Microcapsule Application of *Kecombrang* Flower Extract: Effects of Concentration, Types of Fraction, pH of Medium, and NaCl on Microbiological Properties of Minced Beef

R Naufalin* and SR Herastuti

Department of Food Science and Technology, Jenderal Soedirman University, Purwokerto, Indonesia
* Corresponding author email: rnaufalin@yahoo.co.id

Abstract. *Kecombrang* (*Nicolaia speciosa* Horan), in addition to flavor and medicinal uses, it has potential as antimicrobial substances. The bioactive components in *kecombrang* are alkaloids, flavonoids, polyphenols, steroids, saponin and atsiri oils. This research was aimed to study the effectiveness of microcapsule from flower extract of *kecombrang* added to minced beef; the effects of pH interaction and NaCl addition on antimicrobial activity. The research used a Randomized Completely Block Design factorial pattern with 24 treatment combinations and 2 replications. The tested factors were type and microcapsule concentration (control, ethanol extract 5%, etanol extract 10%, ethyl acetate extract 5% and ethyl acetate extract 10%), pH medium (pH 4 dan pH 7), and NaCl concentration (0% 2,5% and 5%). The results showed the microcapsule of flower extract of *kecombrang* that have antimicrobial activities in boiled minced beef was etyl acetate extract of 10% concentration under pH 4 and 5% NaCl .

Keywords : Microcapsule, *kecombrang*, natural preservative, minced beef.

Abstrak. Kecombrang (*Nicolaia speciosa* Horan) selain sebagai pemberi cita rasa dan berkhasiat obat, juga berpotensi sebagai antimikroba. Komponen bioaktif yang terdapat dalam kecombrang yaitu alkaloid, flavonoid, polifenol, steroid, saponin dan minyak atsiri. Penelitian ini bertujuan untuk mengetahui efektivitas mikrokapsul dari ekstrak bunga kecombrang yang ditambahkan pada daging sapi giling, pengaruh perlakuan pH dan penambahan NaCl terhadap potensi antimikroba. Penelitian ini menggunakan Rancangan Acak Kelompok (RAK) factorial dengan 24 kombinasi perlakuan dan 2 kali ulangan. Faktor yang diteliti adalah jenis dan konsentrasi mikrokapsul yaitu (kontrol, ekstrak etanol 5%, ekstrak etanol 10%, ekstrak etil asetat 5% dan ekstrak etil asetat 10%), pH medium (pH 4 dan pH 7), dan konsentrasi NaCl (0% 2,5% dan 5%). Hasil penelitian menunjukkan bahwa mikrokapsul ekstrak bunga kecombrang yang dapat berfungsi sebagai antimikroba pada daging giling rebus adalah ekstrak etil asetat pada konsentrasi 10% dengan pengaturan pada pH 4 dan pada konsentrasi NaCl 5 %.

Kata kunci : Mikrokapsul, *kecombrang*, pengawet alami, daging giling.

Introduction

Kecombrang (*Nicolaia speciosa* Horan) is one of plants that can serve a source of natural antioxydant and antimicrobial compounds. A study on multi layered extractions of its flower using non-polar solvent (hexane), semi-polar solvent (ethyl acetate) and polar solvent (ethanol) showed that semi-polar solvent produced extract with broad inhibition spectrum on bacteria (both Gram + and -), including spore formation (Naufalin et al., 2005). Meat and meat products are perishable food products, particularly because of high fat content and microbial spoilages during prolonged storage under room temperature. According to Buckle *et al*. (2007), fresh meat can be stored at room temperature for one day only.

Fresh meat is a suitable medium to support microbial growth, including pathogenic bacteria, molds, fungi, and yeast (Dave and Ghaly, 2011). Hence, the application of natural preservatives is becoming imperative as one of ways to prevent microbial growth and prolong the shelf-life of meat and meat products. The previous study showed that pH, NaCl and heat treatment affected the stability of antimicrobial

properties of extract of fresh *kecombrang* flower (Naufalin *et al.*, 2006). The extract contain volatile compounds and very unstable with the presence of light and oxygen. This study presents further investigation on the application of micro-encapsulated *kecombrang* flower extract as natural preservative for minced beef, in combination with different pH condition and NaCl concentration.

Material and Methods

***Kecombrang* flower powder processing (Naufalin, 2008).** The flower was cut and spread on trays and dried with a blower dryer at temperature of 50°C until dry. *Kecombrang* flower which has been dried crushed in a blender until a homogeneous powder and ready to be extracted.

Extraction process of *kecombrang* flower powder (Naufalin, 2008). Extraction process is carried out by extraction multilevel, using the two consecutive solvent with ethyl acetate and ethanol as follows: A total of powdered flowers of *kecombrang* dissolved in ethyl acetate (1:4 w/v), then shaken with a rotation speed of 150 rpm shaker for 2 hours. Then it filtered with filter paper to obtain extract 1 and pulp. Then the extract 1 solvent was evaporated with rotary evaporator and obtained ethyl acetate fraction. Futhermore, Dregs 1 *kecombrang* flower extracted again using ethanol solvent and works the same way as using the ethyl acetate solvent and result the extract 2 and pulp 2. The *kecombrang* flower extracts were then flowed with N2 gases.

Microencapsulation of *kecombrang* flower extract (Naufalin et al., 2011). Microencapsulation process began with making encapsulant in ratio of destilled water with filler materials (1:1) which is the ratio filler materials are gelatin and maltodextrin (1:1), stirred thoroughly and left under room temperature for 12 hours. The next process is *Kecombrang* extract was added to the encapsulant and thoroughly stirred for 5 minutes and spread on trays and dried in a cabinet dryer at 40˚C for 10 hours.

Application on minced beef. Minced beef added by distilled water with ratio 1:2, set pH at 4 and 7 and added by NaCl (0 % , 2.5 % , 5 % , (w/w) then boiled for 10 minutes. Microcapsules (0 % , 5 % and 10 % , (w/w) was added and boiled for 5 minutes. Minced beef total microbes were analyzed on time period of 0, 4, and 8 hours.

Analysis of total microbes. Total microbial analysis was conducted using Total Plate Count by Roberts and Greenwood (2003)

Data analysis. The data obtained from the study were analyzed by variance test (F test) at the 5% level.

Results and Discussion

According to SNI 01-3818-1995 maximum total microbes in beef products are 1 x 10^5 CFU/g. The boiled minced beef without the addition of ethyl acetate or ethanol extract of *kecombrang* flower microcapsules has total number of initial microbes of 2.9 x 10^2 cfu / g. The number of microbes was still increasing at 8 hours observation namely 4.1 x10^4 CFU/g. To maintain the quality of beef remains good and suitable for consumption, it is necessary to add preservatives. The use of *kecombrang* flower extract microcapsules, pH and NaCl treatment can be used as alternative of food natural preservative, especially for beef.

Results of analysis of variance showed that the type and concentration of the microcapsules did not significantly affect microbial growth on boiled minced beef at the observation of 0 and 4 hour, but showed significant effect on the observation of 8 hours. The mean of total microbes in boiled minced beef calculated at the observation of 8 hours with various treatments were ranging from 3.9 X 10^3 CFU/g to 3.3 x 10^4 CFU/g (Figure 1).

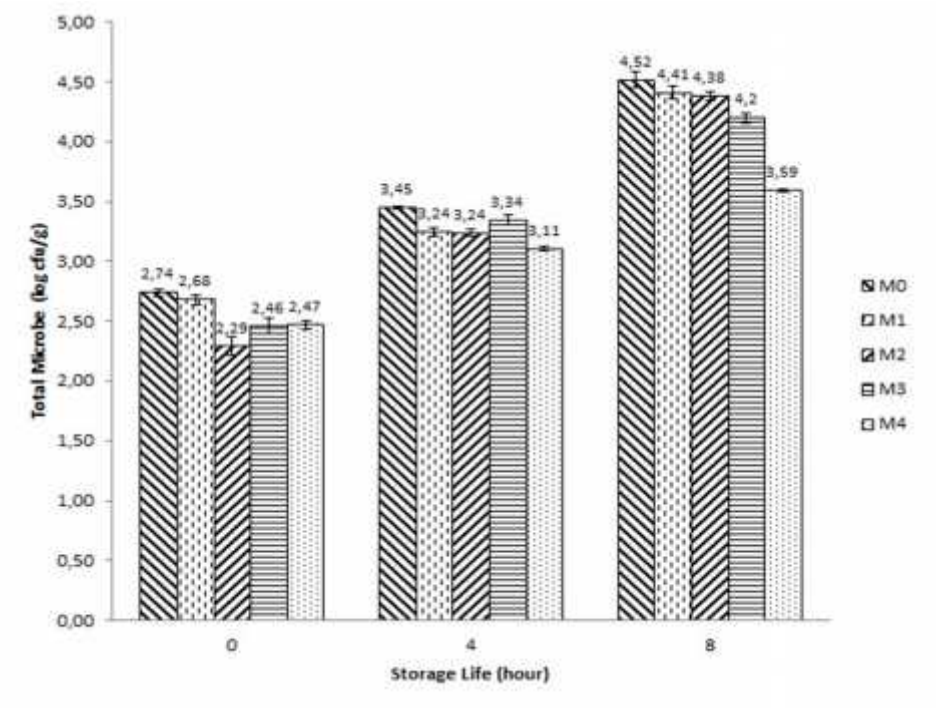
The average pH of minced beef ranged from 7.0 to 7.2. Results of analysis of variance showed that pH had significant effect on total microbes of boiled minced beef with a range of 1.6 x 10^2 to 2.1 x 10^4 cfu/g (Figure 2). NaCl significantly affects total microbes of the boiled minced beef. The average total microbes of boiled minced beef at observation of 0 hour on the addition of NaCl at concentration of 0, 2.5, and 5 % were ranging from 5.8 x10^2 to 7.3 x10^4 CFU/g (Figure 3).

Results showed that ethyl acetate extract microcapsule at 10 % (M4) at observation of 8 hours had significant effect which is reducing as much as 1 log cycle compared the one without microcapsules addition. The higher concentration of the extract, the greater is the number of antimicrobial compounds such as bioactive components thus the penetration of the compound into the cell becomes easier. This was in line with Naufalin et al. (2005) research on *kecombrang* that has extract bioactive components functioning as an antimicrobial, so that it is able to inhibit the growth of microbes including Gram+, Gram- and spore-forming bacteria.

Kecombrang flower microcapsules with ethyl acetate solvent had higher antimicrobial activity than the ethanol solvent. This is probably caused by the optimum polarity of ethyl acetate. According to Kanazawa et al. (1995), a compound having the optimum polarity will have a maximum antimicrobial activity, because the interaction between an antimicrobial compound and bacteria needs hydrophilic-lipophilic balance (HLB: Hydrophilic lipophilic balance). According to Davidson et al (2010), polarity is an important physical property of antimicrobial compounds. The state of hydrophilic is required to ensure that the compounds are dissolved in water phase where the microbes live. In contrast, compounds acting on the hydrophobic cell membrane requires lipophilic state. Thus, antimicrobial compounds require hydrophilic - lipophilic balance to achieve optimum activity.

Boiled minced beef without the microcapsules addition had more total microbes compared to minced beef supplemented with microcapsules. It is suggested that *kecombrang* flower microcapsules of both ethanol and ethyl acetate extracts contain microbes growth inhibiting compounds (antimicrobial compounds). According to Naufalin and Herastuti (2012), the chemical components of *kecombrang* flower are flavonoids, alkaloids, triterpenoids, glycosides, phenolic and saponin. Phenolic compounds, flavonoids, volatile oil, terpenes, plant organic acids, fatty acids, particular fatty acid esters and plant alkaloids are antimicrobial compounds (Naufalin et al., 2005).

Bioactive compounds will react with the proteins on the wall of microbial cell membranes or in the cytoplasm and cause denaturation. Proteins denaturation can be attributed both to phenolic compounds and also to organic compounds that will cause damage to the cell wall and cell membrane. The damage of cell wall and cell membrane is caused by the weakening of the wall structure and cell membranes that become abnormal and the cell pores are enlarged. It causes the cell wall and cell membrane cannot selectively regulate the exchange of substances from and into and cell and ultimately cell lysis occurs. According to Hadioetomo et al. (2008), the mechanism of inhibition of microbes by antimicrobial compounds can be caused in several ways, such as disturbance in cell wall constituent compounds and increased flexibility which leads to loss of cell membrane components of the cell.



Description:

M0 : microcapsule concentration 0%
M1 : ethanol fraction microcapsule concentration 5%
M2 : ethanol fraction microcapsule concentration 10%
M3 : ethyl acetate fraction microcapsule concentration 5%
M4 : ethyl acetate fraction microcapsule concentration 10%

Figure 1. The average total microbes of the boiled minced beef of various concentrations and types of microcapsules at observations of 0, 4 and 8 hours.

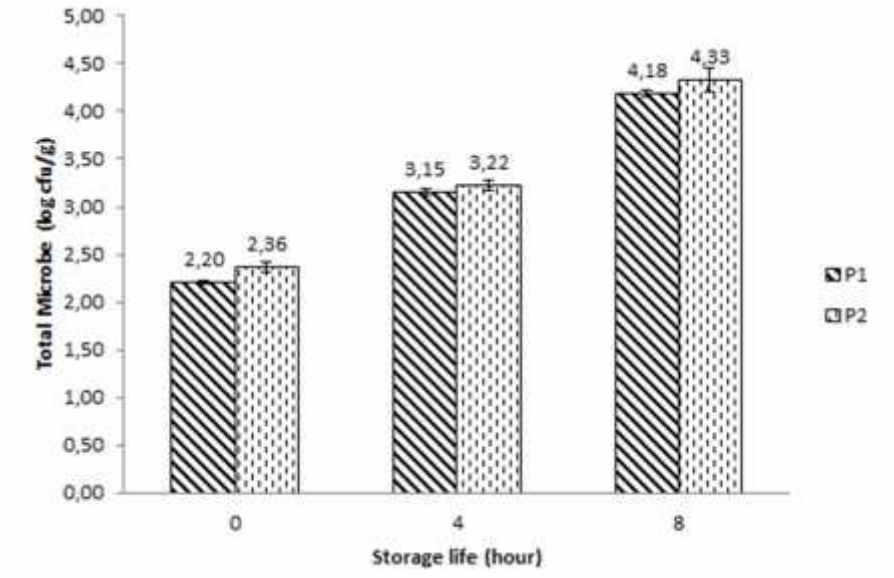


Figure 2. The average value of total microbes on pH condition at 0, 4 and 8 hours under pH 4 (P1) and 7 (P2)

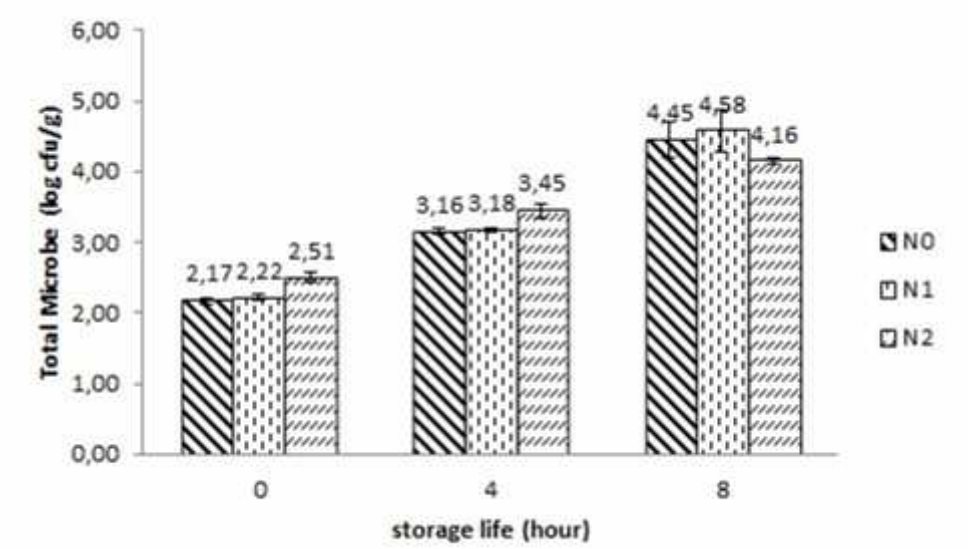


Figure 3. The average of total microbes of boiled minced beef at different NaCl concentrations (N0: 0%; N1: 2.5% and N2: 5%) observed at 0, 4 and 8 hours.

Decreasing pH can inhibit the growth of microbes, this was consistent with the results of Naufalin et al. (2006) research that the effect of pH on the inhibition of ethyl acetate extract of *kecombrang* flowers decreased antimicrobial activity at pH 4 and increased activity of *Bacillus cereus* and *Escherichia coli* at pH 5-7. Medium with pH 4 (acid) has greater microbial inhibition than the medium with pH 7, it is alleged at low pH, microbes will try to maintain a constant pH in the cell so that they require extra energy that will lead interference to the microbes growth. According to Shelef and Seiter (1993), the presence of antibacterial components with hydrochloric acid as halogens causes damage to cell membranes effectively. This is due to Cl^- ion that requires cells to secrete extra energy.

The mechanism of cell inhibition at low pH is due to the cells that try to maintain a constant pH. When the pH is lowered, the high amounts of protons in the medium will enter into the cell cytoplasm (Ray, 2001). It leads to decreased cytoplasmic pH. The decrease of the cytoplasm pH causes the enzymes will work to restore the internal pH of the cells into a normal pH. These protons must be removed to prevent acidification and denaturation of cell components. According to Ekowati et al. (2009), the low pH of the media contains high H^+ ion concentration resulting protonation and enzymes that play a role in growth will binds more with H^+ ions and only a few are bound to the substrate to produce the compounds of cell components. Such conditions may induce the activity of enzymes that play a role in growth is decreasing so that the growth becomes low.

The activity of restoring internal pH into normal requires a lot of energy. When energy required is in high amounts, this will interfere the metabolism, so the cells will die (Roberts and Greenwood, 2003). Kusharyati et al. (2006) adds that the low pH causes an enzyme protonated and loses its negative charge and it ultimately cannot bind to the substrate. This situation will obviously interfere the microbial metabolic pathways and may eventually inhibit growth. According to Naufalin et al. (2006), ethanol and ethyl acetate of *kecombrang* flowers extracts has greatest antibacterial activity at acidic pH (pH 4).

The effectiveness of a natural antimicrobial compounds at low pH on microbial growth inhibition may also be caused by the presence of phenolic components in plant extracts which is more effective at lower pH. The structure of the hydroxyl group of phenolic compounds play

an important role in the antimicrobial activity. At low pH, alkylation and hydroxylation reactions will improve the distribution of phenolic groups in the water and lipid phase of the cell membranes of bacteria (Dorman and Deans , 2005; Puuponen - Pimia , 2001).

NaCl has the ability to inhibit the growth of microbes by inducing plasmolysis or dehydrating microbial cells (Taormina, 2010). According to Jay et al. (2005) that the growth of a number of bacteria were inhibited in the saline concentration of 2 % , but other bacteria, yeast and mold can grow on different concentrations of saline solution. Halophilic bacteria, bacteria that like saline such as Micrococcus and Bacillus type, can grow at a minimum a_w of 0.75 , while the yeast grows well on minimal a_w of 0.88 and mold can grow at a minimum a_w of 0.80 (Soeparno , 2005). Dave and Ghaly (2011) revealed that a slightly halophilic bacteria can grow at 2-5 % saline concentration , halophilic bacteria grow at 5-20 percent saline concentration while the extreme halophilic bacteria can grow on the saline concentration of 20-30 %. Halophilic bacteria, yeast , and mold that may grow on the tested boiled minced beef were causing the effect of saline on preservation to be limited.

According to Kusharyati (2006), the inhibitory effect of NaCl is due to the breakdown of NaCl into ions Na+ and Cl-. The presence of these ions will result in higher environmental fluid concentrations than the inside fluid concentration of the cell, so that the cells undergo plasmolysis. Cl - ions can inhibit microbial growth because it is toxic. The interaction between concentrations of NaCl and antimicrobial agents can exert their influence on the growth of microorganisms. The ability of antibacterial compounds to inhibit the growth of such bacteria is influenced among others by the level of acidity (pH) , temperature, proteins, fats , carbohydrates and water activity (aw) of bacterial growth medium (Nycas and Tassou , 2000) and is influenced by the saline concentration (Brewer, 2000).

Conclusion

Type and microcapsule concentration of *kecombrang* flower are able to inhibit microbial growth on boiled minced beef, at observation of hour 0, 4, and 8. The best treatment was showed on addition of etyl acetate of 10%. The treatment of medium pH of 4 and NaCl of 5% could inhibit microbial growth in boiled minced beef, even in 0, 4 and 8 hour of observations.

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