, yeasts & molds. Several materials can use to prepare edible coatings for cheese.In this research review about the edible coating prepared by using whey protein isolate.Whey protein isolate is a byproduct of cheese manufacturing process and it is rich source of protein.

Other than that it contains several vitamins & minerals. Whey protein can use to prepare edible cheese coatings combine with glycerol, guar gum, sun flower oil & tween twenty (Ramos et al., 2012). Delvocid which is containing 50% of Natamycin can use to inhibit the growth of yeast & molds. Three types of antimicrobial edible coating solutions which had different Delvocid concentrations were used in this research to identify the best delvocid concentration that successfully full fill the industrial requirement. Whey protein isolate has natural lactic acid which was formed by previously added starter culture by degrading the lactose.So it can inhibit the growth of other bacteria species by lowering the pH (Ramos et al., 2012) . This antimicrobial edible coating is minimizing the moisture losses & keeping preferable hardness. Also no need to remove the outer layer of the cheese and therefore it is minimizing the weight loss and wastage. According to that this antimicrobial edible coating which was prepared by using whey protein isolate is a consumable, preferable & economical innovation.

II. METHODOLOGY AND EXPERIMENTAL DESIGN

Whey protein isolate was obtained from Richlife Dairies Ltd as a byproduct of cheese production process.The composition of whey protein isolate was identified as following according to the dry weight basis; Total solid - 6.29% (wt/wt) , Protein - 0.55% (wt/wt), Fat - 0.1 (wt/wt) ,Ash - 0.53% (wt/wt) and Moisture - 93.71% (wt/wt) Also the titratable acidity, pH and Brix value were identifies as following; Titratable acidity - 0.10%, pH - 6.48 (at 200C), Brix value - 70

Glycerin (99.7% Purity) was obtained from Evyap Sabun Malaysia Sdn Bhd .Guar gum was obtained from Shree Ram Gum Chemicals Pvt Ltd.Sunflower oil which has 12% saturated fat and 21% monounsaturated fat and 67% poly unsaturated fat was obtained from Pyramid Wilmar Pvt Ltd. Tween twenty (T-20) was obtained from Taiwan Surfactant. Vitamin E tablets as D- alpha Tocopherys Acetate ph.Eur (400 IU) was obtained from Mega Lifesciences Public Company Ltd. Delvocid was obtained from JK Tradelink PVT Limited.

Rich Life Dairies Ltd, kindly supplied Swiss cheeses without any previous coating, after 30 days of manufacture. Approximately 200g of cheese samples were used for the research. Before applying coatings the Swiss cheese samples were washed well and allowed to drain off the residual water. Then the cheese samples were kept for 12 hours to essentially drying. The composition of Swiss cheese was recognized previously as following according to the dry weight basis;Total solid 54.33%(wt/wt), Moisture 45.47%(wt/wt), Protein 25.70%(wt/wt), Fat 29.60%(wt/wt), Ash 3.76%(wt/wt), Lactose 0.10%(wt/wt).Instead of that ,titratable acidity 0.52%(v/v), pH 5.8.

A. Edible coating solution preparation

Coating forming solution was prepared by as follows:Whey protein isolate was obtained as a byproduct of cheese manufacturing process.Then 84.1% (wt/wt) of whey protein isolate was added into a beaker and 5% (wt/wt) of Glycerol was added into that and the resulted solution was magnetically stirred for approximately 2 hours.Then it was heated in a water bath which was maintained at 80 ± 2_0C for 20 minutes. Then 0.7% (wt/wt) of Guar gum was added in small quantities while continuously stirring and maintaining the temperature at 80 ± 2_0C for 20 minutes. The solution was stirred well to avoid lump formation by Guar gum. Then the solution was kept in an ice bath to cool. After that, the solution was taken out from the ice bath and kept in the room temperature, when it became into the room temperature,10% (wt/wt) sunflower oil was incorporated into the mixture and stirred well for 20 minutes. Then two capsules of Vitamin E were taken and the content of the tablets was added by piercing the tablets. After that, 0.2% of Tween twenty was added and stirred well. Then the solution was homogenized by beater at 1100 rpm for 5 minutes.

B. Antimicrobial edible coating solutions preparation

Whey protein isolate had natural Lactic acid which was formed by Lactic acid bacteria through the lactose degradation. The lactic acid content of whey protein isolate was previously identified as 0.10% (v/v) according to the volume. Delvocid which has 50% of Natamycin was added to inhibit the growth of Yeast and Molds. Three types of Delvocid concentrations were used to recognize the best antimicrobial solution for increase the shelf life of cheese. 0.250g/L, 0.275g/l and 0.300g/L of antimicrobial edible coating solutions were prepared by adding subsequently 0.250g, 0.275g and 0.300g of Delvocid for each 1 L of previously prepared edible coating solutions. Solution 1- 0.250g/L Delvocid added antimicrobial edible coating solution, Solution 2- 0.275g/L Delvocid added antimicrobial edible coating solution, Solution 3- 0.300 g/L Delvocid added antimicrobial edible coating solution. Proximate composition of antimicrobial edible coating solution was identified as following according to the dry weight basis; Total solid :25.34 ± 0.23% (wt/wt), Moisture: 74.66 ± 0.23% (wt/wt),Total Fat:11.87 ± 0.32% (wt/wt),Protein 8.63 ± 0.51% (wt/wt),Lactose 3.80 ± 0.027 % (wt/wt),Total ash 0.81 ± 0.05% (wt/wt),Instead of that, titratable acidity 0.093 ± 0.006% (v/v),Brix value12_0 ± 0.00Antioxidant activity (Scavenging activity) 37.13 ± 0.51.

C. Commercial paraffin coating solution preparation

A paraffin block was taken and it was put into a metallic vessel and the paraffin block was melted by using a burner until it became into 120°C.pH of the paraffin solution was 7.0.

D. Cheese coating

The antimicrobial edible coating solution was adjusted to pH 7.0 by using 0.1 moldm-3 NaOH solution to confirm that the coatings were devoid of any significant antimicrobial activity associated with pH itself therefore any antimicrobial activity observed would be caused by the antimicrobial compounds included in the formulation (Ramos et al.,2012).Coating was applied by dipping cheese samples for 2 minutes until all surfaces were covered, with the residual coating being allowed to drip off. Coating application was performed with appropriate aseptic conditions. Then the samples were left for 8 h at 10°C (85% relative humidity), in a temperature- and humidity-controlled aging room, turning them periodically (every 30 min or so) until the coating was essentially dry (based on visual inspection).For making commercial paraffin coated cheese, the cheese samples were dipped in paraffin wax solution which is in 120 0C. Then, both antimicrobial edible coated cheese and commercial paraffin coated cheeses were stored in aging room for 60 day at 10°C and 85% relative humidity. The coated cheeses were compared with their uncoated (negative control) counterparts which were stored in same conditions(Ramos et al.,2012).

E. Shelf life analysis of antimicrobial edible coating solutions

Prepared antimicrobial edible solutions (0.250g/L, 0.275g/L, 0.300g/L Delvocid concentrated) were poured into the well autoclaved glass bottles in the aseptic chamber and that sample bottles were kept at 80C for 60 days. pH,Total Plate Count (TPC),*Coliform* count and Yeast and Mold count were taken on 5 days intervals of storage period. Triplicated readings were taken from each solution.

F. Cheese analyses

Cheeses were assayed, in triplicate, on 1, 10, 20, 40, and 60day after coating application, for physicochemical properties including moisture, fat, and salt contents, weight loss, pH, texture and color. Microbiological and sensory analyses were also performed.

1)Moisture determination of cheese (A.O.A.C)

About 5g of grated cheese sample was taken from homogenized highest acceptable product in to a cleaned, oven dried and cooled moisture dish of known weight. Then the weight of dish with sample was recorded. Then the moisture dishes which contained sample were placed in an oven (MEMERT) for 16 hours while maintain the temperature at 1050C. Then the moisture dish was removed from the oven and allowed to cool in desiccator. Again the weight of the dish was noted. This procedure was repeated until the difference between two consecutive weights did not exceed one milligram.

$$\%\ Moisture = \frac{m1 - m2}{m1 - m0} \times 100\%$$

m1 = mass of dish + sample before drying (g)
m2= mass of the dish + sample after drying (g)

% of moisture content of the product=100%-TS%

2) Weight loss determination of cheese

Cheese was individually weighed on an automatic electro-balance (Ohaus PA 313) with a precision of ±0.001 g, at the beginning and during the storage period; the relative weight loss was calculated as,

$$\Delta w = \frac{wio - wft}{wft} \times 100\%$$

Where,
Wi0 = initial weight and
Wft = final weight at time t.
Three readings of each cheese sample were produced

3)Fat determination of cheese

About 15 g of Sulphuric acid was added into the Van Gulik butyrometer, closed at the scale end, then 3g ± 0.2g of cheese were added by weighing boat and glass road and filler opening was sealed by using stopper. Then the sealed butyrometer was placed in a 700C-800C water bath with scale pointing upwards and shaken repeatedly until the cheese was dissolved. Then 1ml of amyl alcohol was added followed by Sulphuric acid until it was approximately reached to the 15% mark of the scale. Then the butyrometer was closed and the content was mixed. Then the butyrometer was tempered for 5 minutes in an electronic water bath (RDL-EQP-00204) at 650C and the fat column was adjusted to zero point. The reading was taken from the lower end of the meniscus.

4) Salt determination of cheese (Mohr method)

Procedure followed the method of Sheen and Kahler, (1938) with some modifications.

5% K2CrO4 solution was prepared by dissolving 1.0g of K2CrO4 in 20 ml of distilled water. Standard AgNO3 solution (approximately 0.1 M) was prepared by dissolving 9.0 g of AgNO3 in 500ml distilled water. This solution was standardized against NaCl. Reagent-grade NaCl was dried overnight and cooled to room temperature. 0.250 g portions of NaCl were weighed into

Erlenmeyer flasks and dissolved in about 100 mL of distilled water. In order to adjust the pH of the solutions, small quantities of NaHCO3 were added until effervescence ceased. About 2 mL of K2CrO4 was added and the solution was titrated to the first permanent appearance of red Ag2CrO4 .The grated cheese sample was dried at 110 0C for 1 hour and cooled in a desiccator. About 1g of individual samples were weighed into 250-mL Erlenmeyer flasks and dissolved in about 100 mL of distilled water. Small quantities of NaHCO3 were added until effervescence ceased. About 2 mL of K2CrO4 was introduced and the solution was titrated to the first permanent appearance of red Ag2CrO4. An indicator blank was determined by suspending a small amount of chloride free CaCO3 in 100 mL of distilled water containing 2 mL of K2CrO4.The reactions are:

$Ag + Cl^- \rightarrow AgCl(s)$

$2Ag + CrO_4^{2-} \rightarrow Ag_2CrO_4(s)$

<u>*Calculations for Replicate 1 of standardization*</u>

Molecular mass of NaCl = 58.44 g/mole

Reacted moles of $AgNO_3$ with NaCl = $0.25g/58.44gmol^{-1}$

Molarity of $AgNO_3$ (M) = $0.25g/58.44gmol^{-1}$ / (Reacted volume of $AgNO_3$ with NaCl- Blank volume)Average molarity of $AgNO_3$ was taken by triplicating the titration of reagent grade NaCl with $AgNO_3$

Atomic mass of Cl = 35.45 g/mole

$$\%Cl^- \text{ in cheese} = \frac{\left[M \times (\text{Reacted volume of } AgNO_3 \text{ with cheese sample-Blank volume}) \; 35.45 \times 100\%\right]}{\text{Weight of the cheese sample}}$$

5) pH determination of cheese

The pH value was measured using a pH meter (HANNA-CHI 99161) equipped with a probe for solids inserted directly into the cheese sample at 200C. Three readings of each cheese sample were taken from three places of the cheese sample.

6) Hardness determination of cheese

The textural properties of cheese sample which cut into (20mm×20mm×20mm) identical cubes were evaluated by using a texture analyzer (CT Texture Analyzer, Brookfield) with a 4500g load cell and a 4-mm cylindrical plunger (TA44), at a constant penetration speed of 2 mm/s consisting of double compression test, equivalent to 75% compression, contact force of 3.0g. Three penetrations were performed at the center of three identical cubes per each cheese sample. The software CT Texture Pro V1.8 Build 31(Brookfield Engineering. Labs. Inc.) converted the force deformation readings into hardness values.

7)Colour change determination of cheese

Cheese colour was evaluated using a portable Chroma meter CR-400 (Minolta Chroma, Osaka, Japan). Cubic samples of 2cm edge was used to colour analyse. Changes in colour of the cheese surface were measured using a CIELab colour scale (where L = lightness, a = red-yellow colour, and b = blue-green color) under daylight (D65 illuminant). A standard white plate was used to calibrate the equipment, with colour coordinates

L standard = 97.6, a standard = 0.01, and b standard = 1.60. The total colour difference (ΔE) was calculated as follows,

$\Delta E = *(L - L_0)_2 + (a - a_0)_2 + (b - b_0)_2]_{1/2}$

Where, L0, a0, and b0= The initial values (1 d after coating application) obtained for cheese under each experimental condition. L, a, b= The values measured throughout the storage period. For each cheese sample, 5 readings were made on each side.

8) Microbiological analyses of cheese

Microbiological development on the cheese surface was evaluated via enumeration of *Coliform* count, Yeast and Mold count by 1, 10, 20, 40, and 60 d after application of coatings. All media used in the industry were available in commercial manner. The required amount for prepare required volume of a medium was measured and dissolved in distilled water and heated in a water bath for further dissolving until become a semisolid, prior to autoclaving. For *coliform* enumeration, VRBA (Violet Red Bile Agar) was used and for Yeast and Mold enumeration, PDA (Potato Dextrose Agar) was used. Culture media, Ringer solution containing bottles and sodium citrate solution containing bottles were sterilized by autoclaving for 20 minutes at 121^0C and 15 psi. Glassware including Petri dishes and pipettes were sterilized by placing in an oven for 2 hours at 200^0C.

Pour plate method was used in most of the tests to get a count of existing microorganisms. A portion from the surface of the cheese sample was taken from a spoon which was dipped in 75 % alcohol and flamed and transferred immediately into a sterilized Sodium Citrate bottle. Cheese samples were prepared by dissolving approximately 10 g of the sample in 90 ml of sterile sodium citrate solution (2 % solution warmed to 47 ± 2^0C). For dilution purposes sterile Ringer's solution was used included in Ringer bottles. Sample (1.00 ml) was pipetted and put into sterile Ringer's solution (9.00 ml) to prepare 10 fold dilutions.

For Coliform enumeration this direct plating was done using Violet Red Bile Agar (VRBA) medium. The test sample (1.00 ml) was pipetted out using a sterile pipette and transferred into a sterile Petri dish aseptically. (Test sample were diluted based on the purpose and the sample by using sterile Ringer's solution as mentioned above.) Then sterile VRBA (15.00ml) at the temperature of 45 ± 2^0C was poured into the Petri dish aseptically and the contents were mixed clockwise, anticlockwise, up and down. The plates were allowed to solidify and were

inverted and incubated 48 hours at 36±1^{0}C.After the incubation amount of well separated colonies were counted. Presence of fecal coliforms was checked by the presence of *Escherichia coli* colonies which gives a characteristic greenish metallic sheen. The results were expressed in Colony Forming Units (CFU) per g.

For Yeast and Mold enumeration, Potato Dextrose Agar (PDA) was used and samples were inoculated as described previously, but after solidifying the plates, Para film was used to seal them. Then the plates were inverted and incubated 5 days at room temperature. After the incubation period amount of well separated colonies were counted. The results were expressed in Colony Forming Units (CFU) per g.

9)Sensory analyses of cheese

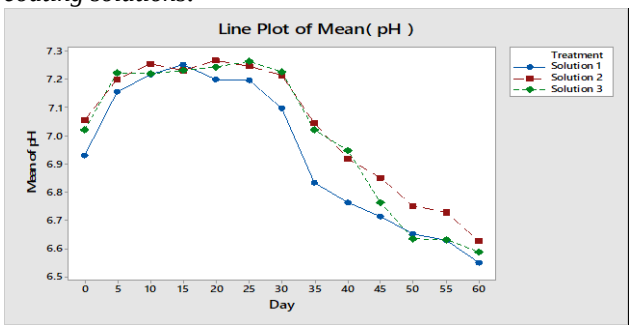
Sensory analyses were carried out in 0, 10, 20, 40, and 60 d after application of coatings. Sensory evaluation was carried out in the Quality assurance lab of the Richlife Dairies LTD by a trained panel of 15 members (both sexes, ages ranging between 26 and 40-year-old), familiar with Riclifes' Swiss cheeses. The panel had previously been screened and selected from among staff of the company. The organoleptic evaluations were carried out by using a test panel of 10 trained panelists. A seven point hedonic scale ranging from dislike very much (1) to like very much (7) to was used to evaluate the degree of liking for the quality attributes namely, odour, colour, surface shininess, hardness, taste and overall acceptability. Cheese samples were cut into cubes approximately 2 cm×2cm×2cm including cheese surface and placed in 500ml individual identical plastic cups. (In paraffin coated cheese, the paraffin coat was removed before cutting the cheese into cubes) The cups were coded using random 3 digit codes and presented in random order to panellists under white fluorescent light. The panellists used water to cleanse their palates between samples. The ballet paper included a "comment" section, in which the panellists were asked to indicate any defects noticed or any descriptors considered useful to better define the coating attributes.

10) Statistical analyses

Analysis of variance was performed to assess the differences between the physicochemical, microbiological, and sensory properties of the cheeses coated with the antimicrobial edible coating solutions or commercial paraffin coating compared with uncoated cheese on 0, 10, 20, 40 and 60 d of storage. The MINITAB 17 software and IBM SPSS statistics 21 were used for analysis. Post hoc test was applied and differences were considered significant at $P < 0.05$.

III. RESULTS AND DISCUSSION

A. Results of shelf life analysis of antimicrobial edible coating solutions.



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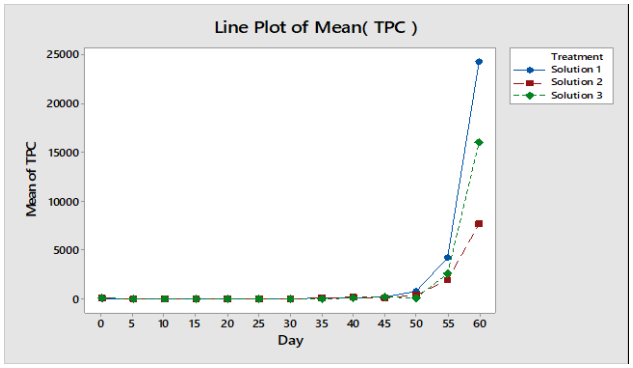

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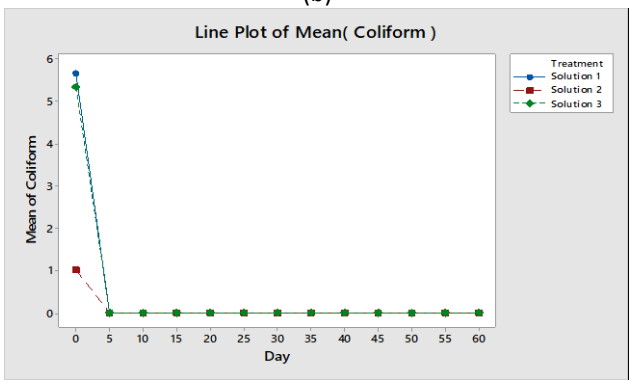

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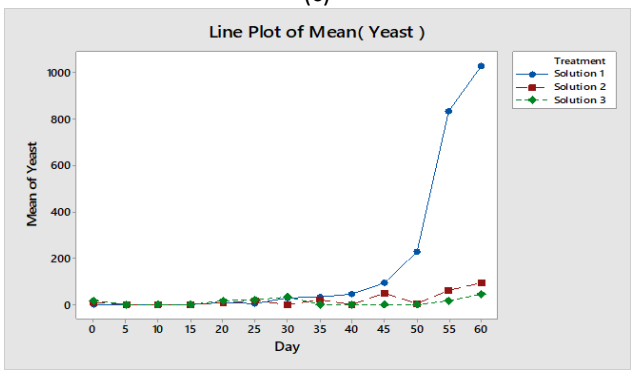

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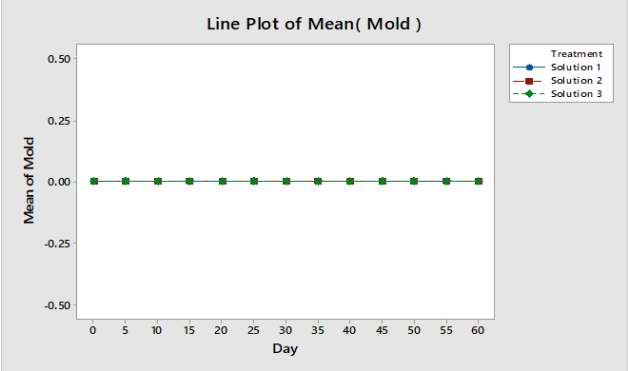


(e)

Figure1. Changes (means ± SD) of (a) pH, TPC(b),Coliform count (c), Yeast count(d) and Mold count (e) of antimicrobial edible coating solutions: Solution 1 (0.250 g/L Delvocid), solution 2 (0.275g/L Delvocid)and solution 3 (0.300g/L Delvocid) during the storage at 80C

pH was measured at the 200C. According to the above results, there was a statistically significant difference ($p<0.05$) of pH among and in between the antimicrobial solutions. pH of all three solutions started from around 7.00 and it had increased from the initial point until it became around 7.26 and after that it gradually decreased until obtained around 6.60. This is already related, when the lactic acid bacterial population increases with the time, then more and more lactic acid formation happen because lactic acid bacteria form lactic acid by degrading the lactose. Thus the solution becomes more acidic. This might be the reason for decreasing the pH of the solution.However the antimicrobial edible solutions were within the low acidic pH range after 60 days of storage period (around 6.6).

There was a statistically significant difference ($P<0.05$) of TPC of all three solutions. TPC of all three solutions increased with the time. But it was within the acceptable level of pasteurized milk products. (<30,000CFU/ml))(SLS 181:1983). According to the above results, that could be assumed increased lactic acid bacterial population might be a reason to decreased pH value during the shelf life.

Coliform count decreased with the time and it became negative over the 5 th day of storage and after that. That can be assumed due to the lactic acid which was produced by lactic acid bacteria might be cause to decrease the pH of the solution and thus it might inhibit the growth of *coliform. Coliform* bacteria are kind of non-spore forming bacteria. In other hand, *Coliform* might suppressed due to the cold storage (80C) without being multiplying because lack of better conditions for growth. However *Coliform* count was constantly negative since 5th day of the storage according to the standards. (<1CFU/g)(SLS 1558-3:2017)

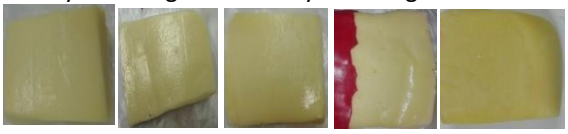
In terms of Yeast count, there was a statistically significant difference ($p<0.05$) among and in between the solutions. Yeast count of all three solutions decreased considerably relative to the initial value due to the inhibition activity of Delvocid and as well as the cold storage might be suppressed the Yeast count. But after 15 days, Yeast count started to increase rapidly. But this test was done for the randomly selected antimicrobial solutions which were stored in sterilized glass bottles.So there might be individual variations of the samples.However according to the results can be assumed that Delvocid effectively inhibit the growth of Yeast and the Delvocid concentration which was used to coating preparation was proportionate to inhibition activity. So Solution 3, which had highest Delvocid concentration was able to inhibit the Yeast population effectively rather than other two solutions. When considering solution 1, at the 45 day of storage, Yeast count exceeded the acceptable level (<100CFU/ml))(SLS 773: 1987).Therefore antimicrobial edible coating solution one is not good enough for the coating solution preparation. The Delvocid concentration (0.25g/L) which was used for the solution one was not good enough for inhibit Yeast for 60 days of storage period. Antimicrobial edible coating solution two exhibited acceptable Yeast count within the storage period. (<100CFU/ml) Therefore the Delvocid concentration which was used for solution 2 (0.275g/L) might good enough to inhibit the growth of Yeast population during the storage period,but solution 3 is better than solution 2 because it inhibited Yeast population effectively rather than solution two.So the Delvocid concentration which was used for solution 3 is the best concentration to inhibit the growth of Yeast throughout 60 days storage period.

According to the graph, the mold count was constantly negative during the shelf life.As well as the initial samples did not contain any molds. Thus can be assumed, there might not any post pasteurization contamination which incorporate molds into the sample.In other hand, the Delvocid concentration which was used for all three solutions might be good enough to inhibit the growth of Molds. In terms of pH, TPC, Coliform, Yeast and Mold that can be assumed, antimicrobial edible coating solution three is the most suitable one for coating preparation.

B. Results of cheese analyses

1) Results of appearance of cheese

The appearance of edible coated cheeses was compared with that of cheese with commercial paraffin coating (positive control) and uncoated cheese (negative control) at 0 day of storage 10 day of storage, 20 day of storage, 40 day of storage and 60 day of storage.



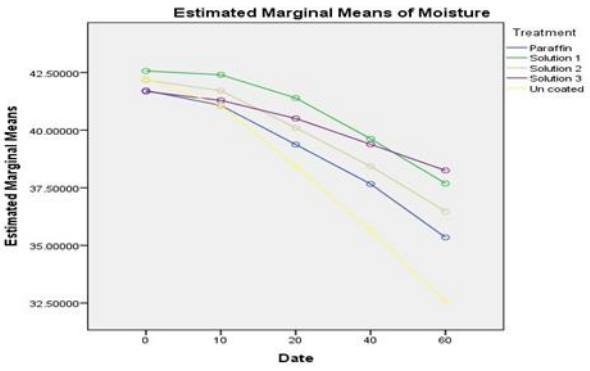
A B C D E

Figure 2. Appearance of cheeses coated with antimicrobial edible coatings incorporated with (A) solution 1(0.250g/L Delvocid) , (B) solution 2 (0.275g/L Delvocid), (C) solution 3 (0.300g/L Delvocid), compared with commercial paraffin coating (D) and uncoated cheese (E) at 0 day storage.

There was a unique shininess or waxy appearance of antimicrobial edible coated.

1) Results of physiochemical analysis

Antimicrobial edible coated cheeses, Paraffin coated cheeses and uncoated Cheeses were assayed, in triplicate on 1, 10, 20, 40, and 60 days after coating application, for moisture, weight loss, fat, salinity, pH, hardness, colour change.



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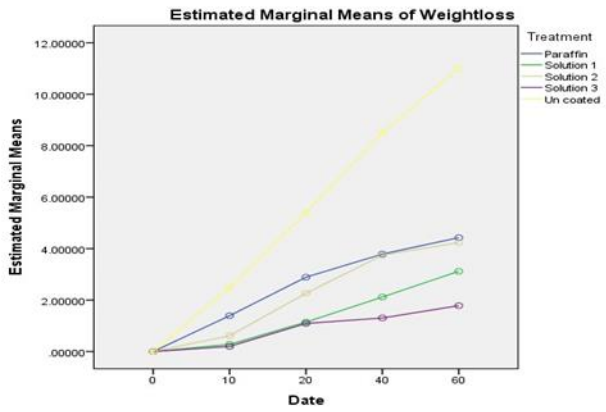

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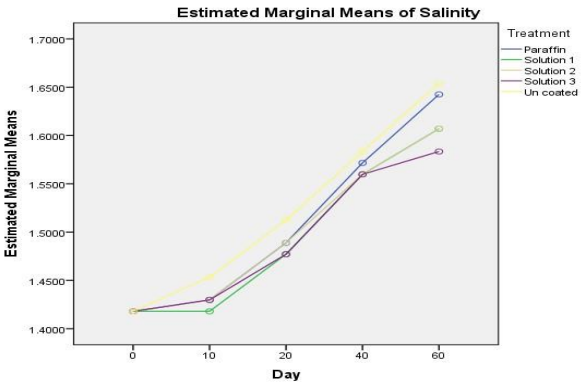

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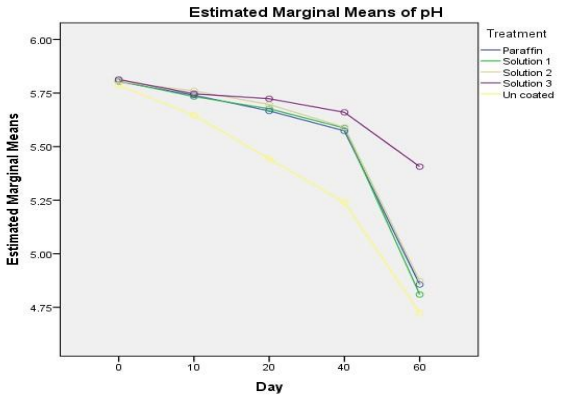

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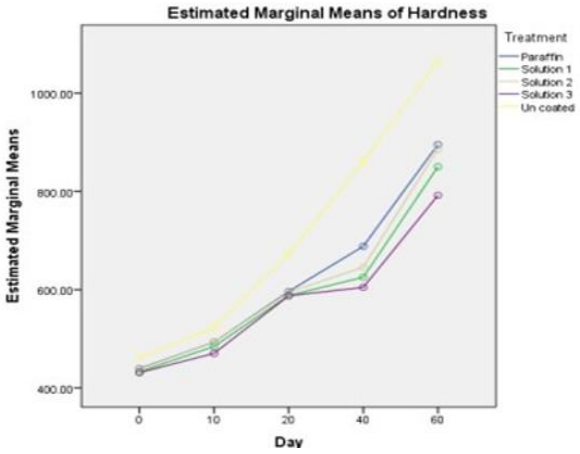
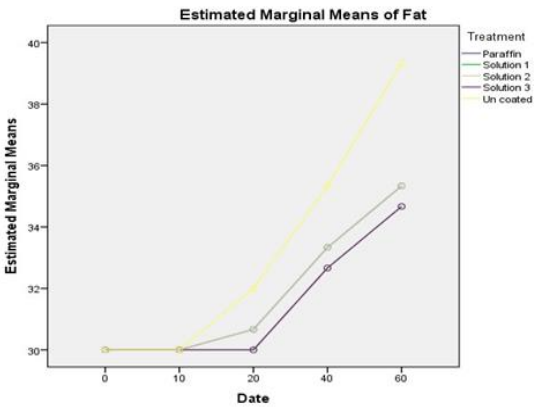
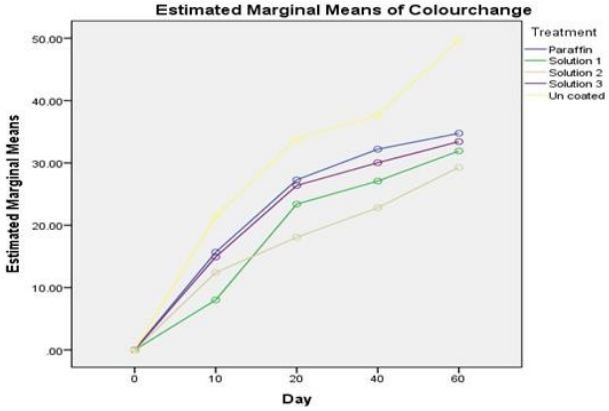






(f)

(g)

Figure 3. Values (means ± SD) of (a) weight loss, (b) moisture, (c) fat content, (d) salt content, (e) pH, (f) hardness, and (g) color change of cheese coated with antimicrobial edible coating solutions: Solution 1 (0.250 g/L Delvocid), solution 2 (0.275g/L Delvocid)and solution 3 (0.300g/L Delvocid) compared with cheese coated with commercial coating and uncoated cheese throughout 60 d of storage at 10°C and 85% relative humidity.

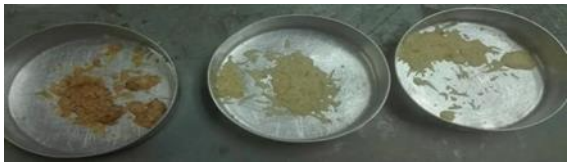


Figure 4. (Left to right) - Moisture analysed dishes of edible coated cheese, paraffin coated cheese and uncoated cheese at 0 day storage.

The brown colour appearance was observed in moisture analysed antimicrobial edible coated cheese due to the lactose caramelization of whey protein isolate. According to the moisture content throughout the storage, there was a statistically significant difference ($p<0.05$) of moisture content among the same cheese categories and in between the different cheese categories, but according to the above graph this moisture decline of the antimicrobial edible coated cheese was lower than both paraffin coated cheese and uncoated cheese and uncoated cheese had highest moisture decrease, so that can be assumed permeability of antimicrobial edible coated cheese for moisture is lower than both paraffin coated cheese and uncoated cheese.

In terms of weight loss, there was a significant difference of all coated and uncoated cheese samples since 10 day of storage ($p<0.05$). The weight loss mean, actually the moisture content which was loss from the cheese samples. According to the graph, moisture loss of the antimicrobial edible coated cheese was lower than other two types.

In terms of fat content, there was not any significant difference ($p>0.05$) of all antimicrobial edible coated cheeses and paraffin coated cheese over the first 20 day of storage, but there was a significant difference ($p<0.05$) of uncoated cheese over the 10 day of storage. Both paraffin coated cheese and antimicrobial edible coated cheese had relatively low increment of fat content.

According to the changes of salinity throughout the storage, the antimicrobial solution 1 and solution 2 coated cheeses exhibited performance with regard to salt content was similar to that of the paraffin coated cheeses ($p>0.05$), but there was a significant difference ($p<0.05$) among the same cheese categories. However increment of salt content is lowest of the antimicrobial solution 3 coated cheese and it is highest of uncoated cheese over the 60 day of storage. According to the overall results can be assumed, that the salt content not considerably changed during the 60 days of storage of all cheese verities.

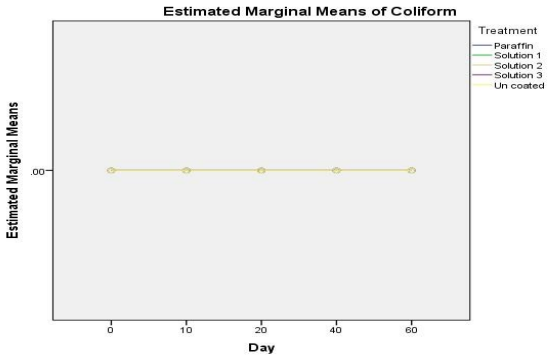
In terms of pH, there was a statistically significant difference ($p<0.05$) among the same cheese categories and in between different cheese categories.However pH decreased for all samples. Such a pH decrease might be due to the activity of ingenious bacteria that metabolize lactose to lactic acid, thus leading to acid production (Ramos et al.,2012). However the pH change among the same cheese category was significantly low of the both solution 2 and solution 3 coated cheeses and pH decrease was comparatively highest in un coated cheese and lowest in solution 3 coated cheese.

According to the changes of hardness throughout the storage, there was a statistically significant difference ($p<0.05$) of hardness among the same cheese categories. Coated cheeses had comparatively lower hardness than uncoated cheese and moreover antimicrobial edible coated cheeses had lowest hardness values. Hardness values directly link to the moisture content.

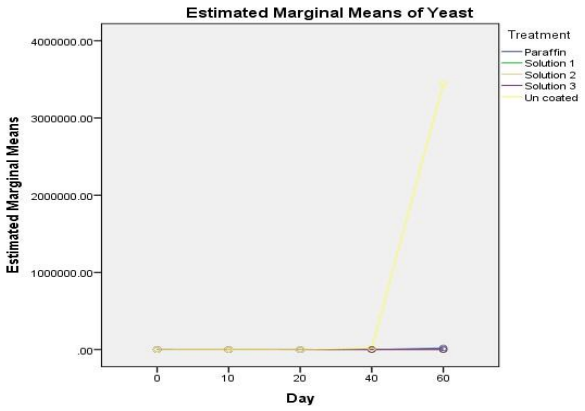
Colour analysis, based on colour change, showed that all cheeses changed colour throughout the storage, and statistically significant difference was observed ($p<0.05$), but colour change among the cheese samples of antimicrobial edible coated cheeses was lower than the colour change among the cheese samples of paraffin coated cheeses. Moreover, antimicrobial edible coated cheeses had statistically significant difference of colour ($p<0.05$), but solution 3 coated cheese had more stable colour than other cheeses throughout the storage. According to the above graph, uncoated cheeses exhibited highest colour change and antimicrobial edible coated cheeses exhibited lowest colour changes, because acidulant power of lactic acid of antimicrobial edible coated cheeses might inhibit the colour change. (Ramos et al, 2012)

The observed change in color can be, at least in part, attributed to cheese oxidation, which is lower in cheeses with a coating, because of protection from oxygen (due to lower oxygen permeability) and light (due to coating opacity). Color change may also be associated with the rate of cheese dehydration, which was lower in coated cheese, thus producing a less dry, and thus less dark, cheese rind (Ramos et al., 2012).

(2) Results of Microbiological analyses



(a)



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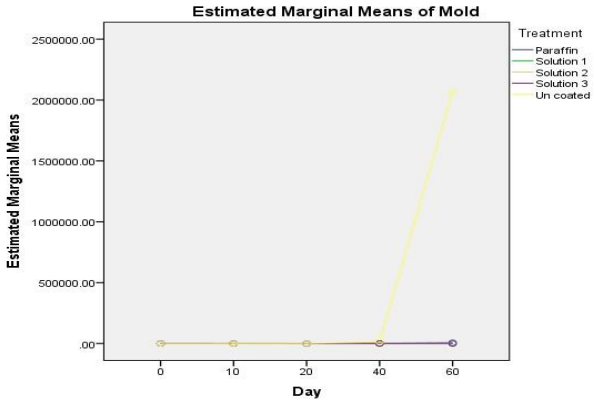

(c)

Figure 5. Coliform,Yeast and Mold count (means ± SD) of cheese coated with edible antimicrobial coatings compared with cheese coated with commercial coating and uncoated cheese throughout 60 d of storage at 10°C and 85% relative humidity.

In terms of Coliform count, it exhibited negative value for all cheeses throughout the storage. Those results confirmed that there was not any contamination of Coliform throughout the storage. Also Coliform count exhibited an acceptable level for consumption (< 1CFU/g) (SLS 773: 1987).

According to the Yeast count, there was a statistically significant difference ($p<0.05$) of all cheeses throughout the storage, but paraffin coated cheeses and uncoated cheeses exhibited comparatively high Yeast count which exceeded the acceptable level for the consumption (<100 CFU/g) (SLS 773: 1987) over the 10 day of storage. Solution one coated cheeses exceeded the acceptable level for consumption over 40 days of storage (<100 CFU/g) (SLS 773: 1987). So the Delvocid concentration which was used for the antimicrobial edible coating solution one was not good enough for inhibit the Yeast count for 60 days of storage period. However antimicrobial solution 2 and solution 3 exhibited better inhibition activity against Yeast. In terms of Mold count, there was a statistically significant difference ($p<0.05$) among the cheese samples during the storage.However all antimicrobial edible coated cheeses exhibited

acceptable Mold count for consumption (<100 CFU/g) (SLS 773: 1987) throughout the storage period, but paraffin coated cheeses and uncoated cheeses exceeded the acceptable level subsequently over the 20 day of storage and over the 0day of storage. According to that the Delvocid concentration which was used for solution one was good enough to inhibit the growth of Molds, but it was not good enough to inhibit the growth of Yeast throughout the storage. Therefore, Delvocid helps to inhibit the growth of the Yeast and Mold effectively during the storage of cheese and its function was relatively proportionate to the concentration.

3) Results of sensory analyses

According to the above results, there was not any significant difference ($p>0.05$) of all cheeses regarding Odour, Colour, Surface Shininess, Hardness, Taste and overall acceptability at 0day storage. Sensory assessments were not performed for uncoated cheese after 0day of storage because it showed large growth (>100CFU/g) of Yeast and as well as it showed visible, largely expanded fungus growth, and thus not suitable for consumption and external sensory attributes evaluation. Sensory assessments were not performed for paraffin coated cheeses after 0 day of storage because it showed relatively larger growth of Yeast (>100CFU/g), and thus might be unsafe for taste evaluation and only external attributes were assessed. According to the panellist the characteristic odour and flavor of sunflower oil was somewhat affect to the taste and odour profiles. This might be due to the unfamiliarity of sunflower oil to the Sri Lankans' consumption pattern. According to the overall results of sensory attributes, the paraffin coated cheeses showed better taste profiles at the 0 day as well as showed better external attributes at the 0 day and 20 day of storage. After 20 day of storage, antimicrobial edible coated cheeses showed better taste profiles as well as better external attributes.

Table 3.1: Sensory evaluation results of cheeses throughout the storage

Day	Cheese sample	Odour	Colour	Surface shininess	Hardness	Taste	Overall acceptability
0	Solution 1	5.10 ± 0.74^{a}	5.40 ± 0.52^{a}	4.90 ± 0.99^{a}	4.90 ± 1.45^{a}	4.10 ± 1.91^{a}	4.70 ± 1.42^{a}
	Solution 2	4.40 ± 1.90^{a}	5.50 ± 0.53^{a}	5.10 ± 0.99^{a}	4.30 ± 1.77^{a}	3.50 ± 1.78^{a}	4.70 ± 1.64^{a}
	Solution 3	4.90 ± 1.20^{a}	5.50 ± 0.85^{a}	4.90 ± 1.52^{a}	5.50 ± 1.51^{a}	4.20 ± 1.40^{a}	4.70 ± 1.34^{a}
	Paraffin	5.10 ± 1.10^{a}	5.70 ± 0.82^{a}	5.20 ± 1.14^{a}	4.60 ± 1.23^{a}	4.70 ± 1.64^{a}	5.60 ± 1.17^{a}
	Un coated	5.00 ± 0.94^{a}	5.60 ± 0.52^{a}	5.50 ± 0.97^{a}	5.40 ± 1.78^{a}	4.80 ± 1.03^{a}	5.20 ± 1.14^{a}
10	Solution 1	4.80 ± 1.32^{a}	5.10 ± 0.99^{a}	5.00 ± 1.16^{a}	4.90 ± 1.10^{a}	5.40 ± 1.43^{a}	4.90 ± 0.88^{a}
	Solution 2	4.20 ± 1.32^{a}	5.60 ± 1.43^{a}	5.50 ± 1.51^{a}	5.30 ± 1.77^{a}	5.60 ± 1.35^{a}	5.10 ± 1.79^{a}
	Solution 3	4.20 ± 1.03^{a}	5.30 ± 1.64^{a}	5.30 ± 1.77^{a}	5.10 ± 1.52^{a}	4.80 ± 1.93^{a}	4.90 ± 1.60^{a}
	Paraffin	5.30 ± 1.06^{a}	5.30 ± 0.95^{a}	5.40 ± 0.97^{a}	5.00 ± 1.89^{a}	-	5.40 ± 1.08^{a}
20	Solution 1	4.90 ± 1.10^{a}	5.40 ± 1.17^{a}	4.80 ± 1.03^{a}	4.90 ± 1.10^{a}	4.40 ± 0.45^{a}	4.30 ± 1.49 ^{a}
	Solution 2	5.40 ± 1.17^{a}	5.30 ± 1.16^{a}	5.40 ± 1.17^{a}	5.50 ± 1.18^{a}	5.00 ± 0.39^{a}	4.90 ± 1.37 ^{a,b}
	Solution 3	5.40 ± 1.27^{a}	6.10 ± 0.74^{a}	5.80 ± 0.79 ^{a}	5.80 ± 1.03^{a}	5.30 ± 1.34^{a}	6.10 ± 0.74^{b}
	Paraffin	5.50 ± 1.08^{a}	5. 80 ± 1.32^{a}	5.20 ± 1.23^{a}	5.70 ± 1.06^{a}	-	5.70 ± 0.68^{b}
40	Solution 1	5.20 ± 1.23^{a}	5.80 ± 0.63^{a,b}	5.40 ± 0.52^{a,b}	5.30 ± 0.82 ^{a}	4.70 ± 1.16^{a}	5.00 ± 0.82^{a,b}
	Solution 2	5.70 ± 1.16 ^{a}	6.20 ± 0.63^{b}	6.00 ± 0.67 ^{b}	5.30 ± 0.68^{a}	5.60 ± 0.70 ^{a}	5.80 ± 0.79^{b,c}
	Solution 3	5.80 ± 0.63^{a}	6.00 ± 0.47^{a,b}	5.60 ± 0.52^{a,b}	5.70 ± 0.68^{a}	5.60 ± 0.84^{a}	5.90 ± 0.57^{c}
	Paraffin	5.30 ± 1.16^{a}	5.10 ± 1.23^{a}	5.10 ± 0.99^{a}	5.20 ± 0.79^{a}	-	4.70 ± 0.68^{a}
60	Solution 1	4.80 ± 1.03^{a}	5.10 ± 1.10^{a}	5.40 ± 0.84^{a}	5.10 ± 0.74^{a}	-	4.60 ± 1.08^{a}
	Solution 2	5.40 ± 0.97^{a}	5.80 ± 0.79^{a,b}	5.50 ± 0.97^{a}	5.70 ± 0.95^{a}	5.10 ± 0.74 ^{a}	5.50 ± 0.85^{a,b}
	Solution 3	5.30 ± 0.95^{a}	6.10 ± 0.32^{b}	5.80 ± 0.79^{a}	5.60 ± 0.84^{a}	5.70 ± 0.82^{a}	5.80 ± 0.79^{b}
	Paraffin	5.30 ± 0.95^{a}	5.70 ± 0.48^{a,b}	5.50 ± 0.53^{a}	5.30 ± 0.68^{a}	-	5.10 ± 0.57^{a,b}

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