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Antimicrobial Activity of Chitosan Based Edible Film Enriched with Red Ginger Essential Oil as An Active Packaging for Food.

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ABSTRACT

The improving of edible film quality on antimicrobial activity can be maintained by incorporation of red ginger essential oil due to their active components of shogaol and gingerol. Shogaol and gingerol are antioxidant and antimicrobial agent which can improve the effectivity of edible film as preservative material for food. The objectives of this research is to study the effect of essential oil addition to the characteristic and antimicrobial activity of chitosan based edible film. This study were carrried out through two steps of procedures. The first step was purification of red ginger oil by using of distillation method at 80°C to separate the ethanol solvent. The second step was preparation of chitosan based edible film by mixing continued with homogenization of red ginger essential oil and chitosan solution. The concentrations of red ginger essential oil in the total mixture were varied of 0.6; 0.9; and 1.2% (w/w). Glyserol was added in the mixture in order to plastisize the film. The obtained solution was then spread on a glass surface and dried to result a thin film. The drying condition were varied of ambient temperature, 70°C, and 80°C by using of the oven. The lowest swelling capacity of 30.5% was resulted from the oven drying method at 80°C temperature and the red ginger oil concentration at 1.2%. The inhibition test of edible film to Escherechia coli (*E.coli*) shows that 1.2% of red ginger oil concentration in the film results the lowest total colonies per 4 cm of the test area (6125 colonies).
Keywords: antimicrobial, chitosan, edible film, essential oil, red ginger.

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INTRODUCTION

Over the last decade, the trends of consumers prefer foods that have good quality, fresh and safe. Therefore, the development of packaging technology is increasing rapidly [1]. It relates to healthy foods and safe from all environmental influences that can damage the quality of the food. One of the packaging technologies that can be used to protect the food was active packaging. Food packaging aims to protect food against oxygen, moisture, UV light, and both chemical and microbial contamination [2].

Active packaging is defined as an intelligent system that involves interaction between the components of the package and the food or the atmosphere and the internal gas in accordance with consumer demand for high quality, fresh-like and safe products[1, 3,]. The main function of active packaging is to control moisture, slowing the loss of moisture that allows the microbes to grow. Controlled humidity makes the shelf life or storage life a product can be long [2].

One of the active packaging is edible film that serves as a barrier to moisture, preventing the loss of aroma; improve physical characteristics and carrier additives. Hydrophobic substances such as resin, wax or protein are a good inhibitor for moisture, but the water-soluble hydrocolloid such as polysaccharides usually provides good mechanical properties such as tensile strength and elongation for edible films and coatings than lipid. Both are inhibitors of oxygen and CO_2which are very good because its hydrogen bonded network structure [4-7].

One of materials commonly used as an edible film is chitosan, because chitosan have a good ability to form the film, biocompatible, biodegradable and non-toxic [8]. Antifungal and antibacterial activity of chitosan derived from natural polycation[9]. Antimicrobials chitosan caused by electrostatic force between the amino groups (NH_2) in chitosan and negative residues on the cell surface [10].Chitosan showed antibacterial activity only in acidic medium, which is usually associated with poor solubility of chitosan above pH 6.5 and positively charged polycation with a strong affinity for the cell [11, 12]. However, it was found that the water-soluble chitosan promote the growth of *Candida albicans* even in an acidic medium in which the dissolved water insoluble chitosan shows the inhibitory effect [12].

The antimicrobial can be further enhanced with the addition of essential oils in edible film as active packaging [13]. Essential oils contain phenolic compounds and highly effective as an antibacterial [14]. The addition of essential oils have additional applications in the food packaging, the addition of antimicrobial agents such as essential oils directly into food packaging is called active packaging (food packaging) [14-16].

Research the addition of essential oils in edible chitosan films have been done as Ojagh et al [15] reported that the addition of cinnamon essential oil in edible film can enhance the antimicrobial activity of chitosan. The incorporation of bergamot essential oil with chitosan films offers the possibilities not only instill antimicrobial activity against *P. Italicum*, but also improve the water vapor barrier properties of the film [17]. Galangal extract can also be added to the chitosan films to further enhance the antimicrobial activity [18]. Ginger essential oil can enhance the antimicrobial activity of the edible film of starch [19], therefore in this study red ginger essential oil is added to the chitosan edible film to further improve the effectiveness of the antimicrobial. Extracts of red ginger contains volatile components and non-volatile such as gingerol[20-22] and shogaol which is formed from dehydration gingerol during the heating process or storage [23-25]. The active component contained in the ginger extract can be used as an antioxidant and a natural antimicrobial [26-28].

Besides the addition of essential oils to enhance its effectiveness as an antimicrobial edible films in active packaging (active packaging), another factor is the drying process. This factor is one of the important factors in the manufacture of edible film, drying methods and under different conditions may affect the properties and function of the resulting edible film [8]. The objective of this research was to study the effect of essential oil addition to the characteristics and antimicrobial activity of chitosan based edible film and to know the effects of some methods of drying (ambient drying and oven) on the ability of chitosan-edible film enriched with red ginger essential oil.

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MATERIALS AND METHODS

Materials

Chitosan food grade from PT. Biotech Surindo with a degree of deacetylation (DD) = 87.2% and a viscosity of 37.10 cps, glacial acetic acid from Merck, technical glycerol from Bratachem, red ginger rhizome, and ethanol 96% from Sigma-Aldrich.

Extraction of red ginger

Dry red ginger entered in glass beaker containing ethanol as a solvent, and then left to stand for 8 hours. Extraction product was separated between red ginger essential oil and solvent.

Preparation of plasticized chitosan solution

Chitosan solution at 2% (w / v) was made by dissolving chitosan in 1% (v / v) of glacial acetic acid and stirred constantly using IKA overhead stirrers RW 20 at room temperature conditions for 4 minutes. A total of 25% glycerol (w/w chitosan) is added to the chitosan solution, and then stirred using an IKA magnetic stirrerhot plate for 1 minute. After completion of the mixing process, the solution is filtered with Whatman filter paper to remove insoluble impurities and bubbles in the solution.

Preparation of chitosan edible film

This method refers to Mayachiew, et al.[8]. Each of red ginger essential oil concentrations are 0.6, 0.9 and 1.2 g/100 g included in in plasticized chitosan, and then homogenized using a mixer for 1 minute. The mixture was then molded with a size of 5 x 5 cm. edible film drying process carried out by three methods: at room temperature (30 °C), 70 °C and 80 °C. Chitosan-based edible films were analyzed for swelling, and antimicrobial activity

Gas chromatography mass spectrometry (GCMS) analysis

Gas chromatography mass spectrometry (GCMS) was used for the analysis of the components contained in the red ginger essential oil. GCMS analysis was performed using a Shimadzu QP 2010S with AGILENTJ% W column HP-5, 30 m long with a diameter of 0.25. The condition operation of GCMS as follows: Column Oven Temp. : 60.0 °C, Injection Temp. : 300.00 °C, Injection Mode: Split, Flow Control Mode: Pressure, Pressure: 13.0 kPa, Total Flow: 80.7, mL/min, Column Flow: 0.51 mL/min, Linear Velocity: 26.2 cm/sec, Purge Flow: 3.0 mL/min.

Swelling analysis

The swelling capacity of gel was determined according to a tea bag method [29]. Powdered hydrogel (0.1 g) were immersed in distilled water (100mL) at room temperature. NaCl and urea were also used in this swelling study to find the hydrogel performance in the salt solutions. At 30 seconds time intervals, the samples were removed from the swelling medium and blotted on a filter prior to weighing to remove excess surface moisture.

The swelling ratio was calculated according to the following expression:

$$\text{Swelling capacity (g/g)} = \frac{W_t - W_0}{W_0} \qquad (1)$$

Where Wt = weight of the swollen gel at time t (g) and W0 is the initial weight of the dried gel (g). The studies were carried out for 180 s.

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Antibacterial Analysis

Evaluation of antibacterial activity of the hydrogel was prepared by using of ring method. The hydrogel at various compositions of chitosan were placed in petridishes with *Esherechia coli* and the Agar Nutrient inside. After 24 hours, the ring was taken off from petridish and the inhibition area was measured. Some procedures including preparation of agar, growth of *Escherechia coli*, micro test preparation, and inhibition zone analysis were done in this study.

RESULTS AND DISCUSSION

Analysis of red ginger essential oil

Red ginger essential oil contain active components that can be used as antioxidants, antimicrobials, etc. Red ginger essential oil component from extraction process can be seen in Figure 1.

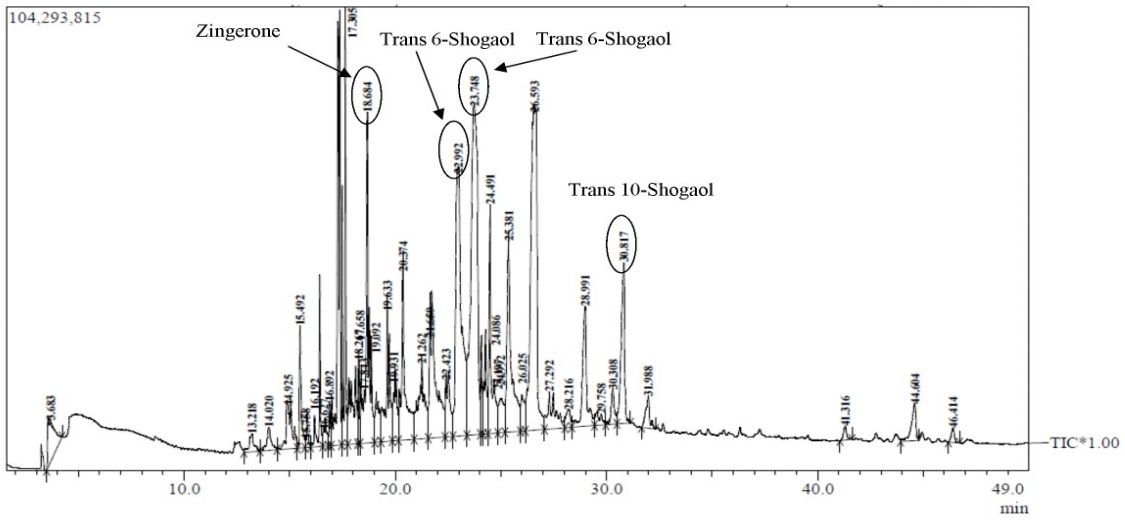

Figure 1: GCMS chromatogram of red ginger essential oil.

GCMS analysis results indicate that the target components of essential oils that could be detected are shogaol and zingerone, while gingerolcould not be detected. This could be due to the content of gingerol in ginger was dehydrated during the drying process or during storage[25]. Components that are detected in the red ginger essential oil were 45 components. Table 1 shows the components analyzed by GCMS

Table 1: Red ginger essential oil compounds

No	Name of the compound	Concentration (%)
1	trans-6-shogaol	12.54
2	2-(3,7-dimethyl-octa-2,6-dienyl)-4-methoxy-phenol	11.69
3	cis-6-shogaol	9.20
4	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (CAS) ar-Curcumene	6.34
5	Zingerone [4-(4-hydroxy-3- methoxyphenyl)-2-butanone	5.67
6	Cyclohexene, 4,5-diethyl-1,2-dimethyl-, CIS-	5.02
7	2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)- (CAS) Zingerone	4.83
8	Hexadecanoic acid (CAS) Palmitic acid	3.56
9	nerolidol Z and E	3.54
10	2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)- (CAS) Zingerone	3.13

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Chitosan-based edible film Enriched with red ginger essential oil

Chitosan and red ginger has the same function are as an antimicrobial agent, so when it was combined in the edible film as active packaging will generate maximum antimicrobial activity than without the addition of red ginger essential oil. Chitosan-based edible film which has added red ginger essential oil can be seen in Figure 2.

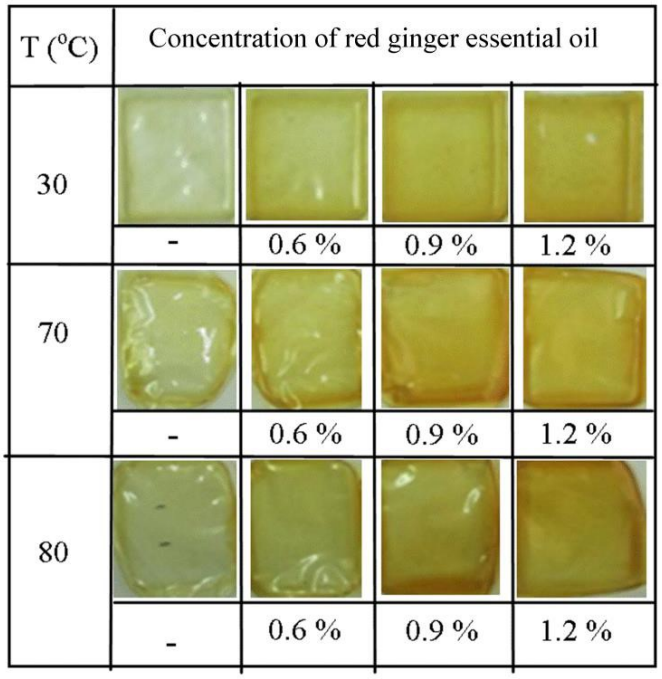

Figure 2: Chitosan-based edible film enriched red ginger essential oil at various concentrations

Figure 2 shows the effect of adding red ginger essential oil at different concentrations. Increased concentration of essential oils made the color of the edible film of dark brown, this was related to the concentration of essential oils was increasing, while the drying temperature did not have a significant influence on the color change of edible film.

Swelling analysis

The durability of the film to water is characterized by swelling. Figure 3 shows the effect of different concentrations of red ginger essential oils and different methods of drying on the degree of swelling. The results showed that the swelling percentage of chitosan-edible film enriched with red ginger essential oil on drying at ambient temperature of 103%.

Swelling analysis showed that the addition of red ginger essential oil affects the degree of swelling. Increasing the concentration of red ginger essential oils were added in edible film, the degree of swelling was decrease. The addition of essential oils of ginger might affect the hydrophobic properties [8]. Incorporation between chitosan and essential oils could improve the interaction between molecules of chitosan and essential oil which causes a decrease in degree swelling.

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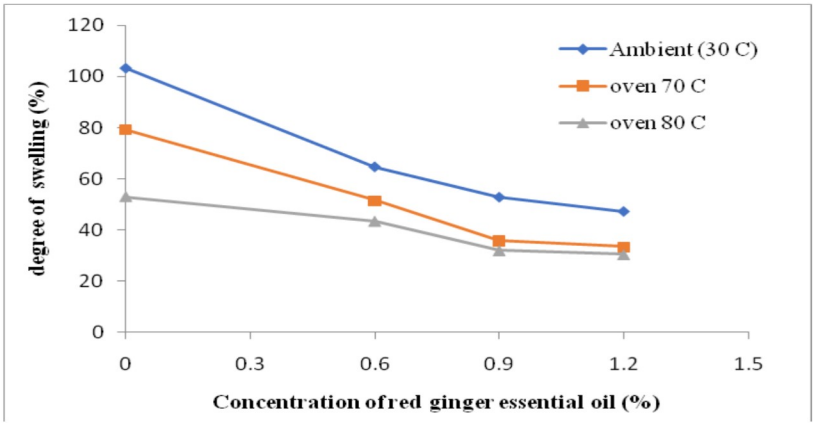

Figure 3: Effect of the red ginger essential oils concentration against the film swelling

The drying temperature also affects degree of swelling, in figure 3 shows that increasing the drying temperature, degree of swelling decreases. The increasing temperature might allow the cross link that makes the edible film more rigid. The heating chitosan edible film causes a reduction in the hydrophilic group, thereby decrease the swelling ofedible film [8]. The degree of swelling is an indication of degree cross-protein. The degree of swelling decreases because film solution heat denaturation temperature and time increased [30].

Edible films of chitosan have a good resistance against O_2 and CO_2 gas, improve physical strength, but the resistance to water vapor is very low due to the properties of hydrophilic. Edible film consists of only one material component only cannot provide satisfactory results in comparison with a mixture of several materials [31]. The addition of red ginger extract can increase the resilience of the film for H_2O.

Antibacterial analysis

Chitosan edible film is applied as a coating material of food so that the necessary observation of the ability edible film which has been added the red ginger extract against pathogenic bacteria in food. One way to determine the antimicrobial activity of the edible film is to use the tools of *EasicultCombi* to observe bacterial contamination of the dip slides that contain bacteria that as a growing medium.

Figure 4 shows the dip slides that have been coated with chitosan solution added red ginger extract 1.2%, 0.9% and 0.6%, and water (from the left to right). Dip slide was coated with water began to look the growth of bacteria on the third day which was marked in red on the dip slide, while the dip slides from the chitosan solution + red ginger essential oil still have not seen the growth of bacteria. It is proved that the addition of red ginger extract in a solution of chitosan can increase the antimicrobial activity was to suppress the growth of bacteria and microorganisms.

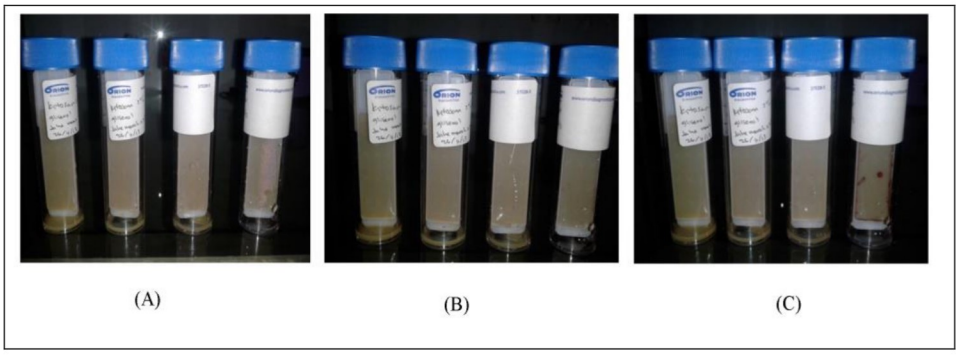

Figure 4: The growth of bacteria in the dip slides on (a) the first day, (B) the second day, and (c) the third day

Another way to know the existence of antimicrobial activity in edible film is by using the Standard Plate Count method, which wasbreeding the bacteria on an agar and placing a sample of chitosan enriched with red ginger essential oil.

Tabel 2: The total percentage of *Escherichia coli* colonies

No	Sample	1st day	2nd day
1	Control	0 colony/4 cm^2	0 colony /4 cm^2
2	0%	15240 colony /4 cm^2	15288 colony /4 cm^2
3	0.6%	7224 colony /4 cm^2	7224 colony /4 cm^2
4	0.9%	6448 colony /4 cm^2	5455 colony /4 cm^2
5	1.2%	6125 colony /4 cm^2	6125 colony /4 cm^2

The growth of bacteria in samples of chitosan-based edible film enriched with the red ginger essential oil with the highest concentration (1.2%) showed the number of bacteria that were smaller than the chitosan-based edible film without red ginger essential oiladdition or from the lower concentration of red ginger essential oil (0.6% and 0.9%).The data shows the interaction betweenthe red ginger essential oil with the bacteria of *E. coli*. Moreover, this indicates that the red ginger essential oil contains anti-bacterial that can prevent the growth of *E. coli* bacteria.

Phenolic compounds contained in red ginger essential oil can attack the cell membrane of phospholipids, cause the increasing of permeability and leakage of the cytoplasm [8]. Edible films with the addition of antimicrobial agent cause damage on the cell wall and the cell membrane of bacteria, this implies that the antimicrobial agent can move from film to aqueous systems [8]. The results of cell damage were correlated with a decreasing in the total number of *E. coli* colonies along with the addition of red ginger essential oil. The change of bacterial growth in the second day on a sample of chitosan-based edible film with red ginger essential concentration of 0.9% due to the death of *E. coli* bacteria found in the sample.

CONCLUSION

Based on the research, production of chitosan-based edible film obtain some conclusions such as the quality of chitosan-based edible film enriched with red ginger essential oil is better than chitosan edible film without the addition of red ginger essential oil. The iincreasing of red ginger essential oils concentration added in the mixture cause the decreasing of degree of swelling and increasing of antimicrobial activity. Increasing of the drying temperature cause the decreasing of degree of swelling. The lowest swelling degree obtained in this research was30.5% and was reached in the use of red ginger essential oil concentration of 1.2% and a drying temperature of 80 °C.The antibacterial test against*E. coli* resultedthe lowest bacterial growth on the red ginger essential oil concentration of 1.2% that was equal to 6125 colonies/4 cm2.

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REFERENCES

[1] Ozdemir M, and Floros JD. Crit Rev Food Sci Nutr 2004;44:185-93
[2] Prashad P, and Kochhar A. J Environ. Scie, Toxic. Food Tech 2014; 8: 1-7
[3] Floros JD. Dock LL, Han JH. Food, Cosmet. Drug Packaging 1997; 20:10–17.
[4] Nussinovitch A. In: Kasapis S. Norton IT, Ubbink JB. (Eds.). Modern biopolymer science: Bridging the dividebetween fundamental treatise and industrial application. Academic Press, 2009, pp. 295–326
[5] Arvanitoyannis IS. Irradiation of food commodities: techniques, applications, detection, legislation, safety and consumer opinion, Academic Press, 2010, pp 609-630
[6] Atarés L, Pérez-Masiá R, Chiralt A. J. Food Eng. 2011; 104:649–656

[7] Bonilla J, Atarés L, Vargas M, Chiralt A. J. Food Eng. 2012; 110: 208–213
[8] Mayachiew P, Devahastin S, Mackey BM, Niranjan K. Food Res. Int. 2012; 43: 125–132
[9] Kim KW, Thomas RL, Lee C, Park HJ. J. Food Prot.2003; 66: 1495–1498
[10] Tsai GJ, Su WH, Chen HC, Pan CL. Fisheries Scie.2002; 68: 70–177
[11] Rabea EI, Badawy MET, Stevens CV, Smagghe G, Steurbaut, W. Biomacromolecules. 2003; 4:1457-65
[12] Qin C, Li H, Xiao Q, Liu Y, Zhu J, Du Y. Carbohydr. Polym.2006; 63: 367–374
[13] Sung SY, Sin LT, Tee TT, Bee ST, Rahmat AR, Rahman, WAWA, Tan AC, Vikhraman M. Trends Food Scie. Tech. 2013; 33: 110-123
[14] Seydim AC, and Sarikus G. Food Res Int.2006;39:639–644
[15] Ojagh SM, Rezaei M, Razavi SH, Hosseini SMH. Food Chem. 2010; 122: 161-166
[16] Elsabee MZ, Abdou ES. Mater SciEng C Mater Biol Appl. 2013; 33: 1819-41
[17] Sánchez-González L, Cháfer M, Chiralt A, González-Martínez, C. Carbohyd. Polym. 2010; 82: 277-283
[18] Sebti I, Martial-Gros A, Carnet-Pantiez A, Grelier S, Coma V. J. Food scie.2005; 70: 100-104
[19] Miksusanti, Herlina, Masril KI.Int. J. Bioscie. Biochem. Bioinform.2013; 3: 354-356
[20] Lun LC, Derong T, Le L. Int J Food Scie. Tech. 2008; 43: 517–525.
[21] Onyenekwe PC. Mol Nutrition Food Res. 2000; 44: 130–132
[22] Nwaoha M, Elizabeth I, Okafor, Ifeanyi G, Veronica AO. African J.Microbio. Res. 2013; 7: 4981-4989
[23] Wohlmuth H, Leach, DN, Smith MK, Myers S. J. Agric. Food. Chem. 2005 53: 5772-5778.
[24] Bhattarai S, Tran VH, Duke CC. J Pharm Biomed Anal. 2007 30;45:648-653
[25] Badreldin HA, Gerald B, Musbah OT, Nemmar A.Food Chem Toxicol. 2008;46:409-420
[26] El- Ghorab AH, Nauman M, Anjum FM, Hussain S, Nadeem M. J. Agric. Food. Chem. 2010; 58: 8231–8237
[27] Oboh G, Ayodele JA, Adedayo OA. ExpToxicolPathol. 2012; 64:31-36
[28] Yeh HY, Chuang, CH, Chen, HC, Wan CJ, Chen TL, Lin LY. LWT - Food Sci Technol. 2014; 55: 329-334
[29] Erizal. Indo J Chem. 2012; 12: 113-118
[30] Perez-Gago MB and Krochta JM. J. Agric.Food Chem. 2001; 49: 996-1002.
[31] Wong DWS, Camirand WM, Pavlath AE. In: Krochta JM. Baldwin EA, Nisperos-Carriedo MO. (Eds). Edible coatings and films to improve food quality. Lancaster, PA: Technomic Publishing Company, 1994, pp. 65-88