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Microemulsion Design to Enhance Antibacterial Activity of Cinnamon Essential Oil for Food Preservatives

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Abstract: The objective of this research was to analyze the capability of cinnamon essential oil as antibacterial agent which is further applied as natural food preservative agent in food products. The cinnamon essential oil was obtained by using steam distillation. Furthermore, the water based microemulsion of cinnamon essential oil was developed by using several types of emulsifiers. In this investigation, four different emulsifiers were tested, namely Tween 20, 80, soya lechitin and Carboxymethyl Cellulose (CMC) to stabilize the essential oil in water. The emulsifiers were used at 2% w/w while the essential oil concentrations were varied at 0.5, 1 and 1.5%. Moreover, the microemulsion produced here was tested for particle size, storage stability, antibacterial activity and sensory evaluation once it is incorporated as preservative for Indonesian beef meat balls. The droplet sizes of the cinnamon essential oil microemulsion were found to range from 30-100 nm and after one week of storage at room temperature, the microemulsion solution did not undergo changes in droplet size which showed the emulsion stability during storage. The antibacterial activity of cinnamon essential oil showed satisfactory results toward food borne bacteria, namely *Staphylococcus aureus* and *Escherichia coli*. The results were measured from the zone of inhibition diameter formed by the microemulsion solution. Among the four types of emulsifier, CMC (Carboxy-Methyl-Cellulose) showed the best results as an emulsifying agent. Furthermore, in this study the micro emulsion was applied as a food preservative agent for meatball, known locally as the beef bakso. CMC and cinnamon essential oil (0.5, 1 and 1.5%) showed satisfactory results in delaying the spoilage of the meatballs. Among the four types of emulsifier, CMC showed the best emulsifying agent in terms of antibacterial activity. After the microemulsion solution was applied to meatballs. CMC and cinnamon essential oil (0.5, 1 and 1.5%) showed satisfactory results in delaying the spoilage of the meatballs. From the sensory evaluation, the overall acceptance from meatball with addition of CMC and essential oil were higher compared to meatball without treatment.

Key words: Microemulsion, antibacterial, cinnamon, essential oil, food preservatives, meatball

INTRODUCTION

In the food preservation process, the extension of shelf life of food has become an obligation or requirement. Throughout the food preservation process, the microorganisms which damage the food is prevented from growing. There are a number of food preservation techniques that can be employed in order to decrease the risk of outbreak of food poisoning for example, through heating, refrigeration and the addition of antimicrobial compounds (Iraj, 2007). Furthermore, food industry employ chemical food preservative agent to decrease microbial spoiling. However, consumers have a variety of health concerns regarding the safety of chemical based food preservatives which encourage the usage of natural green alternatives to extend product shelf-life (Djenane *et al*., 2013; Ebrahimi and Darani, 2013; Santas *et al*., 2010). Therefore, there has been a constant increase in the exploration of alternative and more efficient compounds to be utilized in food preservation, aiming at total or partial replacement of chemical additives.

Although, spices and aromatic herbs are usually used to improve food tastes, these materials can also be used as food preservative agents due to their antimicrobial activity. Among natural antimicrobials, Essential Oils (EOs) have been widely studied due to their antimicrobial (anti-fungal, antibacterial), antioxidants, antimutagenic and anticarcinogenic properties (Iraj, 2007; Djenane *et al*.,

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2013; Ebrahimi and Darani, 2013; Santas *et al*., 2010). Now a days the society appears to be experiencing a trend of "green consumerism" which desires fewer synthetic food additives and product with smaller impact on the environment and health. Hence, antibacterial compounds based on essential oil have shown some renewed interests, both from researchers as well as the industries particularly true in a tropical country rich in herbs such as Indonesia.

The chief significance of this study was therefore to enhance the antimicrobial activity of a formulated microemulsion design based on cinnamon oil as an alternative to chemical preservatives, so as to minimize their side effects and simultaneously improving the shelf life of the food products. Cinnamon is a natural preservative and flavoring substances that are not harmful when consumed in food products. In this study, cinnamon Eos was combined with the selected type of emulsifier to create a water based system which will later, be applied as a food preservative agent. In this research meatballs was selected as the product. In Indonesia there have been some serious problem encountered by law enforcement agency in which home industries were using ilegal and dangerous type of chemical food preservatives such as formalin or borax.

MATERIALS AND METHODS

The main materials used in this experiment were cinnamon essential oil derived from the barks of *Cinnamomum burmanii* and was obtained by using steam distillation method. Food borne bacteria, namely *Staphylococcus aureus* and *Escherichia coli* and four types of emulsifier (Soya Lecithin, CMC, Tween 80 and 20) were used in the antibacterial tests and in the formulation of stable microemulsion that would remain active as antibacterial system. It is noted during the preliminary studies that different types of emulsifiers would affect the antibacterial strength differently.

Formulation of microemulsion solution: Emulsifier agents would prevent the oil droplets or fat from coalescing and separating from the opposing liquid. Schubert *et al*. (2006) presumed that emulsions with sufficiently small droplets which are prevented from aggregating or coalesing by the use of suitable emulsifiers, exhibit great physical stability. Thus in this research, 4 type of different emulsifiers were used with the intention to find out the best type of emulsifiers (Table 1).

Table 1: Formulation of emulsifier and essential oil

Formulation (150 mL)		
Microemulsion solution	Volume of cinnamon essential oil (mL)	Volume of water with 2% (w/v) emulsifier (mL)
Blank / Control	0	150
Essential Oil 0.5%	0.75	149.25
Essential Oil 1%	1.5	148.5
Essential Oil 1.5%	2.25	147.75

Liquid phase antibacterial properties of microemulsion design: Using disc diffusion method, liquid phase antibacterial properties were performed. In this research, the type of media culture that was used was Mueller-Hinton agar. In each of petri dish, 4 paper discs were applied on the top of the Mueller-Hinton agar. The 3 paper discs containing the extract from the same type of emulsifier but with different concentration and 1 paper disc containing Dimethylsulfoxide (DMSO) as a negative control were used. Subsequently, each of the plate was observed antimicrobial susceptibility testing (Li *et al*., 2014), after 16-18 h of incubation. If the plate was acceptably streaked and the inoculums were correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. Furthermore, the diameters of the zones of complete inhibition are measured, including the diameter of the disc. Zones of inhibition were measured to the nearest whole millimeter, using sliding calipers which was held on the back of the inverted petri plate.

Application to meatball making: There were 3 types of meatball that was made those are meatball with 0.5% cinnamon essential oil, meatball with 1% cinnamon essential oil and meatball with 1.5% cinnamon essential oil. Firstly, 200 g of fresh beef meat and 20 g of ice cube were blended until the meat became smooth and the color of the meat become pink. Then, 500 μL emulsifier that contains 0.5% cinnamon essential oil was put into the egg white. The egg white and the meat were blended for about 5 min until the meat became a little bit soft. The dough will have soft pink color. Salt, pepper, garlic and 1 tablespoon of corn flour were added into the dough. The dough was mixed up until it became a little bit sticky and not too soft. The dough was made into meatball. Moreover, 500 mL of water was boiled and then the meatball dough was put into the boiling water. After the meatball floated, it could be taken. The floating meatball indicated that the meatball has completely well done cooked. The whole procedure was repeated to make meatball with 1% cinnamon essential oil and meatball with 1.5% cinnamon essential oil.

RESULTS AND DISCUSSION

Preliminary research: The intention of preliminary research was to discover the best method for enhancing antibacterial activity of cinnamon essential oil by using micro emulsion design. The research consists of determining the formulation of micro emulsion solution, learning the method of aseptic transfer, moving inoculants to nutrient broth and discovering the technique for antibacterial susceptibility testing. Prior to the main research, a stable micro emulsion solution must be created. It could be said that an emulsion is a stable mixture of two liquids that do not normally mix such as oil and water. Generally, in order to form a stable mixture of two repelling liquids an emulsifier agent is needed. It is well known that the addition of emulsifier agents and the usage of high shear mixer or a homogenizer will help to make the emulsion steady.

Emulsifier agents will prevent the oil droplets or fat from coalescing and separating from the opposing liquid. Schubert *et al.* (2006) presume that emulsions with sufficiently small droplets which are prevented from aggregating or coalescing by the use of suitable emulsifiers, exhibit great physical stability. Thus in this research, 4 types of different emulsifiers were used with the intention to find out the best type of emulsifiers, so that, the micro emulsion design will exhibit great physical stability, yet still possessed good antibacterial activity. Each type of emulsifiers have different chemical properties, the biggest difference were shown in the value of viscosity. The carboxymethyl cellulose has the highest viscosity among the entire emulsifiers. Prior to the main research, it was expected that Carboxymethyl Cellulose (CMC) would produce the best result. From the literature, it was known that CMC is used primarily as food additives because it has high viscosity is non-toxic and is non-allergenic and could improve texture of the food.

The preliminary research led to final decisions on methods to be performed in this research. The initial antibacterial susceptibility tests led to a few conclusions as follows: *Escherichia coli* were chosen as the representative of Gram-negative bacteria and *Staphylococcus aureus* were chosen as the representative of Gram-positive bacteria. Both ba Dimethylsulfoxide cteria were representative of food-borne bacteria, (DMSO) was used as a negative control and it has no antibacterial activities compared to the mixture of emulsifier and cinnamon essential oil, the emulsifiers have no antibacterial activities compared to the essential oil, 6 mm paper disc could only fully absorb 10 μL solution, 5) direct dropping of micro emulsion solution on the top of agar media on the Petri dish would produce unclear ZID (Zone of Inhibition Diameter), therefore paper disc must be applied, 6) Incubation for 48 h did not create any changes for ZID, 7) Incubation of blank (Mueller-Hinton agar) did not promote any bacterial growth.

Table 2: Comparison of *Cinnamomum burmanii* essential oil chemichal compounds

Compound found in GCMS Spectra in this research	Antibacterial compound of *Cinnamomum burmanii* as found by Li *et al.* (2014), Raesi *et al.* (2015), Peng *et al.* (2012) and Imelouane *et al.* (2009)
β-linalool	-
α-Terpineol	√
Trans-Cinnamaldehyde	√
Eugenol	√
Copaene	-
β-Caryophyllene	-
Caryophyllene oxide	√
Camphor	√
Borneol	√

Emulsifying agents perform two duties. They coat the oil droplets with like charges and lessen the surface tension of the water. This reduces the waters ability to repel oil and reduces the oils ability to recombine to form larger oil droplets. In contrast to a common emulsion where the droplets slowly coalesce and the phases ultimately separate, the oil droplets in a microemulsion are in a highly dynamic state, yet phase separation never occured.

The content in cinnamon essential oil was investigated using GCMS (Gas Chromatography Mass Spectroscopy) technique. The main components of the essential oils found here were summarized in the Table 2 and it was compared with the compounds found in the literature.

From the GCMS result, it was found that the main compound of the cinnamon essential oil was Cinnamaldehyde (66.09%) and Eugenol (1.77%). Both of the compounds possessed antibacterial activities against Gram-positive and Gram-negative bacteria (Gupta *et al.*, 2008).

Primary research: In liquid phase antibacterial susceptibility test, initially the number of desired initial blank bacteria was planned to be approximately 300 bacteria colonies. Some of the experiments using 100 and 200 colonies were also done for comparison. For liquid phase antibacterial susceptibility test, disc diffusion method was chosen, since, it is the most practical method and this method will produce the most satisfactory result. From all of the media culture available, the Mueller-Hinton agar was considered as the best routine susceptibility testing for non-fastidious bacteria.

In each of petri dish, 4 paper discs were applied on the top of the Mueller-Hinton agar. The 3 paper discs containing the extract with the same type of emulsifier but

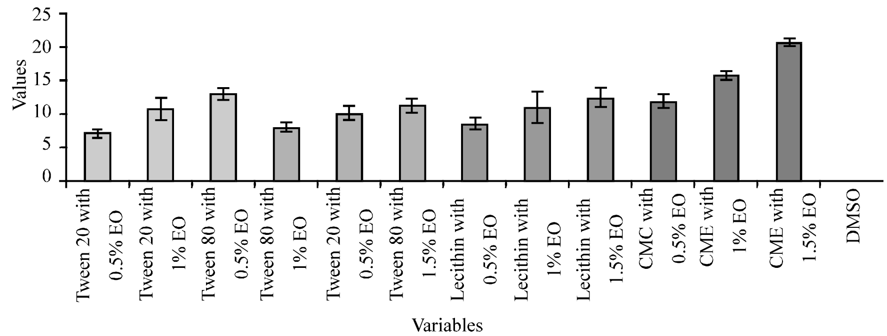

Fig. 1: Result of liquid phase antibacterial for *Staphylococcus aureus* in which CMC yielded the best antimicrobial results

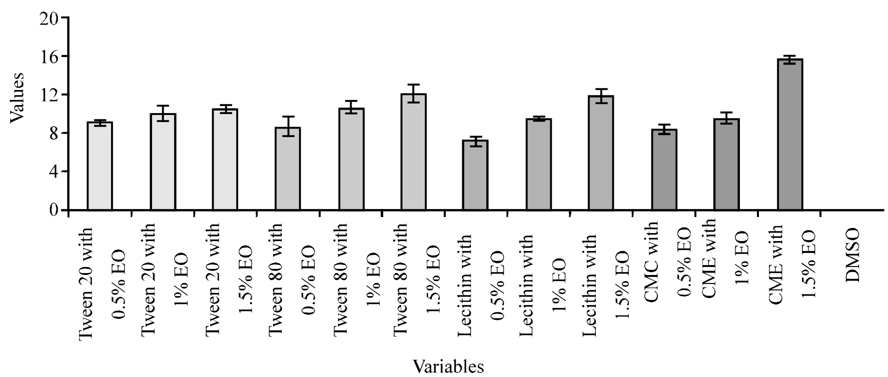

Fig. 2: Result of liquid phase antibacterial for *Escherichia coli* in which CMC yielded the best antimicrobial results

with different concentration and 1 paper disc containing Dimethylsulfoxide (DMSO) as a negative control were applied. The experiments were repeated to obtain 4 data needed for statistical analysis. In accordance with the performance standards for antimicrobial susceptibility testing (CLSI., 2014), either the discs were placed individually or with a dispensing apparatus, the paper disc must be distributed evenly so that they were no closer than 24 mm from center to center. Generally, one 150 mm plate should contain no more than 12 discs or on a 100 mm plate no more than 5 paper discs.

Subsequently, based on literature by performance standards for antimicrobial susceptibility testing (CLSI., 2014), after 16-18 h of incubation, each of the plate was observed. If the plate was acceptably streaked and the inoculums were correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies are obvious, the inoculums were too light and the test must be repeated. Furthermore, the diameters of the zones of complete inhibition were measured, including the diameter of the disc. Zones were measured to the nearest whole millimeter, using sliding calipers which was held on the back of the inverted petri plate. Then the data obtained was statistically analyzed. Just as expected from the preliminary research, the cinnamon essential oil possesses antibacterial activity. In this research, the antibacterial properties of cinnamon essential oil started at the concentration of 0.5% essential oil in 150 mL emulsifier solution.

From the Fig. 1 and 2, it could be seen that the cinnamon essential oil was more effective in inhibiting the growth of *S. aureus* rather than *E. coli*, since, Gram-negative bacteria has a double cell wall (Chatterjee and Chaudhuri, 2012). The double cell wall prevented the ease of diffusion of hydrophobic compounds such as cinnamon essential oils through lipopolysaccharide layer. The hydrophobicity of essential oil is responsible for the disruption of bacterial structures that can increase permeability due to an inability to separate the essential oils from the bacterial cell membrane. The cell membranes have permeability barrier that is indispensable to many cellular functions, including maintaining the energy status of the cell, membrane-coupled energy-transducing processes, solute transport and metabolic regulation (Nazzaro *et al*., 2013).

Application of micro emulsion solution as food preservatives: From the previous experiment, it was noted that Carboxymethyl Cellulose (CMC) combined with

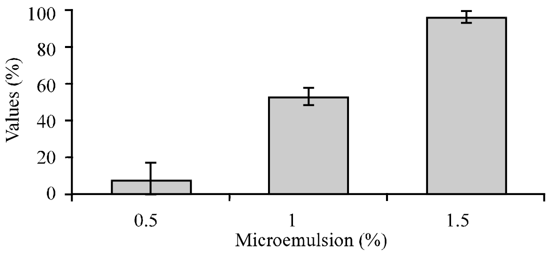

Fig. 3: Effect of cinnamon essential oil and CMC toward reduction of food-borne bacteria in meatball

essential oil possess the strongest antibacterial activity toward the food-borne bacteria in this case *S. aureus* and *E. coli*. Other reasons why CMC was selected as the best type of emulsifier in this research was that from the literature, it was known that CMC has been used as food additives because it has high viscosity is non-toxic and is non-allergenic. Furthermore, CMC could improve texture of food, so, it was suitable to be used as food additives since the emulsion was applied to Indonesian beef meatball where enhancement of texture could be achieved by the addition of CMC. There were 4 types of meatball that were made, blank meatball, meatball with 0.5% essential oil in CMC, meatball with 1% essential oil in CMC and meatball with 1.5% essential oil in CMC, respectively. The following graph in the Fig. 3 showed the antibacterial activity of cinnamon essential oil with Carboxymethyl Cellulose (CMC) in meatball.

From the graph above, it is shown that the percentage of food borne bacteria found in the meatball decreased along with the increasing amount of essential oil in CMC. The highest percentage reduction value of bacteria occurs in CMC with 1.5% essential oil. The percentage reduction was 96.04%. This was the highest percentage reduction value of bacteria compared with the CMC with 0.5% essential oil and CMC with 1% essential oil.

Sensory analysis: Sensory analysis test is a test to verify specific differences among samples and describe briefly each attribute of the sample which will describe the acceptability of the product to future consumers. There were 4 attributes that were tested in this research, namely Foreign taste, Foreign odor, texture and overall acceptance. The meatball with CMC and cinnamon essential oil had higher value for foreign taste compared to the untreated meatball sample. The flavors and smells that the panelists experienced were a result of chemical interacted with their taste receptors. Cinnamon consisted of one component that responsible for this characteristic flavor and odor, namely cinnamaldehyde. When the meatball was mixed with cinnamon essential oil, the cinnamaldehyde remained in the meatball dough causing

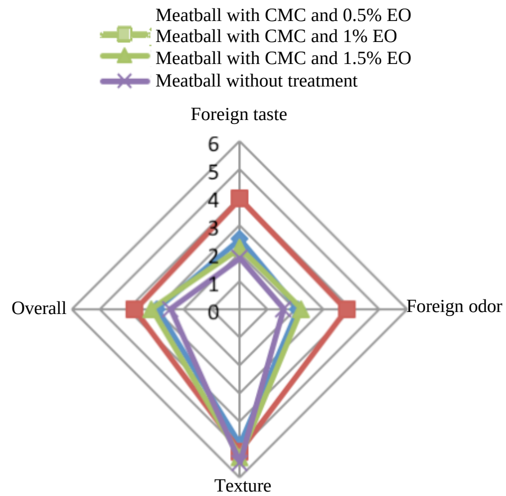

Fig. 4: Qualitative descriptive analysis result

the meatball to carry the cinnamaldehyde flavor. Nevertheless, changing the method of cooking could reduce the foreign taste of cinnamaldehyde. In this experiment, the meatball was only boiled, however when the meatball was cooked with spices or even fried, the cinnamaldehyde would have less impact on the flavor and odor of the meatball. Therefore, the addition of cinnamon as preservative agent in meatball would not significantly obstruct the original taste and aroma of meatball (Fig. 4).

In this research, the cinnamon essential oil acted as the preservatives agent, since, it could decrease the number of bacteria found in the meatball. Meanwhile, the Carboxymethyl Cellulose (CMC) has a tendency to act as an emulsifier agent. Although, the ANOVA results showed that there was no significant difference in texture attributes among meatball with CMC and 0.5% essential oil, meatball with CMC and 1% essential oil, meatball with CMC and 1.5% essential oil, meatball without treatment, the average value of texture from meatball with CMC was not differ much than the meatball without treatment which means that the meatball added with CMC and essential oil possessed similar texture compared to meatball without treatment. The addition of thickening agents will increase the consistency of meatball because the moisture did not vaporize easily in this case when CMC was added into the meatball dough.

CONCLUSION

Cinnamon *(Cinnamomum burmanii)* bark extract is known to have antibacterial properties. Based on the GCMS data, the essential oil of Cinnamon *(Cinnamomum burmanii)* bark extract used in this study was found to contain cinnamaldehyde and eugenol which are known to possess strong antibacterial activities. In this research Cinnamon essential oil was shown to have higher activity in inhibiting *Staphylococcus aureus* (Gram-positive, endospore-forming bacteria) compared to *Escherichia coli* (Gram-negative bacteria). It is noted that the microemulsion was active as antibacterial agents against both types of microbes studied here.

In this investigation, four different emulsifiers were tested to stabilize the essential oil in water, namely Tween 20, 80, soya lechitin and Carboxymethyl Cellulose (CMC). The emulsifiers were used at 2% w/w while the essential oil concentrations were varied at 0.5, 1 and 1.5%. Among the four types of emulsifier, CMC showed the best results in terms of its antibacterial activities.

Furthermore, in this study the micro emulsion was also applied as a food preservative agent for Indonesian meatball. CMC and cinnamon essential oil (0.5, 1 and 1.5%) showed satisfactory results in delaying the spoilage of the meatballs. Moreover, based on sensory analysis, meatballs that were added with CMC and cinnamon essential oil have better overall acceptance and better resistance against microbes compared to meatball without treatment.

REFERENCES

CLSI., 2014. Performance Standards for Antimicrobial Susceptibility Testing. 22nd Edn., Clinical and Laboratory Standards Institute, USA., ISBN: 9781562388973, Pages: 226.

Chatterjee, S.N. and K. Chaudhuri, 2012. Outer Membrane Vesicles of Bacteria. Springer, Berlin, Germany, ISBN:978-3-642-30525-2, Pages: 161.

Djenane, D., J. Yanguela, P. Roncales and M. Aider, 2013. Use of essential oils as natural food preservatives: Effect on the growth of salmonella enteritidis in liquid whole eggs stored under abuse refrigerated conditions. J. Food Res., 2: 65-78.

Ebrahimi, M. and K.K. Darani, 2013. Essential Oils as Natural Food Preservatives: Antimicrobial and Antioxidant Applications. In: Antimicrobials from Nature: Effective Control Agents for Drug Resistant Pathogens, Doughari, J.H. (Ed.). Transworld Research Network Publisher, Kerala, India, ISBN:978-81-7895-603-9, pp:15-37.

Gupta, C., A.P. Garg, R.C. Uniyal and A. Kumari, 2008. Comparative analysis of the antimicrobial activity of cinnamon oil and cinnamon extract on some food-borne microbes. Afr. J. Microbiol. Res., 2: 247-251.

Imelouane, B., H. Amhamdi, J.P. Wathelet, M. Ankit and K. Khedid *et al.*, 2009. Chemical composition and antimicrobial activity of essential oil of thyme (*Thymus vulgaris*) from Eastern Morocco. Intl. J. Agric. Biol., 11: 205-208.

Iraj, R., 2007. Food preservation: A biopreservative approach. Food, 1: 111-136.

Li, L., C. Shi, Z. Yin, R. Jia and L. Peng *et al.*, 2014. Antibacterial activity of á-terpineol may induce morphostructural alterations in *Escherichia coli*. Braz. J. Microbiol., 45: 1409-1413.

Nazzaro, F., F. Fratianni, L. de Martino, R. Coppola and V. de Feo, 2013. Effect of essential oils on pathogenic bacteria. Pharmaceuticals, 6: 1451-1474.

Peng, W.L., S. Zhong, Y. Yan and M. Jiang, 2012. Study on the antimicrobial activity of essential oils from *Cinnamomum camphora* wood. Proceedings of the IEEE International Conference on Biomedical Engineering and Biotechnology (iCBEB), May 28-30, 2012, IEEE, Macao, China, ISBN:978-1-4577-1987-5, pp: 1742-1744.

Raesi, M., H. Tajik, A. Yarahmadi and S. Sanginabadi, 2015. Antimicrobial effect of cinnamon essential oil against *Escherichia coli* and *Staphylococcus aureus*. Health Scope, 4: e21808-e21812.

Santas, J., M.P. Almajano and R. Carbo, 2010. Antimicrobial and antioxidant activity of crude onion (*Allium cepa* L.) extracts. Int. J. Food Sci. Technol., 45: 403-409.

Schubert, H., R. Engel and L. Kempa, 2006. Principles of structured food emulsions: Novel formulations and trends. Proceedings of the 13th World Conference on Food Science and Technology, September 17-21, 2006, IUFoST publisher, Torontoý, Canada, pp: 1343-1343.