3rd International Student Conference on Food Science and Technology

Greening The Food Industry:
Inovation for Sustainability

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The Committees of 3rd International Student Conference
On Food Science and Technology

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Greening The Food Industry: Inovation for Sustainability

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PREFACE

Food Production and processing, starting from the crop production untill the food is ready to be consumed, requires a lot of energy and resources. Green technology in food production and processing is one of the possible solution for the upcoming problems. Green technology is intented to minimize the effect of human activity, i.e. food production and processing on the environment. Producers answer the consumers demand by identifying and using greener technologies for food productions and processing. Some producers have started usage of organic inputs in the processing, usage of recyclable and environmentally-friendly packaging, and usage of minimal-energy processing.

The Faculty of Agricultural Technology, Soegijapranata Catholic University, organized this 3rd International Conference on Food Science and Technology "Greening the Food Industry: Innovation for Sustainability". This conference is purposed to be a media for exchanging knowledge and perspective between students, researchers and practitioners, especially in the area of green technology in food production and processing in order to enhance food sustainability worldwide

This publication includes papers writen by oral presenters and poster presenters which categorized in three parallels session, i.e. Food Product Development (FPD), Food Safety and Ecotoxicology (FSE), and Food Nutrition and Culinary Technology (FNCT).

The organizing committee is grateful to all honorable speakers, participants and sponsors, for joining this gathering and for their valuable contribution on the conferences.

Semarang, October 2014

Dr. Victoria Kristina Ananingsih, ST, MSc
Dean
Faculty of Agricultural Technology
Soegijapranata Catholic University

RULES OF CONFERENCE

1. Plenary Session

a. Every speaker will present his/her paper for maximum 20 minutes.
b. All participants are prohibited to use any electronic device that produces loud sound during speakers presentation.
c. After all presentations, there will be a discussion session
d. The audience should state their personal identity before giving question, suggestions, etc.

2. Parallel Session

a. The parallel session will be conducted by the chairman of that session (moderator).
b. Speakers should give a short note with their name, institution, and curriculum vitae before their presentation.
c. Duration of presentation is maximum 15 minutes and followed by 5 minutes disccussion.
d. The audience should state their personal identity before giving question, suggestions, etc.

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SUSTAINABLE FOOD PACKAGING: POSSIBILITY OR PIPEDREAM?

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University of Queensland &Food•Packaging•Environment
Brisbane, Australia

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ABSTRACT

Sustainability is generally considered to have three dimensions or pillars: economic, environmental and social. Sustainable packaging has been defined by the U.S. Sustainable Packaging Coalition as packaging that is sourced, manufactured, transported, and recycled using renewable energy; optimizes the use of renewable or recycled source materials; is manufactured using clean production technologies and best practices; is made from materials healthy throughout the life cycle; is physically designed to optimize materials and energy; and is effectively recovered and utilized in biological and/or industrial closed loop cycles.Rather than trying to define sustainable packaging, U.S.-based Wal-Mart Stores, the world's largest retailer, is promoting itself as a leader in sustainable packaging through initiatives such as their packaging scorecard, a measurement tool that allows suppliers to evaluate themselves relative to other suppliers, based on specific metrics.The European Organisation for Packaging and the Environment (Europen) believes it makes much more sense to talk about packaging and sustainability rather than sustainable packaging which in their view cannot be an end in itself.Trying to reconcile "sustainable packaging" and "packaging and sustainability" is difficult if not impossible. How can the food industry expect the public to understand what they are doing to become more sustainable when the language and metrics are so confusing? Reaching a broad consensus on what may constitute sustainable packaging would provide the packaging industry with a platform from which to influence regulation as well as customer and consumer attitudes and expectations. This presentation will discuss innovations in sustainable packaging and suggest reasons why it may be some time before sustainable packaging becomes widely available.

1.SUSTAINABLE DEVELOPMENT

The most widely-accepted definition of Sustainable Development is the one that appeared in the report of the World Commission on Environment and Development (also known as the Brundtland Commission) in 1987 entitled *Our Common Future*:

"Humanity has the ability to make development sustainable – to ensure that it meets the needs of the present without compromising the ability of future generations to meet their own needs."

In other words, sustainable development is the level of human consumption and activity which can continue into the foreseeable future, so that the systems which provide goods and services to humans persist indefinitely. The practical implications of this definition are diverse, ranging from the consumption of resources with respect to their rate of renewal, the efficiency of resource use, and the equity of their use across societies and generations, with different emphases according to discipline and political ideology.

Contained within the Brundtland definition are two key concepts: that of needs, and in particular the essential needs of the world's poor to which overriding priority should be given, and the idea of limitations imposed by the state of technology and social organisation on the environment's ability to meet present and future needs. But the Brundtland report also contained a further statement that is often overlooked:

"Sustainable development is not a new name for environmental protection: it is a new concept of economic growth"

The London-based International Institute for Environment and Development (IIED: www.iied.org) defined sustainable development as an activity that can be maintained indefinitely because it is:

- socially desirable
- economically viable, and
- ecologically sustainable

These three parameters are sometimes referred to as People, Profits and Planet.

2.SUSTAINABILITY

The word sustainable means *"to maintain or keep going continuously"* and has been used in connection with forest management for over a century. The US Environmental Protection Agency (www.epa.gov/sustainability) defines sustainability as:

"The ability to achieve continuing economic prosperity while protecting the natural systems of the planet and providing a high quality life for its people"

They state that this requires balancing the three E's: Economy, Environment and (social) Equity. Thus sustainability

can be thought of as consisting of three pillars: Economy, Environment and Society.

The implications of the above definitions for sustainable packaging are that:

- Consumption of resources must match their rate of renewal
- Use of non-renewable resources such as petroleum-based plastics (and metals) is unsustainable
- The focus must be on renewable biobased materials
- Resources must be used efficiently
- There must be equity of resource use across societies and generations

A major problem is that the public generally tends to have a poor understanding of energy (especially embodied energy) and environmental impacts as the following example illustrates:

1 U.S. gallon (3.79 L) of gasoline comprises about 131.7 MJ of energy. A LCA of plastic water bottles showed that it requires about 92.6 MJ of energy to make the plastic, transport and convert it into one hundred 10 g PET water bottles. Thus, the energy equivalent of 1 gallon of gasoline could potentially create about 142 water bottles. If a car travels 30 miles per gallon, that is the equivalent of about 4.7 bottles per mile. When we drive our cars, the exhaust from the burned-up fuel "disappears" out of the tailpipe, and we never see it. 10 gallons of gasoline produces about 88 kg of CO_2e, the equivalent footprint of 1,940 water bottles. If we had to dispose of, or recycle, 88 kg of material every time we burned 10 gallons of gasoline, many people would think very differently about driving. In theory, the plastic bottles can be recycled to reduce material needed for the next generation of bottles. The burned-up gasoline is lost forever.
(from Amcor Rigid Plastics blog at http://amcorforwardaction.com/blog/).

For a packaged food as a generalisation, 90% of the life cycle impacts are caused by the food and 10% by the package. Of the 10% packaging impacts, 10% arise during the disposal/recycling/incineration stage. Despite this, the public (and many governments) focus their attention on packaging disposal which accounts for only 1% of total life cycle impacts of packaged food. Fortunately, increasing attention is now being given to food waste which is a much more important issue than packaging waste. In June 2014 FAO released a report by the High Level Panel of Experts on Food Security and Nutrition of the Committee on World Food Security entitled *Food losses and waste in the context of sustainable food systems* (www.fao.org). We must always remember

that packaging prevents waste and that the developing world needs more and better packaging – not less.

Of course, mere improvement in resource productivity and eco-efficiency of processes won't fully address environmental impacts from increased consumption as the world's population rises. We must move away from a material-intensive consumer culture towards sustainable consumption. However, no company has yet addressed the thorny issue of sustainable consumption – and how might they? But sustainable consumption must be part of any sustainable business model.

3.SUSTAINABLE PACKAGING

Although sustainable packaging is widely discussed at conferences and in the packaging media, many in the packaging industry are confused as to what it actually means. Consumers are also very confused and the possibility exists for unscrupulous companies to market packages as "sustainable" when they are not and thus mislead consumers.

Sustainable packaging has been defined by the U.S. Sustainable Packaging Coalition (SPC: www.sustainablepackaging.org/) as packaging that is sourced, manufactured, transported, and recycled using renewable energy; optimises the use of renewable or recycled source materials; is manufactured using clean production technologies and best practices; is made from materials healthy throughout the life cycle; is physically designed to optimise materials and energy; and is effectively recovered and utilised in biological and/or industrial closed loop cycles. Today there are no packages on the market that would qualify as sustainable under the SPC definition.

However, a single definition of sustainable packaging is unfeasible, as the sustainability of a packaging material intrinsically depends on aspects specific to its life cycle such as its manufacturing process, the length of its supply chain, its use and finally its disposal options. Some would even argue that there is no such thing as "sustainable packaging" but rather there are improvements that can be made to the packaging's attributes and its manufacturing process in order to reduce its life cycle impacts. But what about the social dimension of sustainability?

The European Organisation for Packaging and the Environment (Europen: www.europen-packaging.eu/) believes it makes much more sense to talk about packaging and sustainability rather than sustainable packaging which in their view cannot be an end in itself.

Trying to reconcile "sustainable packaging" and "packaging and sustainability" is difficult if not impossible. How can the food industry expect the public to understand what

they are doing to become more sustainable when the language and metrics are so confusing? Reaching a broad consensus on what may constitute sustainable packaging would provide the packaging industry with a platform from which to influence regulation as well as customer and consumer attitudes and expectations.

In 2012, PricewaterhouseCoopers (PwC) - a leading professional services firm - published a report in the UK entitled "*Sustainable packaging: myth or reality*?" They concluded that sustainable packaging as a term is no longer relevant today as it is too broad a term to be useful at a practical level. No one can come up with a single meaningful definition of sustainable packaging. Rather, in their view, sustainable packaging has been substituted with a more balanced view of efficient packaging, characterised by the use of minimum resources; minimising product waste; transport and display efficiency; and effective after-use disposal and recycling.

4.BIOBASED PACKAGING MATERIALS

These are defined as materials derived from primarily annually renewable sources – and they are not necessarily biodegradable. This definition excludes paper-based materials since trees generally have renewal time of 25-65 years (but paper is obviously biobased). The main driving force is a desire for renewable resources and the current interest in sustainability is driving development of biobased packaging materials.

Although Nature produces 170 billion metric tonnes per year of biomass by photosynthesis, only 3-4% of these compounds are used by humans for food and non-food purposes. Biomass carbohydrates are the most abundant renewable resources available (75% of this biomass) and are currently viewed as a feedstock for the green chemistry of the future (including plastics).

The public believes that biodegradable packaging will solve the solid waste problem plus the litter problem. To many consumers biodegradation appears 'natural' – it is what Nature does so it must be good! Petroleum-based plastics when incinerated (or biodegrade in some cases) release CO_2 that was fixed millions of years ago. Biodegradable plastics when they degrade release 'new' CO_2 and are classed as 'carbon neutral' (Narayan, 2012). Biobased, biodegradable plastics have been classed as 'sustainable packaging' by some people and organisations.

To realize the benefits of biodegradable plastics, municipal composting facilities must be available. However, few cities have such facilities or the capacity to collect green waste separately. This is a major drawback to the expansion of biodegradable

plastics. The biggest benefit of composting is avoidance of methane production in landfills. It is worth noting in passing that metal, glass and most petroleum-based plastics do not biodegrade!

It can be confusing when people talk about bioplastics. This is because bioplastics can be biobased or biodegradable or both. Biobased plastics are derived from biobased materials and may (or may not) be biodegradable. Biodegradability does not depend on the origin of the raw materials - only on their chemical composition.

Biodegradable polymers can be made either from renewable biobased or petroleum-based raw materials.

Biobased packaging materials can be classified as shown below:

Biobased (Renewable)

	BioPET	3	TPS	1	
	BioPE		PLA		
Not biodegradable	PEF		PHA		Biodegradable
	LDPE/HDPE 4		PCL	2	
	PP & PA		PBT		
	PET & PS		PBS		
	PVC		PGA		

Petroleum-based (Non-renewable)

1. Biobased and Biodegradable

Thermoplastic starch (TPS) is typically 70% starch plus plasticisers (glycerine and polyols), fillers and PCL or PVOH (both petroleum-based). TPS can be extruded into sheet for thermoforming into trays; it can also be expanded into foams for cushion packaging. To improve barrier and mechanical properties, nanoclays can be added.

TPS products normally retain the hydrophilic characteristics of starch and readily degrade in home composters. They are suitable for low moisture products such as confectionary and biscuit trays, and short service life foams for transport packaging. However, TPS is not really a viable alternative to most petroleum-based plastics. It has also been blamed (unfairly) for the rise in food prices – bioethanol is real culprit.

The Melbourne-based manufacturer Plantic sells ecoPlastic™ High Barrier film that provides exceptional gas barrier and excellent sealing with existing petroleum-based lidding films. These films are suitable for packaging fresh produce including meat, chicken, fish and seafood, small goods, fresh pasta and cheese. Although they have 89% renewable biobased content (LDPE and PP skins) they are not compostable or recyclable. Their cost is comparable to conventional films

Polylactic Acid (PLA) is manufactured by NatureWorks LLC at their 140,000 t/yr plant

in Nebraska, USA using GM corn. Purac, a Dutch lactide producer, uses sugar beets in Europe and commissioned a 75,000 t/yr plant in Thailand in 2012 using sugar cane and tapioca to produce lactic acid. There are also manufacturers in China and Japan.

The major application of PLA in food packaging is as a rigid bottle or tub. PLA also finds some use in food packaging as a film. The biggest problem is its high WVTR as discussed by Cairncross *et al*., (2005). It can also contaminate the PET recycling stream and will not decompose in home composters as it requires higher temperatures that are only found in industrial composters (Kolstad *et al*., 2012).

In June 2009, Fritolay launched a 33% PLA bag for their SunChips in the USA. A 100% PLA bag was launched in March 2010 but removed in October 2010 due to complaints about noise! A Facebook group called *"Sorry But I Can't Hear You Over This SunChips Bag"* gathered more than 44,000 fans. They relaunched a 100% PLA bag for plain chips in February 2011 (90% by weight biobased). It was 6 layers and 20 µm thick and consisted of PLA-print-adhesive-mPLA-PLA-sealant. It employed a different adhesive to bind the inner and outer layers and this reduced the noise considerably. In 2013 they reverted to their original petroleum-based PET bag.

Although not a biobased plastic material as it will not melt under heat and pressure, regenerated cellulose film (in effect transparent paper) has been used for packaging since the 1920s but is always coated with petroleum-based polymers to improve its barrier and sealing properties. In March 2011 Boulder Canyon Natural Foods launched a compostable package for its chips consisting of a metallised RCF film with a coating of PVdC copolymer (Saran). It is certified by the Biodegradable Products Institute to meet ASTM 6400 standards for compostability.

Poly(hydroxyalkanoates) or PHAs are microbial polyesters such as poly(hydroxybutyrate) (PHB). They are produced by many bacterial species as intracellular particles that function as energy and carbon reserves. The bacteria *Cupriavidus necator* ferments sugars to random copolymers of HB & HV (hydroxybutyrate and hydroxyvalerate). PHAs have high performance properties including excellent strength and toughness, as well as resistance to heat and hot liquids. Suggested applications include cold and hot cups, cup lids, yogurt containers, tubs and trays for meats and vegetables, and disposable food packaging.

Mirel™ was released in 2010 by Telles, a joint venture of Metabolix and ADM. A 50,000 tonne per annum plant was

constructed in Clinton, Iowa; there are also manufacturers in China and Japan. However, it is too expensive to find widespread use in food packaging.

Polyglycolic Acid (PGA) has been used for decades for sutures and recently Kureha established a manufacturing facility in the US to produce PGA for packaging. Although presently made from petroleum-based raw materials, it could be made from biobased resources. It has excellent barrier properties and is likely to find application as a barrier layer in PET bottles.

3. Biobased but not Biodegradable

Bioethylene can be produced by the catalytic dehydration of bioethanol, followed by normal polymerisation to produce polyethylene (Koopmans, 2014). It is not biodegradable and has the same properties, processing and performance as polyethylene made from natural gas or oil feedstocks. To date most biopolyethylene is produced in Brazil which is a large producer of bioethanol from sugar cane. The major producer Braskem claims every tonne of bioPE captures 2.5 tonne of CO_2.

In August 2011, Danone in Brazil launched 150 g Activia yogurt in bioHDPE. From 20102 Tetra Pak is using 5000 t/yr of bioHDPE in the production of plastic caps and closures. Coca-Cola uses bioHDPE for their Odwalla fruit juice bottles.

In May 2009, Coca-Cola announced the release of PlantBottle™ made from a blend of petroleum-based materials and up to 30% plant-based materials (ethylene glycol (EG) from molasses), leading to a 25% reduction in carbon emissions. In February 2011, Coca-Cola and H.J. Heinz announced a strategic partnership that enables Heinz to produce its ketchup bottles using Coca-Cola's PlantBottle™ packaging; Volvic will also use PlantBottle™.

In March 2011, Pepsico unveiled plans for a bioPET bottle made from switch grass, pine bark, corn husks and other materials to be piloted in 2012. Ultimately, they plan to also use orange peels, oat hulls, potato scraps and other leftovers from their food business. They have not yet announced from where they will source the terephthalic acid or the result of their pilot plant trials.

In December 2011, Coca-Cola signed an agreement with Virent in the USA to develop 100% biobased bottles. Virent is partly owned by Cargill, Shell and Honda and can produce *para*-xylene (BioFormPX™) from a wide variety of feedstocks, including sugar cane, corn and woody biomass. *Para*-xylene can be converted to terephthalic acid and reacted with ethylene glycol from molasses

to produce 100% biobased PET.

In December 2011, Coca-Cola also signed an agreement with Gevo in the USA to develop 100% biobased bottles. Gevo's Integrated Fermentation Technology®

(GIFT®) converts biomass into isobutanol that can be used to make *para*-xylene. On 24th May 2012, the world's first commercial biobased isobutanol production plant started up in Luverne, Minnesota. When cellulosic biomass processing technology is ready for commercialisation, Gevo plans to deploy cellulosic butanol technology.

In December 2011, Coca-Cola also signed an agreement with Avantium in the Netherlands to develop 100% biobased bottles made of polyethylene furanoate (PEF) which has better gas barrier properties than PET. In March 2012 Danone signed an agreement with Avantium to develop PEF bottles. Avantium's patented technology (YXY) converts biomass into furanics building blocks such as 2,5-furan dicarboxylic acid (FDCA) that can replace TA and be polymerised to PEF. Avantium was founded in 2000 by an international consortium of industrial companies including Royal Dutch Shell, Eastman Chemical, Akzo-Nobel and Pfizer.

PEF has superior barrier properties:

- PEF O_2 barrier is 10 times better than PET
- PEF CO_2 barrier is 4 times better than PET
- PEF H_2O barrier is 2 times better than PET PEF also has more attractive thermal properties:
- The T_g of PEF is 86°C compared to the T_g of PET of 74°C
- The T_m of PEF is 235°C compared to the T_m of PET of 265°C PEF is likely to replace PET for carbonated beverages by 2020.

Are Biobased Plastics Sustainable?

The best way to try and answer this question is using LCA (life cycle assessment). Many LCAs have been published but there is only time to consider six recent ones.

Weiss *et al.*, (2012) addressed the environmental impacts of biobased materials in a meta-analysis of 44 LCA studies. Biobased materials save primary energy and greenhouse gases (GHGs) but may increase eutrophication and stratospheric ozone depletion. Most impacts are caused by the application of fertilisers and pesticides during industrial biomass cultivation. Loss of biodiversity, soil carbon depletion, soil erosion and deforestation, as well as GHG emissions from indirect land use change, were not quantified in the

LCAs.

Alvarez-Chavez *et al*., (2012) reported that none of the biobased plastics currently in commercial use or under development are fully sustainable. They noted that when deciding to substitute conventional petroleum-based plastics with biobased plastics, it is important to understand the flow of these materials and their adverse impacts in all parts of their life cycles in order to select a material that is more sustainable.

Hottle *et al*., (2013) reviewed sustainability assessments of biobased polymers. Studies that included end of life (EOL) options reported much higher global warming potential (GWP) results than those that limited the scope to resin or granule production. Including EOL in the LCA provides more comprehensive results but simultaneously introduces greater amounts of uncertainty and variability. Little LCA data is available on the impacts of different disposal options yet such data is critical for sustainability assessments.

Yates and Barlow (2013) reviewed existing LCAs on PLA, PHA, and starch-based polymers. While reductions in non-renewable energy use (NREU) and GWP can be achieved, there were higher impacts in other categories. Definitive conclusions were difficult to draw although the studies reviewed suggest that these biopolymers may not necessarily be more environmentally friendly than the petroleum-based polymers they could replace.

Pawelzik *et al*., (2013) reported that although the internationally agreed LCA standards provide generic recommendations on how to evaluate the environmental impacts of products and services, they do not address details that are specifically relevant for the life cycles of biobased materials. Treatment of biogenic carbon storage is critical for quantifying the GHG emissions of biobased materials in comparison with petroleum-based materials.

Eerhart *et al*., (2012) presented a process analysis, energy and GHG balance if petroleum-based PET was replaced with biobased PEF. They found that production of PEF can reduce NREU 40% to 50% and GHG emissions by 45% to 55% compared to PET on a cradle to grave basis. These reductions are higher than for other biobased plastics such as PLA or PE. Given that annual production of PET bottles is 15 mio. tonnes, substitution by PEF would save 440-520 PJ (petajoules) of NREU and reduce GHGs by 20-35 t of CO_2e. GHGs could be reduced further by a switch to lignocellulose feedstocks but more research is required before this route can be commercialised.

5.QUANTITIES OF BIOBASED MATERIALS

Global production of petroleum-based plastics is around 250 mio. tonnes/year of which plastics packaging is 100 mio. tonnes/year. Biobased plastics production in 2005 was 80,000 tonnes of which starch was 45,000 tonnes and PLA 35,000 tonnes. In 2011, biobased plastics production was 1.2 mio. tonnes and it is estimated to reach 5.8 mio. tonnes in 2016 of which bioPET will be 4.6 tonnes. Strongest growth will be led by biobased, non-biodegradable bioplastics such as bioPE and bioPET which are dubbed "drop-in" solutions. BioPET already accounts for 40% of the global bioplastics production capacity.

6.INNOVATION

Invention is the creation of a new idea, concept, device or process, whereas innovation is turning a new concept into commercial success: the introduction of change via something new. It is not an innovation until a customer says it is! Thus innovation involves both invention plus exploitation and typically takes 3-8 years. While the patent literature is full of inventions, few ever qualify as innovations.

Innovations in Sustainable Packaging

Time only permits mention of a few such innovations. A major one involves lightweighting. This has been going on for decades, driven primarily by economics. Just when you think the limit has been reached, a new low is achieved. For example, Sidel has recently launched their RightWeight PET bottle which weighs just 7.95 g for a 500 mL bottle. While the industry average is 12 g, back in 1985 the weight was 28 g. This new bottle also has 32% more top-load performance. There have also been spectacular reductions in aluminum can body thickness over a decade. Lightweighting of packaging means less material is used and therefore environmental impacts are reduced.

Wal-Mart recently redesigned their gallon milk jug to improve transport & display efficiency. The new jug requires no crates or racks for shipping and storage (which saves on water to clean the racks and crates). The jugs are self-stacking because the spout is flatter and each jug can rest on another during transport, as well as while on display. The squared-off design allows the jugs to be palletised; 9% more of them fit on a truck. However, the packaging engineers ignored consumers when redesigning the jug and it attracted a lot of negative comment because it is almost impossible to pour from without spilling milk.

LiquiGlide™ was founded in 2012 from research at MIT. LiquiGlide™ coatings allow viscous liquids to move easily due to permanently wet slippery surfaces. Coatings consist of two layers: a porous solid layer

and an impregnating liquid layer. The first consumer products with LiquiGlide™ coatings are expected to hit shelves in 2015, and likely products include mayonnaise, ketchup and toothpaste. Benefits include reducing waste, increasing consumer value and eliminating the need for complicated pump modules and dispensing/closure systems.

7.FUTURE DIRECTIONS

The packaging industry must continue to innovate or it will stagnate because consumers want innovation and value novelty. There are several drivers for packaging innovations. One is the fast-changing social trends and the increasing consumer demand for convenience and safety. Another is the growing environmental awareness of consumers, while profitability and differentiation are also important for food companies seeking to attract consumer attention. Sustainability will receive increasing attention and a plethora of labels such as carbon footprint and paper from sustainably managed forests will indicate how companies are performing in this area. Material substitution and lightweighting will continue. Biobased but not biodegradable plastics will become commonplace over the next decade and ensure that the packaging industry becomes more sustainable.

8.SUSTAINABLE FOOD PACKAGING: POSSIBILITY OR PIPEDREAM?

To answer the question posed by the title of this presentation, a lack of consensus over what constitutes sustainable packaging means that different companies and individuals will answer the question in different ways. What some might consider sustainable will be viewed as unsustainable by others. But what is clear is that eventually petroleum-based polymers will be replaced by biobased polymers and these will not be biodegradable. Meanwhile, the food and packaging industry must continue to decrease environmental impacts throughout the package life cycle, and efforts to recycle post-consumer packaging must by increased. Even greater results will be achieved by reducing impacts from food production and minimising food waste.

REFERENCES

Alvarez-Chavez, C.R., Edwards, S., Moure-Eraso, R., Geiser, K. 2012. Sustainability of bio-based plastics: general comparative analysis and recommendations for improvement. *Journal of Cleaner Production* 23: 47-56.

Avantium 2013. http://avantium.com/yxy/products-applications/fdca/PEF- bottles.html.

Cairncross, R.A., Becker, J.G., Ramaswamy, S., O'Connor, R. 2005. Moisture sorption, transport, and hydrolytic degradation in polylactide. *Applied Biochemistry and Biotechnology* 129–132: 774–785.

Eerhart, A.J.J.E., Faaij, A.P.C., Patel M.K. 2012. Replacing fossil based PET with

biobased PEF; process analysis, energy and GHG balance. *Energy and Environmental Science* 5: 6407-6422.

Hottle, T.A., Bilec, M.M., Landis, A.E. 2013. Sustainability assessments of bio-based polymers. *Polymer Degradation and Stability* 98: 1898-1907.

Kolstad, J.J., Vink, E.T.H., De Wilde, B., Debeer, L. 2012. Assessment of anaerobic degradation of Ingeo™ polylactides under accelerated landfill conditions. *Polymer Degradation and Stability* 97: 1131-1141.

Koopmans, R.J. 2014. Polyolefin-based plastics from biomass-derived monomers. Chpt. 14 in *Bio-Based Plastics: Materials and Applications*, ed. S. Kabasci, pp. 295-310. John Wiley & Sons, Ltd., Chichester, England.

Narayan, R. 2012. Biobased & biodegradable plastics: Rationale, drivers, and technology exemplars. Chpt. 2 in *Degradable Polymers and Materials: Principles and Practice*, eds. K. Khemani & C. Scholz, 2nd ed, pp. 13-31. ACS Symposium Series; American Chemical Society: Washington, DC.

Pawelzik, P., Carus, M., Hotchkiss, J., Narayan, R., Selke, S., Wellisch, M., Weiss, M., Wicke, B., Patel, M.K. 2013. Critical aspects in the life cycle assessment (LCA) of bio-based materials – Reviewing methodologies and deriving recommendations. *Resources, Conservation and Recycling* 73: 211-228.

PwC PricewaterhouseCoopers 2012. Sustainable packaging – myth or reality? www.pwc.co.uk/forest-paper-packaging/publications/sustainable-packaging-myth- or-reality.jhtml.

Robertson, G.L. 2009. Sustainable food packaging. Chpt. 11 in *Handbook of Waste Management and Co-Product Recovery in Food Processing* Vol 2, ed. K.W. Waldron, pp. 221-254. Woodhead Publishing, Cambridge, England.

Robertson, G.L. 2013. Edible, biobased and biodegradable food packaging materials. Chpt. 3 in *Food Packaging: Principles and Practice*, 3rd ed. pp. 49-90, CRC Press, Boca Raton, FL.

Robertson, G.L. 2014. Biobased but not biodegradable. Is this the new paradigm for sustainable food packaging? *Food Technology* 68(6): 61-70.

Weiss, M., Haufe J., Carus M., Brandão, M., Bringezu, S., Hermann, B., Patel, M.K. 2012. A review of the environmental impacts of biobased materials. *Journal of Industrial Ecology* 16: S169-S181.

Yates, M.R., Barlow, C.Y. 2013. Life cycle assessments of biodegradable, commercial biopolymers - A critical review. *Resources, Conservation and Recycling* 78: 54-66.

ORAL PRESENTATION

FOOD PROCESSING AND PRODUCT DEVELOPMENT

OPTIMIZED FERMENTATION OF SORGHUM (*Sorghum bicolor* L.) FLOUR FORTIFIED WITH SOY PROTEIN USING *Lactobacillus plantarum* 3704

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ABSTRACT

Optimization of sorghum flour fermentation has been conducted to determine the optimal condition of fermentation based on soluble protein content measured by spectrophotometer at 550 nm using Biuret reagent. Sorghum flour was fermented by *Lactobacillus plantarum* 3704. The data of soluble protein contents of sorghum flour were analyzed using Factorial Treatment Design 3 × 3 × 3 with Randomized Complete Block Design. The first factor was concentration of soy protein concentrate (1%, 2%, and 3%). The second and third ones were concentration of *L. plantarum* suspension (0.25%, 0.5%, 0.75%) and the duration of the fermentation (24 h, 36 h, and 48 h), respectively. Data were assessed by Tukey's HSD (Honestly Significant Difference) test with a significance level of 95%. Result showed that the optimum condition of fermentation was 2% soy protein concentrate, 0.25% *L. plantarum* suspension, and 36 h of fermentation. The soluble protein content in the optimum fermentation condition was 11.52%.

Keywords: sorghum *flour, soluble protein, soy protein concentrate, Lactobacillus plantarum*

1.INTRODUCTION

Indonesian food security continues to face various problems such as insufficient food production, low competitiveness of agricultural product, shrinking of arable land in Java island (100,000 acre/year), lack of infrastructures, climate change, undeveloped food diversification based on local product, population growth, and increasing consumption per-capita (RISTEK, 2013).

The increasing population of Indonesian results in dependence on imported wheat. Volume of imported wheat in 2011 reached 5.4 million metric tons and increased to 6.2 million metric tons in 2012 (Theresia, 2013).

Sorghum is a local Indonesian crop with various local names. In Java, sorghum is called *"canthel"*. Sorghum can be grown well in Indonesia. Moreover, sorghum is considered as the most important crop in the world followed by wheat, rice, corn, and barley (FAO, 1997).

The protein content of sorghum is 11%, but it will decrease because of washing and further processes (Puspaningsih, 2013). Sorghum contains protein in the form of albumin, globulin, prolamine, and glutelin (Skoch *et al.*, 1970). The high protein content in sorghum does, unfortunately, not come along with variation of amino acids (Andayani in Puspaningsih, 2013). The poor amino acid variation reduces the digestibility of the protein (Puspaningsih, 2013).

Sorghum contains tannin and phytic acid that inhibit the absorption of carbohydrates and proteins (Osman, 2004). *L. plantarum* has proteolytic (Mugula *et al.*, 2003) and amilolytic (Songre-Ouattara *et al.*, 2009) activities thus increasing the digestibility of carbohydrates and proteins.

Fermentation of sorghum flour using *L. plantarum* and soy protein fortification would improve the quality of the sorghum flour by increasing the protein content and digestibility. Thus, the aim of this study was to investigate the optimum fermentation condition of modified sorghum flour based on soluble protein content.

2.MATERIALS AND METHODS

The main materials in this study were sorghum (*Sorghum bicolor* L.) obtained from Surakarta, soy bean (*Glycine max* (L.) Merr.) obtained from Grobogan, and *Lactobacillus plantarum* 3074 obtained from PAU Pangan UGM. The chemicals and medium used in this research were purchased from Merck, Germany. They were $CuSO_4.5H_2O$, NaOH, BSA, KNa-tartrat, and MRS (deMann, Rogosa and Sharpe) medium.

2.1.*Lactobacillus plantarum* suspension preparation

Suspension containing 10^8 CFU/ml determined using McFarland scale by means of spectrophotometer UV/VIS (Optizen UV 2120, South Korea).

2.2.Soy Protein Concentrate

The pH of soy milk was adjusted to 4.8 using citric acid to separate soy protein from the milk. The protein precipitate was dried in drying cabinet for 24 h in 50°C. Then, it was defatted by Sohxlet extractor using diethyl ether as solvent.

2.3.Sorghum Flour Fermentation Optimization

Sorghum was germinated for 5 days and dried in drying cabinet for 48 h. Sorghum was milled into 60 mesh flour, and it was fortified with soy protein concentrate (1%, 2%, 3%). Fermentation was carried out by mixing 25 g fortified sorghum flour and 50 mL PPS (peptone physiological salt) solution containing *L. plantarum* (0.25%, 0.5% and 0.75%). The slurries were allowed to be fermented at 37°C for 24 h, 36 h, and 48 h in closed plastic cups. The slurries were dried in a drying cabinet at 50°C for 48 h. The dried samples were milled using food processor and stored in plastic bags at 4°C. All kinds of the flour were analyzed for their soluble protein content using Biuret reagent measured at 550 nm (AOAC, 1995).

2.4.Data analysis

The data of soluble protein content were analyzed using Factorial Treatment Design 3 × 3 × 3 with Randomized Complete Block Design. There were three factors, namely concentration of soy protein concentrate (1%, 2%, and 3%), concentration of *L. plantarum* suspension (0.25%, 0.5%, 0.75%), and duration of the fermentation (24 h, 36 h, and 48 h). Data were assessed by Tukey's HSD (Honestly Significant Difference) test with a significance level of 95%.

3.RESULT AND DISCUSSION

The optimization of fermentation condition data analysis were followed by a review of the interaction between each variable (*L. plantarum* concentration, soy protein concentrate addition, and duration of fermentation). The ANOVA analysis showed significant difference of fermentation duration (**Table 1**), soy protein concentrate addition (**Table 2**), interaction of bacterial suspension and fermentation duration (**Table 3**), soy protein addition and fermentation duration (**Table 4**).

Table 1. *Soluble protein content ($\bar{x} \pm SE$, %) of fermented sorghum flour in every duration of fermentation*

	24 h	**36 h**	**48 h**
$\bar{x}$ ± SE	9.87±0.82	10.77±0.95	9.77±0.70
W = 0.96	(a)	(ab)	(a)

W is the honest significant difference of 5%, and different alphabets indicate significant difference between treatment, this applies to all tables represented in this paper.

Table 1 shows that the highest soluble protein content is in 36 hours of fermentation with significant difference marked with (ab). Thus, the optimum duration of fermentation was 36 hours.

Table 2. *Soluble protein content ($\bar{x} \pm SE$, %) of fermented sorghum flour in every addition of soy protein concentrate*

	1%	2%	3%
$\bar{x} \pm$ SE	9.06±0.84	10.80±0.88	10,56±0.68
W = 0.96	(a)	(b)	(b)

Table 2 indicates a significant difference between soy protein concentrate addition. The highest soluble protein content is in 2% and 3% soy protein concentrate additions, so the lowest addition is chosen.

Table 3. *Soluble protein content ($\bar{x} \pm SE$, %) of fermented sorghum flour in soy protein concentrate addition and duration of fermentation*

	24 h	36 h	48 h
0.25%	10.27±1.36(a)	11.90±1.61(b)	9.65±1.43 (a)
	(a)	(b)	(a)
0.50%	9.83±1.43 (a)	9.27±1.74 (a)	10,28±1.35(a)
	(a)	(a)	(a)
0.75%	9.53±1.91 (a)	11.13±1.76(b)	9.39±1.24 (a)
	(a)	(ab)	(a)

W=1,64

Table 3 shows that the highest soluble protein content is in 0.25% bacterial suspension for 36 h fermentation. Thus, the optimum fermentation condition is in 0.25% bacterial suspension and duration of 36 h.

Table 4. *Soluble protein content ($\bar{x} \pm SE$, %) of fermented sorghum flour in various concentrations of bacterial suspension and durations of fermentation*

	24 h	36 h	48 h
1%	8,60±1,51 (a)	8,79±2,05 (a)	9.79±1,08 (a)
	(a)	(a)	(a)
2%	11.62±1,48(b)	11.72±1,28 (b)	9.05±1,74 (a)
	(b)	(b)	(a)
3%	9.41±1,06 (a)	11.80±1,41(b)	10.48±0,99(a)
	(a)	(ab)	(a)

W= 1,64

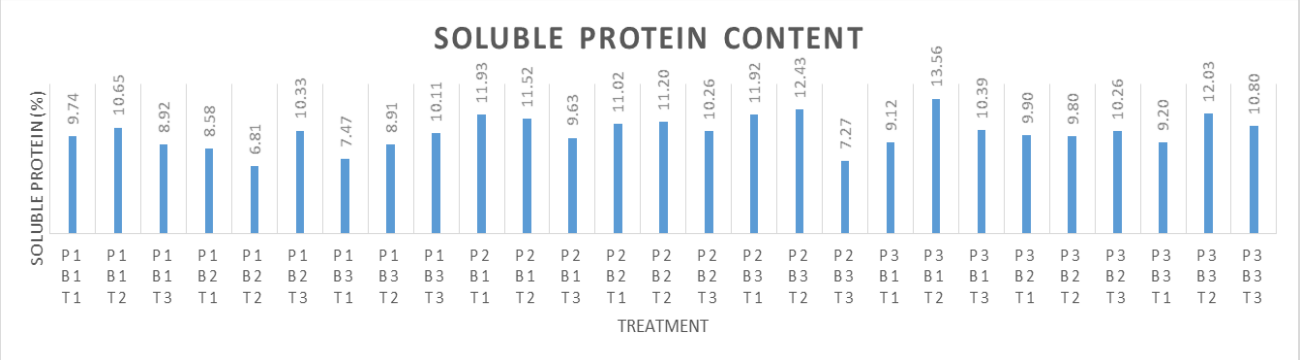

Figure 1 The soluble protein contents of all samples showing optimum at P2 B1 T1 (P1=1%, P2=2%, P3=3% of soy protein concentrate addition; B1=0.25%, B2=0,50%, B3=0,75% *L. plantarum addition*; T1=24h, T2=36h, T3=48h of fermentation duration)

Table 4 shows that the highest soluble protein content is obtained by fermentation for 24 h with 2% soy protein concentrate addition. The condition has been chosen based on the highest soluble protein content obtained at lower concentration of addition and shorter time.However, according to **Table 1** and **Table 3** showing that the optimum duration of fermentation is obtained at 36 hours of fermentation, thus 2% soy protein concentrate addition and 36 h of fermentation time in combination with 0.25% bacterial suspension is more likely to be the optimum fermentation condition instead of that condition with 24 h of fermentation time.

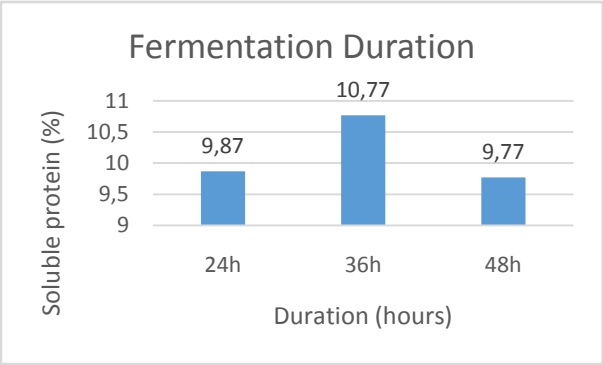

Figure 2 Interaction within duration of fermentation. The graph shows that the soluble protein content peaks at 36 h of fermentation

The optimum soluble protein content was achieved at fermentation for 36 h. The result of this study corresponds to the research conducted by Pranoto (2013) that the highest IVPD (In vitro protein digestibility) value of sorghum flour fermented with *L. plantarum* bacteria peaked at 36 hours of fermentation. Thus, increasing number IPVD indicated that the soluble protein in this sorghum flour also increased. The *L. plantarum* has proteolytic activity (Mugula *et al.*, 2003) that could break down long protein chains into smaller protein unit thus increasing the IPVD and soluble protein content.

Soy protein concentrate fortification increases the quality and quantity of protein in modified sorghum. Puspaningsih (2013) conducted a study of sorghum fortification using peanut. Peanut only contain 25.3% total protein and 7.92% soluble protein compared to soy protein isolate that contain 60.05 soluble protein. The research showed that at the optimum fermentation condition using *Rhyzophus oligosporus* 2.5% , in which the peanut addition was 5%, the soluble protein content reached only 6.36%, lower than soluble protein content (11.52%) in modified sorghum flour fermented by *L. plantarum* at an optimum condition utilizing 2% soy protein concentrate . Apart from the distinguished cultures which might affect the fermentation processes thus causing the different values of protein content, soy protein concentrate addition is probably more effective than peanut addition because the protein contained in soy protein concentrate is more higher.

4.CONCLUSION

Depending on the statistical analysis, it could be concluded that the optimum condition for fermentation was 0.25% *L. plantarum*, 2%

soy protein concentrate, and 36 hours of fermentation.

5.ACKNOWLEDGEMENT

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REFERENCES

Andayani, P. 2008. *Isolasi dan Identifikasi Mikrob dari Tempe Sorghum Coklat (Sorghum bicolor) Serta Potensinya Dalam Mendegradasi Pati dan* Protein. Universitas Brawijaya, Malang.

FAO, 1997. *Production Yearbook.* Rome: Food and Agriculture Organization of the United Nations.

Mugula, J. K., Sørhaug, T. & Stepaniak, L., 2003. Proteolytic activities in togwa, a Tanzanian fermented food. *International Journal of Food Microbiology*, Issue 84, pp. 1-12.

Pranoto, Y., Anggrahini, S. & Efendi, Z., 2013. Effect of natural and Lactobacillus plantarum fermentation on in-vitro protein and starch digestibilities of sorghum flour. *Food Bioscience*, Issue 2, pp. 46-52.

Puspaningsih, V., 2013. *Analisis dan Identifikasi Asam Amino dan Asam Lemak Tak Jenuh Sorgum (Sorghum bicolor L.) Terfortifikasi Kacang Tanah (Arachis hypogaea) Sebagai Pangan Fungsional.* Salatiga: Universitas Kristen Satya Wacana.

RISTEK, 2012. *Kementrian Riset dan Teknologi.* [Online] Available at: http://ristek.go.id/index.php/module/News+News/id/12015[Accessed 30 10 2013].

Skoch, L. V. et al., 1970. Protein Fractination of Sorghum Grain. *Cereal Chemistry*, Issue 47, pp. 472-481.

Songre-Ouattara, L. T. et al., 2009. Potential of amylolytic lactic acid bacteria to replace the use of malt for partial starch hydrolysis to produce African fermented pearl millet gruel fortified with groundnut. *International Journal of Food Microbiology*, Issue 130, pp. 258-264.

Theresia, A., 2013. *Tempo.co Bisnis.* [Online] Available at: http://www.tempo.co/read/news/2013/07/24/090499391/Indonesia-Didesak-Kurangi-Impor-Gandum[Accessed 30 10 2013].

THE EFFECT OF ADDITION OF VARIATION DRYING AGENT ON THE CHARACTERISTIC OF RED BEET POWDER (*Beta vulgaris* L) BY SOLAR TUNNEL DRYER (STD)

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ABSTRACT

Healthy natural dyes are needed to produce a good quality and attractive food products. One of the natural red dyes which can be used as a food colorant is red beet (*Beta vulgaris* L). Solar Tunnel Dryer (STD) is one method to produce red beet powder. To improve the quality of dried foods, drying agents (maltodextrin and arabic gum) are commonly applied. This drying agents could speed up the drying process and can also retain the nutrients of products. The purpose of this study is to investigate the effect of drying agent on the physicochemical properties of red beet dried by STD. Physico-chemical properties of red beet powder analyzed were antioxidant activity, moisture content, bulk density, color intensity and wetting ability. Maltodextrin and arabic gum were added in the red beet extract at concentrations of 20%, 40%, and 60%. Red beet extract was adjusted at pH 4. STD was conducted for 200 minutes. The results showed that the use of gum arabic as drying agent with a concentration of 60% had the higher antioxidant activity (90.10%) than that of maltodextrin (79.17%). Moreover, application of 60% arabic gum showed the brighter color (48.22) and higher a* value (22.99) compared to that of maltodextrin. In addition, the highest bulk density of red beet powder (0.721) was achieved by using 60% gum arabic. In conclusion, application of 60% arabic gum is the optimum condition to produce a good quality of red beet powder.

Keywords: *red beet, arabic gum, maltodextrin, Solar Tunnel Dryer*

1. INTRODUCTION

1.1. Background

Currently many food and drink products that use additional food dyes. Color is very important in food industry because it could affect the mind consumers of food products. Food dyes that are used include natural and artificial dyes. Dyes from natural sources is very rare due to the expensive price. In Indonesian, this triggered the use of non-food chemical dyes that are harmful to health like Rhodamin B and Metanil yellow. Both the ingredient is actually coloring matter textil but often used as food coloring since their prices appertain cheap and easily acquired. Data from the *Pengawas Obat dan Makanan* (POM) about extraordinary events (of the Outbreak) food poisoning from 2001-2006 shows an increase in both the number of events or the number of victims who fell ill and died. Nevertheless, the victim dies of suspected might be just 1% only in accordance with the World Health Organization (WHO) estimates. POM data in 5 provinces in 1999-2001 shows that about 89,8% food products contain food additive consisting of 35.6% food products contain boraks, 41.2% contain formalin, 10.4% contain Rodhamin B and 1.9% containing amaran.

To answer that problems is by developing the potential cheap natural dye. Natural pigment of plants get more attention to replacing synthetic dye food coloring which proved to give effect to the human and environmental health. One of the natural Red dyes that can be used as a food coloring derived from Red beet (*Beta vulgaris* L). Beets contain betalain pigments which are complex. These Betalains has attracted to utilized in applicative due to its use as a food coloring and the presence of radical scavenging and antioxidant properties as a protection against the nuisance caused by certain oxidative stress. Pigment color red-purple on beets is derived from betasianin which is called betanin. Beets contain betanin reach 200 mg/100 g. betasianin, like most of betanin in the metabolism of molecules called 3,4-di-hydroxyphenylalanine (L-DOPA).

1.2. Research Purposes

This research aims to know the use of drying agent (Maltodextrin and Gum Arabic) on physicochemical changes n methods of drying using STD including antioxidant activity, moisture content, bulk density, intensity of color and red beet powder wetting ability.

2. MATERIALS AND METHODS

2.1. Materials

The equipment used for the drying of beet red powder are slicer, analytic scales, spoon, measuring cup, a beaker glass, blender, pH meters, moisture balance, spectrophotometer, Chromameter CR-400. The materials used in the manufacture of red beet powder

maltodextrin DE 10, gum arabic, ascorbic acid.

2.2. Methods

2.2.1. Sample Preparation

Fresh red beets are separated and washed to clean then peeled the skin. Red beets that have been peeled sliced with uniform thickness using the slicer.

2.2.2. Immersion in Drying Agent

Red beet slices dipped into the drying agent. Drying agent that used was maltodextrin and gum arabic each with a concentration of 20%, 40%, and 60% in aquades. Added Ascorbic acid until the pH reach 4.

2.2.3. Drying with STD

After dipped with drying agent, red beets are styled in the tray that is found in STD. Drying is performed until the red bits moisture content below 10%.

2.3. Analysis

2.3.1. Moisture Content Analysis

Moisture content of red beets each treatment is calculated using moisture balance. Then dried using STD. weight of red beets with analytic scales weighed every 20 minutes to know water levels decrease until it reaches below 10%. Data results weighing heavily red bit is inserted into the formula.

$$\text{Solid Content 1} = 100 - \text{initial water content}$$

$$\text{Solid Content 2} = \left(\frac{\text{Solid Content 1}}{100}\right) x\ sample\ weight\ t_0$$

$$\text{Water Content } t_n = \frac{weight\ t_n - solid\ content\ 2}{weight\ t_n}$$

2.3.2. Antioxidant Activity Analysis

Samples about 0,5 grams of red beet powder dissolved in 5 ml of aquades. The sample as much as 0.1 ml is reacted with 3.9 ml solution of DPPH (2,4 mg in 100 ml of methanol DPPH). After it is stored in a dark room for 30 minutes at room temperature and protected with aluminum foil. T then the sample measured using a spektofotometer with a wavelength of 515 nm. DPPH solution standard stored at a temperature of 4^{o}C and protected with aluminum foil to keep it from the influence of the light effect. The antioxidant activity was calculated as % discoloration by using the formula below:

$$\%\ discoloration = \left[1 - \frac{At_{30}}{At_{\ 0}}\right] x100\%$$

Notes: At_{30} is absorbance at 30 minutes and control absorbance is At_0

(Apriyantono *et al.*, 1989).

2.3.3. Bulk Density Analysis

Bulk density analysis done by inserting samples into a container of known volume to the brim then weighed. Bulk density is measured by comparing the mass of powder sample bit with volume container (bulk). Bulk density is expressed in units of g/cm3 (Sharma et al, 2000).

Weight of material = (weight of the container + material)

$$\text{Bulk Density} = \frac{\text{weight of the container}}{\text{volume container}}$$

Volume Container = $\pi.R^2.t$

2.3.4. Wetting Ability Analysis

This measurement is used to determine the ease of the powder is moistened expressed in units of seconds. A number of 150 ml water in a glass cup 600 ml wide mouth and closed. As many as 1 gram beetroot powder added to the surface of the water is calm and then records the time formed the starting count since pouring until all the powder is wet (Hartomo & amp; Widiatmoko, 1993).

2.3.5. Color Analysis

Color analysis done using the MINOLTA Chromameter 200 series (CR-200). Chromameter calibrated in advance by firing on a white plate. Once calibrated, chromameter is used to measure the color samples. Red beet powder sample is placed on the clear plastic then measured with a chromameter. Units of color emerges is L *, a *, and b *. L value indicates the level of brightness of the sample, i.e., a value of 0 means the absolute black and 100 means white. The value of b is one attribute that indicates with a scale (-70) to 70. The value of b is negative indicates the degree of bluish while the positive values of b indicates the degree of yellowish. The value of a shows a reddish or greenish sample (Hutching, 1999).

3. RESULTS AND DISCUSSION

3.1. Moisture Content of Red Beet Powder

Table 1. Moisture Content of Red Beet Mixed Maltodekstrin

Time	Control	Maltodextrin 20%	Maltodextrin 40%	Maltodextrin 60%
t-20	79.69 ± 1.30	73.58 ± 0.73	70.81 ± 1.11	67.36 ± 0.80
t-40	73.77 ± 3.10	69.63 ± 0.87	66.71 ± 1.23	62.96 ± 0.82
t-60	67.34 ± 2.99	63.77 ± 1.52	61.22 ± 1.86	57.17 ± 1.11
t-80	59.66 ± 3.36	56.56 ± 1.50	53.77 ± 1.75	49.55 ± 1.20
t-100	46.18 ± 8.34	48.40 ± 1.88	41.75 ± 4.93	37.34 ± 1.80
t-120	33.90 ± 5.87	32.71 ± 1.10	28.47 ± 3.41	22.51 ± 0.59
t-140	26.62 ± 6.23	19.09 ± 2.28	17.57 ± 2.49	11.13 ± 3.14
t-160	17.28 ± 4.79	12.59 ± 2.60	9.47 ± 1.40	7.54 ± 2.05
t-180	12.39 ± 2.46	9.76 ± 2.33	5.54 ± 1.50	4.06 ± 1.27
t-200	9.05 ± 0.41	7.04 ± 1.58	3.26 ± 2.00	2.09 ± 1.59

Description:
All values are the average values ± standard deviation

This research use the drying method of Solar Tunnel Dryer (STD) until the moisture content below 10% and counted the water level every 20 minutes. The temperature recorded during the drying process of red beets are 70±5^{o}C. Test results on the water content of red beets with maltodekstrin contained in table 1, it can be known that drying by using a maltodekstrin 40% and 60% is the quickest drying, but water levels decrease red beets with maltodekstrin 60% is faster.

Table 2. Moisture Content of Red Beets Mixed Gum Arabic

Time	Gum Arabic 20%	Gum Arabic 40%	Gum Arabic 60%
t-20	71.80 ± 1.00	68.99 ± 0.51	66.27 ± 0.12
t-40	68.25 ± 0.93	65.22 ± 0.86	62.54 ± 1.05
t-60	63.83 ± 1.53	60.60 ± 1.48	57.85 ± 1.98
t-80	57.21 ± 1.92	53.62 ± 3.07	51.13 ± 2.99
t-100	46.42 ± 3.79	42.19 ± 5.56	40.40 ± 4.77
t-120	34.11 ± 6.32	29.70 ± 8.34	28.77 ± 7.17
t-140	24.20 ± 2.63	20.19 ± 3.27	19.16 ± 4.18
t-160	17.67 ± 1.54	16.15 ± 2.81	11.80 ± 1.65
t-180	13.14 ± 0.92	9.45 ± 0.28	6.77 ± 1.29
t-200	8.08 ± 0.89	6.22 ± 1.70	4.41 ± 2.38

Description:
All values are the average values ± standard deviation

Based on data of table 1 and 2 about the decrease in moisture content of red beets can be seen that the use of maltodextrin has a faster drying time compared to using gum arabic. This happens because the gum arabic is heteropolimer compact so it can withstand stronger material water. Maltodextrin has a lower molecular weight and molecular structures are much simpler, so that water can easily evaporated when the drying process takes place either in the form of the free water, bound to physically and chemically bound. While at the gum arabic has a higher molecular weight and molecular structure is more complex, there is a large amount of starch in them so that their nature is more hygroscopic and consequently the complex is water on material more difficult evaporated is stuck causing the drying time of red beets to be longer (Dickinson 2003).

3.2. Antioxidant Activity of Red Beet Powder

Table 3. Antioxidant Activity of Red Beet Powder with Drying Agent

Concentration	% *Inhibition*	
	Maltodextrin	Gum Arabic
0%	80.02 ± 2.72^{c}	80.02 ± 2.72^{c}
20%	79.17 ± 4.13^{c}	81.90 ± 4.89^{c}
40%	52.15 ± 3.24^{b}	85.89 ± 3.06^{d}
60%	46.88 ± 2.29^{a}	90.10 ± 0.88^{e}

Description:
1. All values are the average values ± standard deviation
2. Values with different superscript on each line shows that there is a significant difference between the treatment on a confidence level of 95% (<0.05) using test Duncan.

The highest antioxidant found in red beet powder with 0% maltodekstrin or control, while using maltodekstrin can be seen that higher concentration used the lower the % Inhibition or its antioxidant activity. While the use of gum arabic can be known that the use of the concentration of 60% in the real effect on antioxidant activity of red beet powder. Antioxidant activity of red beet powder with gum arabic is so much better than maltodextrin. This happens because the gum arabic can improve stability with increased viscosity. The type of thickener is also heat resistant to process that uses heat but it is better if the heat is controlled to shorten the heating time, given the gum arabic can be degraded slowly and lacked efficiency emulsification and viscosity, so the constituent components of antioxidants like betalain is not damaged by warming.

Decreased antioxidant activity that occurs in the red beet powder added with maltodextrin with the higher concentration, caused by the increasing number of total solids contained in the material is maltodextrin so the total phenolic the less measurable. The level of antioxidant activity in red beet due to the content of phenolic compounds. The content of flavonoids and phenolic acids in red beet is very low (Czapski et al., 2009).

3.3. The Color Red Beet Powder

Table 4. Red Beet Powder Color Using a Drying Agent

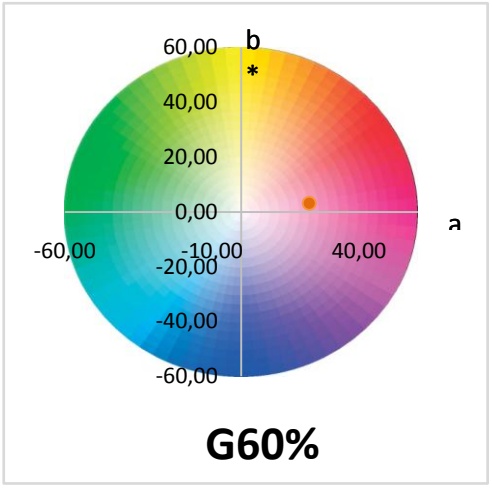
Treatment	Color		
	L*	a*	b*
Control	43.82 ± 0.85^{ab}	14.59 ± 0.98^{a}	3.35 ± 0.15^{b}
Maltodextrin 20%	45.52 ± 1.65^{b}	18.22 ± 0.37^{bc}	3.01 ± 0.32^{ab}
Maltodextrin 40%	43.50 ± 3.79^{ab}	16.44 ± 3.30^{ab}	3.58 ± 0.99^{b}
Maltodextrin 60%	41.42 ± 3.98^{a}	16.02 ± 3.71^{ab}	3.10 ± 0.61^{ab}
Gum Arabic 20%	44.08 ± 1.96^{b}	19.55 ± 2.96^{c}	3.08 ± 1.09^{ab}
Gum Arabic 40%	45.93 ± 2.25^{bc}	20.27 ± 1.94^{c}	2.65 ± 0.32^{a}
Gum Arabic 60%	48.22 ± 1.27^{c}	22.99 ± 0.60^{d}	3.29 ± 0.15^{ab}

Description:
1. All values are the average values ± standard deviation
2. Values with different superscript on each line shows that there is a significant difference between the treatment on a confidence level of 95% (< 0.05) using test Duncan.

The largest value of L* in powder gum Arabic concentration bit with 60% indicating the color of the powder is getting brighter. An a* and b* are also contained on the highest of the powder with a drying agent gum Arabic with a concentration of 60%, this indicates that the color red beet powder tends to be more red compared to using maltodextrin. While the color control powder tends toward dark red color.

The use of drying agents such as maltodextrin and ascorbic acid will lower the pH of the product on the network, so it will inactivate the polyphenol oxidase enzyme that can cause discoloration and decrease in antioxidant activity (Winarno, 2002). The theory is supported by the statements above Sunarmani and Soedibyo (1992), in the manufacture of flour Siam oranges, use the smaller concentrations are 5.0% better than a higher concentration is 7%, due to the higher concentration of filler material, the color products will be further away from the original color.

This causes the brightness of color red beet powder is inversely proportional to the concentration of maltodextrin, which means the higher maltodextrin given as a drying agent, then the color of the powder will be getting darker. Color of maltodextrin is also affect the color of the white pigments. In other words, the color of the products would be further away from the original color. This also causes the color of the powder with a drying agent gum arabic brighter than maltodextrin. A layer of film maltodextrin has the proportion of solids that are higher than those of gum arabic, because of the degree of conversion is lower than the gum arabic (Stephen, 1995).



Figures 1. a* and b* Values of Red Beet Powder with Gum Arabic

3.4. Bulk Density of Red Beet Powder

Table 5. Bulk Density of Red Beet Powder Using Drying Agent

Concentration	Bulk Density	
	Maltodexrin	Gum Arabic
0%	0.498 ± 0.011^{a}	0.498 ± 0.011^{a}
20%	0.549 ± 0.038^{b}	0.642 ± 0.012^{d}
40%	0.571 ± 0.029^{bc}	0.678 ± 0.009^{e}
60%	0.583 ± 0.028^{c}	0.721 ± 0.026^{f}

Description:
1. All values are the average values ± standard deviation
2.Values with different superscript on each line shows that there is a significant difference between the treatment on a confidence level of 95% (< 0.05) using test Duncan.

Based on the observations in the table 5 it can be seen that the use of gum arabic produced powders with bulk density better than using maltodextrin. The addition of gum arabic powder causes a higher bulk density than the bulk density of the powder with the addition of maltodextrin. Maltodextrin film coating solids have a higher proportion than gum arabic, because the degree of conversion is lower than gum arabic (Stephen, 1995). Gum arabic is a natural polymer composed mostly of polysaccharides with high molecular weight and contain calcium, magnesium, potassium and high potassium, which produces arabicinose, galactose, glucuronic acid ramnosa and after hydrolysis (Almuslet, et.al, 2012). While maltodextrin is usually distinguished by the value of DE (Dextrose Equaivalency). The value indicates multiplicity DE sugar reduction calculated as dekstrosa (Hui, 1992). DE value is inversely proportional to molecular weight, maltodextrin with the lowest score is usually non-hygroscopic whereas DE maltodextrin with higher (lower molecular weight) is hygroscopic. This is also the effect on the higher concentration of drying agent the greater bulk density are also produced.

3.5. Red Beet Powder Wetting Capability

Table 6. Red Beer Powder Wetting Capability Using Drying Agent

Concentration	Wetting	
	Maltodextrin	Gum Arabic
0%	46.69 ± 3.89^{b}	46.69 ± 3.89^{b}
20%	45.78 ± 8.61^{b}	61.28 ± 10.50^{d}
40%	25.98 ± 4.30^{a}	54.12 ± 2.69^{c}
60%	20.63 ± 3.55^{a}	49.73 ± 1.63^{bc}

Description:
1. All values are the average values ± standard deviation
2.Values with different superscript on each line shows that there is a significant difference between the treatment on a confidence level of 95% (< 0.05) using test Duncan.

Measurement of wetting capability aims to determine the solubility of red beet powders produced at different drying agent. The solubility of red beet powder to be applied in determining the ease with which the food product is easily soluble powder would be easy to make and produce texture and good color uniformity. Unlike the other tests, the wetting ability by using maltodextrin having wetting time is faster than using gum arabic. The structure of each granule types of binders are also affecting the solubility of red beet powder. Gum arabic has a molecular structure that is more complex than the maltodextrin, and has a number of starch in it, so the nature of gum arabic is more hygroscopic. Starch re-polymerization process with the help of acid at the time dekstrinisation make maltodextrin molecules

are split into smaller sizes with more hygroscopic component, so that when subjected to heating by low pressure, damaged starch particles. As a result of water easily switch to it while releasing components that are easily soluble in water, hence the high solubility (Loksuwan, 2006).

In addition to the mass of powder solubility in water is influenced by the water content of the mass of material that is dissolved. Moisture content of materials of high mass causes a mass of such material becomes difficult to spread or dispersed in water, because the materials tend to be sticky. Thus no pore formed and the mass of the material is not able to absorb large amounts of water. In addition, the mass of material with a higher moisture content has a narrow surface to moistened, because large particle mass so that the sticky side of the particle mass. Solubility level also determines the amount of mass diffusion mass exists in the component materials. The higher the degree of solubility in water then the diffusion components into a mass of material the higher (Straatsma *et al*., 1999). According to McDonald's (1984), maltodextrin has less hygroscopic properties, less sweet, have a high degree of solubility and tend not to form a color reaction browning.

4. CONCLUSION

Drying agent for the manufacture of beet red powder the most optimum use is gum Arabic with a concentration of 60%, which can increase antioxidant activity, the brightness and the color intensity is higher, as well as bulk density is the highest compared to maltodextrin**.** Maltodextrin advantages compared to gum arabic are faster drying time and red beet powder wetting capability increasingly rapidly.

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6. REFERENCES

Adawyah, R., 2007. Pengolahan dan Pengawetan Ikan. Bumi Aksara, Jakarta.

Ali, A. Maqbool, M. Alderson, P.G. Zahid, N. 2012. *Effect of gum arabic as an edible coating on antioxidant capacity of tomato (Solanum lycopersicumL.) fruit during storage*. Postharvest Biology and Technology 76 (2013) 119–124.

Almuslet, N. A., Elfatih A. H., Al-Sayed A. A. and Mohamed G. A. M., 2012. Diode Laser (532 nm) Induced Grafting of Polyacrylamide onto Gum Arabic. Journal of Physical Science, University of Science Malaysia. Vol 23. Hal 43.

Cahyadi, W., 2006. Bahan Tambahan Pangan. Bumi Aksara, Jakarta.

Dickinson E. 2003. Hydrocolloids at Interfaces and the Influence on the

Properties of Dispersed Systems. Food Hydrocolloids, 17: 25-39.

Dumas, Y., Dadomo, M., Di Lucca, G., Grolier, P., 2003. Review: effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. Journal of the Science of Food and Agriculture 83, 369–382.

Earle, R. L., 1982. Satuan Operasi Dalam Pengolahan Pangan. Penerjemah Z. Nasution. Sastra Hudaya, Jakarta.

Fardiaz, D., 1989. Hidrokoloid. Lab Kimia dan Biokimia Pangan, PAU Pangan dan Gizi, IPB, Bogor.

Fellows, P. 1990. Dehydration. In Encyclopedia of Food Science and Technology. Volume 1. Jhon Willey and Son, Inc. New York.

Foodreview Indonesia vol III/ No.6 / Juni 2008.

Francis, F.J. 2002. Food Colorings. Dalam Color in Food Improving Quality. Macdougall, D.B.(Ed), 297-327. CRC Press, Boca Raton.

Glicksman. 1983. Food Hydrocolloids. CRC Press. Boca Raton FL.

Gustavo, V and Barbosa-Canovas. (1999). Food Powders : Physical Properties, Processing, and Functionality. Springer publiser. Texas.

Hernani dan Rahardjo, Mono. 2005. Tanaman Berkhasiat Antioksidan. Penebar Swadaya. Depok.

Hui, Y. H. 1992. Encyclopedia of Food Science and Technology. Volume II. John Willey and Sons Inc, Canada.

Imeson, A. 1999. Thickening and Gelling Agent for Food. Aspen Publisher Inc, New York.

Iradiasi. 1991. Iradiasi Pangan : Cara Mengawetkan dan Meningkatkan Keamanan Pangan. Terjemahan Hermana. ITB. Bandung.

Klose, R. and M. Glicksman. 1968. Gums. CRC Press Inc., Boca Raton, Florida.

Kujala, T.S.; M.S. Vienola; K.D. Klika; J.M. Loponen & K. Pihlaja. (2000). Betalain and Phenolic Composition of Four Beetroot (*Beta vulgaris*) Cultivars. European Food Research and Technology Vol. 214 No. 6.

Kuntz, L. A. 1998. Bulking Agent : Bulking up While Scalling Down. Weeks Publising Company.

Kuntz. Lynn A. 1997. Making The Most of Maltodextrins. Http://www.foodproductdesign.com.

Lachman, J.; A. Hejtmankova; M. Orsak; V. Pivec. (2005). Natural Antioxidants – Important Food Constituens in Human Nutrition for Healthy Life in Beginning Century. Departement of Chemistry Chzech University of Agriculture. Prague.

Loksuwan J. 2006. Characteristics of Microencapsulated β-carotene Formed by Spray Drying with Modified Tapioca Starch, Native Tapioca Starch and Maltodextrin. Food Hydrocolloids, 21: 928-935.

Marecik RB, Czapski J. The effect of selected compounds As inhibitors of enzymatic browning and softening of minimally processed apples Acta Sci. Pol., Technol. Aliment. 2007; 6(3): 37-49.

Mastuti, Retno., 2010. Pigmen Betalain pada Famili Amaranthaceae. Basic Science Seminar VII,FMIPA, UB, Malang.

Mc Donald, P., A. Edwards and J. F. D. Green Haigh. 1994. Animal Nutrition. 4th Ed. Longman Scientific and Technical. Copublishing in The USA

with John Wiley and Sons. Inc. New York.

Motlagh, S., Ravines, P., Karamallah, K.A., Ma, Q., 2006. The analysis of Acaciagums using electrophoresis. Food Hydrocolloid 20, 848–854.

Palvov Atanas, Petia Kovatcheva, Vasil Georgiev, Irina Koleva and Mlandenka Ilieva. (2002).

Biosynthesis and Radical Scavenging Activity of Betalain During The Cultivation of Red Beet (Beta vulgaris). Hairy Root Cultures. Bulgaria.

Rabah dan Abdalla, 2012. Decolorization of Acacia Seyal Gum Arabic. Annual Conference of Postgraduate Studies and Scientific Research Hall, Khartoum, Republic of Sudan. Vol 1. Hal 34.

Reid, M., S. Jack., L. Paul., and R.E.Young., (1980), Effects Of pH And Ethephon on Betacyanin Leakage From beet root discs, Plant Physiol.

Satuhu, S. dan A. Supriadi. 1994. Budidaya Pisang. Pengolahan dan Prospek Pasar. Penebar Swadaya. Jakarta.

Sitinjak, K dan D.J. Saragih. 1995. Teknologi Hasil Pertanian. Fakultas Pertanian Universitas Sumatera Utara. Medan.

Stephen, A. M. and S. C. Churms. 1995. Food Polysaccarides and Their Applications. Marcell Dekker, Inc, New York.

Straatsma J, Van Houwelingen G, Steenbergen AE, De Jong P. 1999. Spray Drying of Food Products: 2. Prediction of Insolubility Index. Journal of Food Engineering, 42: 73-77.

Subeki. 1998. Pengaruh Cara Pemasakan terhadap Kandungan Antioksidan Beberapa Macam Sayuran serta Daya Serap dan Retensinya pada Tikus Percobaaan [Disertasi]. Bogor: Sekolah Pascasarjana IP

PRODUCT INNOVATION (*LIDAH KUCING* PUMPKIN COOKIES AND PUMPKIN JAM) TO STRENGTHEN SMALL FOOD INDUSTRIES BASED ON PUMPKIN

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ABSTRACT

Product innovation is commonly regarded as a major success factor in aggressive and competitive food markets for both small and big food industries. As food technology, becoming a social responsibility to help small food industries to grow and face the competitive of food market. Pumpkin is one plant widely grown in Indonesia. The benefits and potential of pumpkin has been widely researched and proven contain high antioxidant, vitamin and mineral. This research have purpose to help small bussines food industries based on pumpkin in Getasan, Kopeng to develop a new inovative product. The objective is to make inovative product from pumpkin with simple method, easy to aplicate, but have highly added value. There are two innovation developed in this project, they are *lidah kucing* pumpkin cookies and pumpkin jam. This new product have great economic value, they can be sold at a good price range, and providing good profit for the small industries. The method used in this research are formulation of *lidah kucing* pumpkin cookies and pumpkin jam product. Also create marketing strategy to promote this product. Later, to reach national market acceptance can be set as the next goal, by utilizing modern marketing strategy, such as using social media for promoting, benchmarking, etc. Through those marketing strategies, in the long term, producing pumpkin food products can become the right arrow, to put a real step in food industry business. From this innovation product is then can enrich the product diversity and develop the entrepreneurship of local residents.

Keyword : *pumpkin, product innovation, cookies, jam, marketing*

1.INTRODUCTION

Small industries are the root of economic system in Indonesia. There are many small business, from garment, souvenir, until food business, which support and play important role in Indonesia's economic system. So that helping those small business to grow, is as same as helping strengthen economic system in Indonesia. As food technologist, becoming a social responsibility to help small food industries to grow and face the competitive of food market.

Pumpkin (*Cucurbita maxima Duch*) is local fruit from Indonesia which has high potential to contribute in food diversification. It has high nutrition such as protein, fat, carbohydrate, vitamin A, vitamin B, vitamin C, magnesium, and phosphor. Thus, this fruit has good effect for health especially for diabetic, hypertension, and alergic. Acceptable price and long shelf life of this pumpkin (Sudarto, 1993). It is another reason for processing it into functional product such as cookies and jam. Pumpkin is high in β-carotene, which gives it yellow or orange color. β-carotene in plants that have a pleasant yellow-orange color is a major source of vitamin A (Lee, 1983).

Getasan, one of a subdistrict in Semarang is located near tourism sites, Desa Wisata Kopeng. This site attracts local residents to participate and use the opportunity for industry, and food industry become one option. Getasan is also well known with its agriculture crop product. One of its biggest commodity is pumpkin. Because of that, processing pumpkin into a new creative and innovative products is potential to increase local residents' wealth.

Lidah kucing cookies is one of favorit in Indonesia. This cookies have a form like a cat's tongue, so called *lidah kucing* in bahasa. Innovation on lidah kucing cookies it what the market needs at this time. This is because the time is close with Eid al-Fitr Day, and also perfect time to launch new cookies product. And mostly people like this cookies.

Besides cookies, consuming bread with jam for breakfast now being a new habit for Indonesian. Therefore, production of pumpkin jam will provide food product for better taste and maintain people's health because the nutrition content. Jam was made from fruit and sugar (Margono, 1993). The aim of this research is giving an innovative way to produce pumpkin jam and *lidah kucing* pumpkin cookies with simple technology which is expected have high competitiveness.

2.MATERIALS AND METHODS

2.1.The Program Target

This project is included in Service Learning Program in Getasan Village, Semarang. This program's aim is to help local residents develop their local commodity, to encourage

entrepreneurship, and then give impact to their increasing income. This program was conducted in 2 local entrepreneur. The first is Ibu Nurdjanah. Ibu Nurdjanah has a home industry named Rumah Waloeh Rizky. This industry produce many kinds of creative pumpkin products. Now, this small industry has 11 kinds of product. The second one is Ibu Nanik, she also has a pumpkin based food home industry. Both of them distributed their product only in local areas.

2.2.*Lidah kucing* Pumpkin Cookies Making

Combine the butter and sugar in the bowl of a mixer, process until light and fluffy. Add egg whites, one at a time, mixing well after each. Add the flour, pumpkin and egg yolk, then mix it well. Put the batter into a piping bag, and pipe onto the sheet in a small and long shape. Bake them for 10 minutes (200 ^{0}C) until the cat's tongues are light golden brown. Cool the cookies on the baking sheet for seconds, and then transfer them to a wire rack to cool. Small sensory test are used to know the consumer acceptance for this product. In this research, preparation of pumpkin through three variables they are steamed, grated and use juicer then squeezes. And to find the best pumpkin cookies formulation, there are three formulation of each variable, the different is on the ratio between pumpkin and flour.

2.3.Pumpkin Jam Making

For making yellow pumpkin jam, firstly the pumpkin was mashed with blender and mixed with sugar then heated. After that, another material was added and stirred until dissolved.

Formulation 1 : Firstly the 1 kg pumpkin was mashed with blender and mixed with sugar then heated. Then, 2 g pectin and 1 kg sugar was added, stirred and put 3 g citric acid to heat together until boiled.

Formulation 2 : 167 g yellow pumpkin was mashed with blender and heated for 15 minutes together with 50 ml water. Then, 100 g sugar, 1,5 tsp citric acid, and 1 tsp agar powder was added gradually.

Formulation 3 : 250 g of yellow pumpkin shredded and heated. Then, 250 g sugar was added and stirred until dissolved. When the mixture started to thick, agar powder and lemon juice was added.

3.RESULTS AND DISCUSSION

In this research the small food industry is a pumpkin based food industry. This small business produce many traditional food products from pumpkin. For example *geplak*, syrup, stick, and so on. This business is started by small family since years ago, and now become quite popular in central java province. However, looking at the 12 years business history, we can say that their

achievement is still less satisfactory. After years build this business, their market are still limited to central java province only.

We tried analyzing the problem, and found that there are 2 major problems in their business, which are :

1. They tend to lack in marketing aspect.
2. They aren't productive in making new pumpkin product innovation

First, regarding marketing problem, their business lacks in strategy. Their marketing strategy up until now, is just utilizing mouth-to-mouth strategy, and sometime through local magazine, which isn't give much. Local magazine only can reach central java market, because it only being sold in that area. While for mouth-to-mouth is worse, because it only effective to inform the society that live near the production site. As the result, their market is stagnant, only circle around central java area for 12 years.

This is such a pity, to see that they cannot reach bigger achievement, while their products are good quality and affordable. They have what is needed to be success, but they just don't know how. Nowadays, internet and social media is a very powerful tool to inform the society. Because everyone can easily have access to them, whenever, and wherever they are. Realizing this fact, its good to decide upgrade their marketing strategy, by utilizing those two items. Internet and social media (using website and facebook application). Their record to promote business are already known and proven by other people before. Moreover, promoting through them don't require much amount of money. So in other word, tehy're effective and affordable. Very good and suitable combination for small business, which don't have much capital to begin with.

Second regarding new pumpkin food innovation. They only have some traditional products which are same and not developing since years ago. For example *geplak*, syrup, stick, and so on. As the matter of fact, those traditional foods actually are delicious. However, the problem here is the market being bored, because only meet same products from time to time. When there are another parties that produce more modern foods from pumpkin, their traditional food products start to lost in the market competition. For solving that problem, we tried to look for new pumpkin food product innovation. In this research, devided into two innovated product, they are *lidah kucing* pumpkin cookies and pumpkin jam.

3.1.*Lidah kucing* Pumpkin Cookies

Innovation *lidah kucing* cookies choose because this cookies liked by mostly people. And when this research did, its close by Eid al-Fitr Day. People who celebrate Eid al-Fitr Day, will need to buy delicious cookies to serve their family member, who come to their

home to strengthen their family bound. Looking at this moment, we believe that *lidah kucing* pumpkin cookies have big opportunity to be well accepted by society. It's extraordinary, delicious, full of nutrition, but still affordable one for serving even big family member. This cookies also can eat at normal day, because it is have a good taste, can became snack or can serve if guest come to our house.

For innovate *lidah kucing* pumpkin cookies, the flour will substitusied with pumpkin, so it would reduce the amount of flour used. In addition, pumpkin also give spesific unique flavor to the cookies. In this research, preparation of pumpkin through three variables they are steamed, grated and use juicer then squeezes. And for find the best formulation through three formulation combine with three variables of pumpkin preparation. Steamed, grated, and use juicer is choosen because all of that method is easy to aplicate, and doesnt need complex method to do, compare with pumpkin pumpkin powder making. Pongjanta *et al.*(2003) revealed that pumpkin powder produced by juice extraction and cabinet drying then ground with pin mill and sifted through an 80 mesh sieve was cheap to produce.

Formulations of variable using juicer and squeezed can successfully due to the way of water removed with better than any other way. Then using juicer, the pumpkin has a smooth pieces so the products can be slightly crunchy texture and strong pumpkin flavor (Table 1). After the formulation of the cookies, so followed by a small survey to inquire about the overall taste of the cookies. This survey also done by asking Ibu nanik and her husband about the overall taste of the final cookies.

3.2.Pumpkin Jam

Jam is a mixture of fruit that contains pectin and acid, also sugar added to make a thick and sticky but easy to spread (jam-like texture) mixture (BPTP Yogyakarta, 2013). Formulation became an important step to make the best quality and well accepted jam.

Table 1. Result of *lidah kucing* pumpkin cookies formulation

	Material	Formulation 1	Formulation 2	Formulation 3
Steamed	Margarine	100 gr	100 gr	100 gr
	Powdered sugar	100 gr	100 gr	100 gr
	Flour	50 gr	60 gr	75 gr
	Pumpkin	50 gr	40 gr	25 gr
	Egg whites	2	2	2
	Egg Yolk	1 tsp	1 tsp	1 tsp
Evaluation		Lost of pumpkin flavor, texture not crispy	Lost of pumpkin flavor, texture not crispy	Lost of pumpkin flavor, texture not crispy, but better than formulation 1 and 2
Grated	Margarine	100 gr	100 gr	100 gr
	Powdered sugar	100 gr	100 gr	100 gr
	Flour	50 gr	60 gr	75 gr
	Pumpkin	50 gr	40 gr	25 gr
	Egg whites	2	2	2
	Egg Yolk	1 tsp	1 tsp	1 tsp
Evaluation		Less pumpkin flavor, texture not crispy, there are chewy texture in gourd grate	Less pumpkin flavor, texture not crispy, there are chewy texture in gourd grate	Less pumpkin flavor, texture not crispy, there are chewy texture in gourd grate, but better than formulation 1 and 2
Use Juicer and squeezed	Margarine	100 gr	100 gr	100 gr
	Powdered sugar	100 gr	100 gr	70 gr
	Flour	50 gr	60 gr	75 gr
	Pumpkin	50 gr	40 gr	25 gr
	Egg whites	2	2	2
	Egg Yolk	1 tsp	1 tsp	1 tsp
Evaluation		The pumpkin flavor is tastes, texture crispy	The pumpkin flavor is tastes, texture crispy	The pumpkin flavor is tastes, texture more crispy than formulation 1 and 2, the sweetness good enough, because sugar revised to 70 gr.

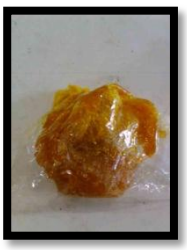

Figure 1. Result of Formulation 1, Formulation 2, Formulation 3

Table 2. Texture Result of Jam Formulas

Formula	Materials Added	Texture
1	Pectin + citric acid	Watery, too soft, less thick
2	Citric acid + agar powder + water	Hard, difficult to spread
3	Lemon + agar powder	Thick, sticky, soft

There were 3 formula tried in this project. The first formula used pectin and citric acid, the second used citric acid, agar powder, and water, and the third formula used lemon and agar powder.The result shows that different formula yield in differences of physical appearance of product (Table 2). The first formula, which used pectin and citric acid had a watery texture, too soft and less thick. This formula didn't show the jam-like texture, which is thick and a little bit sticky. The pectin is basically contained in the fruit, but it is necessary to add more pectin, to use as gelling agent in making the jam-like texture. Meanwhile, the citric acid is used to strenghten the taste of the fruit(BPTP Yogyakarta, 2013).The proper level of acidity is critical to gel formation in making jam. If there is too little acid, the gel will never set but if too much, the gel will lose liquid (Albrecht, 2010). Based on this reference, too much citric acid added can be a reason for the watery texture because the gelling formation is not happen. The second formula used citric acid, agar powder, and water. This mixture yield a hard texture of jam. Agar powder, alginate, pectin, carrageenan, and gelatin is gelling agent type

of hydrocolloids. This type disperse in water to give a thickening or viscosity producing effect. While all hydrocolloids thicken and impart stickiness to aqueous dispersions, a few biopolymers also have another major property of being able to form gels. Gel formation is the phenomenon involving the association or cross-linking of the polymer chains to form a three dimensional network that traps or immobilises the water within it to form a rigid structure that is resistant to flow. The textural and sensory properties also depend on the hydrocolloid employed (Glicksman, 1982). Adding too much agar powder could be the reason of the hardness of the jam.

The third formula shows the best texture and taste. This formula used lemon and agar powder. Citric acid can be replaced with lemon, which is more natural and easier to be found by local residents as Albrecht (2010) says that for fruit that low in acid content, such as pumpkin, material which contain acid ingredient can be used, such as lemon juice.Agar powder can act as gelling agent as well as pectin. The mixture of these more

natural materials yielded in best texture and taste of the jam.

Jam produced from formulation three then packaged into jam bottle. But, before filling the jam, the bottle was sterilized to kill all microorganism and prevent contamination (Winarno, 1994). Thus, the shelf life of jam will increase. This jam has market potential due to its taste and functional content.

4.CONCLUSION

The best formulation of *lidah kucing* pumpkin cookies using variable juicer and squeezed, then the best condition if we add sugar only 70 g. The best of jam making is formulation three, with condition of yellow pumpkin already shredded and heated, added with sugar and stirred until dissolved and started to thick, agar powder and lemon juice was added after that.

5.ACKNOWLEDGEMENT

This service learning base food technology program it just an example to help each other and to aplicate capability of the student. Through identification problem, innovation and marketing product in the real world, may help the student to more understand about society and their knowledge.

REFERENCES

Albrecht, J.A. (2010). Let's Preserves Jams, Jellies, and Preserves. http://ianrpubs.unl.edu/live/ec448/build/ec448.pdf. Accesed August, 22nd2014.

Badan Litbang Pertanian. (2013). Membuat Selai. Kementrian Pertanian Republik Indonesia. litbang.deptan.go.id. Accessed August 21st 2014.

Glicksman, M. (1982). Food hydrocolloids Vol. 1. CRC Publ, Boca Raton, Florida.

Lee, F.A. 1983. Basic Food Chemistry. AVI Publisher, Westport.

Margono, Tri. (1993). Buku Panduan Teknologi Pangan. <http://www.ristek.go.id> Accesed August 20th2014.

Pongjanta, J., Phomphang, U., Manon, T., Isarangporn, R. and Thaiou, T. 2004. The utilization of pumpkin powder in Thai Sweetmeal. Food Journal. Vol. 34 : 80-89.

Sudarto, Y. (1993). Budidaya Waluh. Kanisius. Yogyakarta.

Winarno, F.G. (1994). Sterilisasi komersial produk pangan. Gramedia Pustaka Utama. Jakarta.

FOOD SAFETY AND QUALITY

DEVELOPMENT OF NATURAL VEGETABLE SANITIZER FROM THAI LOCAL HERBS

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ABSTRACT

Nowadays, food safety becomes public concern, especially in fresh produce; fresh vegetables and fruits. The CDC has been reported at least 7 outbreaks from fresh produce in the past 3 years; for example, *Escherichia coli* O121 in raw clover sprouts (2014), *E. coli* O157:H7 in ready-to-eat salads (2013), *Salmonella* Saintpaul in cucumbers (2013), *Salmonella* Typhimurium and Newport in cantaloupe (2012), and *E. coli* O157:H7 in spinach and spring mix salad (2012). Washing, before consuming, is the most important step in order to prevent foodborne outbreak in fresh produces. Therefore, this research was aimed to develop vegetable sanitizer from crude extracts of *Acacia concinna* (Willd.) D.C (Som-poi), *Capsicum annuum* (Chili), *Cymbopogon citratus* (Lemongrass), *Citrus hystrix* (Kaffir lime) and *Chrysanthemum indicum* L. (Chrysanthemum) in order to reduce foodborne bacteria in fresh vegetable. These herbs have been reported for their antibacterial activity (Pitinidhipat and Yasurin, 2012; Utami *et al*., 2012; Saenghiruna and Yasurin, Lazuardi *et al*., 2013; 2013; Piya-isarakul and Yasurin, 2013; Saenghiruna *et al*., 2014; Dung *et al*., 2014). The 18 treatments with 3 different crude extracts percentage (0.5, 1 and 1.5%), 3 different volumes (3, 5, and 10 ml.) and 2 different soaking times (10 and 20 min) were investigated for their antibacterial synergistic activity using *Lactuca sataiva* (Lettuce) as vegetable model. The MPN, total place count on PCA, BHI agar for *Listeria monocytogenes* count, and SS agar for *Salmonella* sp. count were used to evaluate the antibacterial efficiency of vegetable sanitizer. The results showed that treatment 18 (using 10 ml of 1.5% crude extracts with 20 min soaking time) gave the highest bacterial efficiency statistically by vegetable sanitizer comparing with the commercial brand. The MPN number, the total plate count, and the *L. monocytogenes* count were 1.34 ±0.19 MPN/ml, 1.62 ± 1.02 log CFU/ml, and 1.31 ± 0.40 log CFU/ml, respectively. The *Salmonella* sp. was not found. The t-test has been done by using SAS on log CFU/ml with $P \leq 0.05$. It was found that crude extract percentage, used volume, and soaking time affected the antibacterial efficiency of this vegetable sanitizer.

Keywords: *Vegetable sanitizer, Thai Local Herb, Crude Extract, Foodborne bacteria*

1.INTRODUCTION

Nowadays, food safety becomes public concern, especially in fresh produce; fresh vegetables and fruits. The Centers for Disease Control and Prevention (CDC) has been reported at least 7 outbreaks from fresh produce in the past 3 years; for example, *Escherichia coli* O121 in raw clover sprouts (2014) [1], *E. coli* O157:H7 in ready-to-eat salads (2013) [2], *Salmonella* Saintpaul in cucumbers(2013) [3], *Salmonella* Typhimurium and Newport in cantaloupe (2012) [4], and *E. coli* O157:H7 in spinach and spring mix salad (2012) [5]. Washing, before consuming, is the most important step in order to prevent foodborne outbreak in fresh produces. Interestingly, Thai herbs has been used for a large range of purposes including medicine, nutrition, flavorings, beverages, dyeing, repellents fragrances, cosmetics, charms, smoking, and industrial uses. Therefore, this research was aimed to develop vegetable sanitizer from crude extracts of Thai herbs.

Acacia concinna (Willd.) D.C (Som-poi), *Capsicum annuum* (Chili), *Cymbopogon citratus* (Lemongrass), *Citrus hystrix* (Kaffir lime), and *Chrysanthemum indicum* L. (Chrysanthemum) have been reported for their antibacterial activity under different extraction conditions. [6] [7] [8][9][10] In addition, these herbs have been reported for their antibacterial activity and also contain innumerable constituents and are valuable sources of new and biological activity molecules possessing antimicrobial properties [11].

Therefore the objective of this research is to develop vegetable sanitizer from Thai local herbs; *A. concinna* (Willd.) D.C (Som poi), *C. annuum* (Chili), *C. citratus* (Lemongrass), *C. hystrix* (Kaffir lime), and *C. indicum* L. (Chrysanthemum).

2.MATERIALS AND METHODS

2.1.Preparation of crude extract

Plant samples: *A. concinna* (Willd.) D.C (Som -poi), *C. annuum* (Chili), *C. citratus* (Lemongrass), *C. hystrix* (Kaffir lime), and *C. indicum* L. (Chrysanthemum) were bought from local fresh market on Bangkapi, Bangkok, Thailand. The herbs were cut into small pieces and dried in air oven (Memmert, UM500) at 45°C for 20 hours. Then, dried herbs were blended by using blender to reduce the size of herbs. The herb powder was stored in refrigerator at 4°C until use.

2.2.Preparation of crude extract

The 20 g of herbs powder was weight on Top-loaded balance (1 decimal) (ZEPPER model ES-300) Then, 100 ml 95 % Ethanol was added and soaked for 24 hours at room temperature and shake at 120 rpm by shaking incubator (LSI-3016R model). After that, the liquid part was filtered out. Then, supernatant was evaporated paper by rotary evaporator at 120 rpm, 45°C for 15 minutes until supernatant become slurry crude extract. The crude extract was diluted to 0.2g/ml by using 95% ethanol. It was kept in freezer at -20°C until use.

2.3.Preparation of Som-poi water

The 100g *A. concinna* (Willd.) D.C (Som poi) power was boiled with 500 ml water for 30 minutes, stirred every 10 minutes. The temperature was in range between 95-98°C. Then Som-poi water was cool down to room temperature before using as vegetable sanitizer base.

2.4.Preparation of vegetable sanitizer

For one vegetable sanitize using the 10 ml Polysorbate Tween20 was weighed, mixed with 90ml Som-poi water and stirred for 15 minutes. Then, 0.2g Disodium-ethylenediaminetetraacetate (2NaEDTA) was added and stirred for 15 minutes. Then 4 types of 95% crude crude extracts; *C.*

annuum (Chili), *C. citratus* (Lemongrass), *C. hystrix* (Kaffir lime), and *C. indicum* L. (Chrysanthemum) were added with 3 different percentage (0.5, 1 and 1.5%). The formulas were in the table1.

Table1 The formula of vegetable sanitizer

95% Crude Ethanolic Extract 0.2 g/ ml	Percentage		
	Formula 1	Formula 2	Formula 3
Lemongrass *(C. hystrix)*	0.5	1.0	1.5
Chili (*C. annuum*)	0.5	1.0	1.5
Kaffir lime *(C. citrates)*	0.5	1.0	1.5
Chrysanthemum *(C. indicum* L.)	0.5	1.0	1.5

2.5.Antibacterial Assay

Lactuca sataiva (Lettuce) was used as a vegetable model. The lettuce was soaked. The swap method was used to evaluate antibacterial efficiency. The plate count agar (PCA), Brain Heart Infusion (BHI) agar, and Salmonella-Shigella (SS) agar were used for total plant count, *Listeria monocytogenes* count, and *Salmonella* sp. respectively. The most probable number (MPN) method was used for coliform bacteria count by using Lauryl sulfate tryptose (LST) broth. The 3×3×2 factorial design in the randomized complete block design (RCBD) was applied to this study. There were 3 formulas (formula 1, 2, and3), 3 used volume (3, 5, and 10ml), and 2 soaking time (10 and 20 min), total of 18 treatments. Each treatment was done duplicate 3 times independently. The results were compared with 2 commercial brands; Veggie and Jidrada.

2.6.Statistical Analysis

The data were analyzed using SAS (Statistical Analysis System for 158 Windows, Varsion 9.2, 2006, SAS Institute Inc., Cary, NC).

3.RESULTS AND DISCUSSION:

The vegetable sanitizer antibacterial efficiency is showed in table 2 – 5.

Table2 The MPN/ml

Note: Different superscript show significant different (P≤0.05)

Formula	Used Volume (ml)	Soaking Time (minutes	
		10	20
Formula 1	3	2.95 ± 2.57 b	2.38 ± 0 d
	5	2.50 ± 2.10 d	2.18 ± 0 f
	10	2.18 ± 0 f	1.88 ± 1.46 i
Formula 2	3	2.47 ± 2.16 d	2.380 ± 0 d
	5	2.26 ± 1.72 f	2.05 ± 1.19 f
	10	1.97 ± 0 i	1.75 ± 1.16 i
Formula 3	3	2.34 ± 1.24 d	2.05 ± 1.19 d
	5	2.18± 0 f	2.05 ± 1.19 f
	10	1.85 ± 0.80 i	1.34 ±0.19 j
Un-wash	-	3.04 ± 0.00 a	-
Wash	-	1.66 ± 0.00 c	-
Soak with tap water	-	2.36 ± 1.24 d	2.32 ± 0.00 d
Veggi	6	2.32 ± 0.00 d	1.42 ± 0.46 j
Jidrada	3	2.66 ± 0.00 c	0.92 ± 1.22 j

Table3 The log CFU/ml of total plate count on PCA

Formula	Used Volume (ml)	Soaking Time (minutes)	
		10	20
Formula 1	3	2.39 ± 0.70 b	2.31 ± 1.18 d
	5	2.29 ± 1.26 d	2.05 ± 0.76 f
	10	2.27 ± 0.76 f	2.21 ± 0.81 i
Formula 2	3	2.25 ± 1.40 d	2.15 ± 1 d
	5	2.21 ± 1.02 f	2.18 ± 1.01 f
	10	2.18 ± 1 i	2.11 ± 1.42 i
Formula 3	3	2.04 ± 1 d	2.03 ± 1.06 d
	5	2.24 ± 0.70 f	1.92 ± 1.18 f
	10	2.15 ± 1 i	1.62 ± 1.02 j
Un-wash	-	2.46 ± 1 a	-
Wash	-	2.30 ± 1.30 c	-
Soak with tap water	-	2.39 ± 1.51 d	2.26 ± 1.42 d
Veggi	6	2.04 ± 1.42 d	1.40 ± 0.64 j
Jidrada	3	2.32 ± 1.18 c	1.99 ± 1.32 j

Note: Different superscript show significant different (P≤0.05)

Table4 The Log CFU/ml of *Listeria monocytogenes* count on BHI agar

Formula	Used Volume (ml)	Soaking Time (minutes	
		10	20
Formula 1	3	1.84 ± 0.88 b	1.73 ± 1.02 d
	5	1.87 ± 0.72 d	1.94 ± 1.18 f
	10	1.92 ± 0.76 f	1.60 ± 1 i
Formula 2	3	1.81 ±1.15 d	1.74 ± 1.12 d
	5	1.73 ± 0.46 f	1.61 ± 0.74 f
	10	1.86 ± 1.06 i	1.65 ± 0.67 i
Formula 3	3	1.90 ± 1 d	2.13 ± 0.76 d
	5	1.740 ± 1 f	1.92 ± 0.76 f
	10	1.75 ± 0.76 i	1.31 ± 0.40 j
Un-wash	-	1.78 ± 1.30 a	-
Wash	-	1.73 ± 1.30 c	-
Soak with tap water	-	1.70 ± 11 d	1.71 ± 1.17 d
Veggi	6	1.67 ±0.76 d	1.15 ± 0 j
Jidrada	3	1.57 ± 0.40 c	1.73 ± 1.02 j

Note: Different superscript show significant different (P≤0.05)

Table5 The Log CFU/ml of *Salmonella* sp. count on SS agar

Formula	Used Volume (ml)	Soaking Time (minutes)	
		10	20
Formula 1	3	2.39 ± 0.70 b	2.31 ± 1.18 d
	5	2.29 ± 1.26 d	2.05 ± 0.76 f
	10	2.27 ± 0.76 f	2.21 ± 0.81 i
Formula 2	3	2.25 ± 1.40 d	2.15 ± 1 d
	5	2.21 ± 1.02 f	2.18 ± 1.01 f
	10	2.18 ± 1 i	2.11 ± 1.42 i
Formula 3	3	2.04 ± 1 d	2.03 ± 1.06 d
	5	2.24 ± 0.70 f	1.92 ± 1.18 f
	10	2.15 ± 1 i	1.62 ± 1.02 j
Un-wash	-	2.46 ± 1 a	-
Wash	-	2.30 ± 1.30 c	-
Soak with tap water	-	2.39 ± 1.51 d	2.26 ± 1.42 d
Veggi	6	2.04 ± 1.42 d	1.40 ± 0.64 j
Jidrada	3	2.32 ± 1.18 c	1.99 ± 1.32 j

Note: ND = Not Detectable

From table 2 to 4, un-wash and wash treatment gave the lowest bacterial efficiency statistically. The MPN number, the total plate count, and the *L. monocytogenes* count were 3.04 ± 0.00 a, 1.66 ± 0.00 c MPN/ml, 2.46 ± 1 a, 2.30 ± 1.30 c log CFU/ml, and 1.78 ± 1.30 a, 1.73 ± 1.30 c log CFU/ml, respectively. But use 0.5% crude extract the result show that The MPN number, the total plate count, and the *L. monocytogenes* count it is not different with soak with tap water treatment. When increasing the percentage of crude extract (at 1%) the result different from the 0.5% crude extract.

According to table 2-4, showed that highest crude extracts percentage, volumes, and soaking times gave better results in bacterial efficiency statistically.

This result indicates that crude extract concentration, used volume, and soaking affect on antibacterial efficacy. The result showed that using treatment 18; 10 ml of formula 3 with 20 min soaking time, gave the highest bacterial efficiency statistically. The

MPN number, the total plate count, and the *L. monocytogenes* count were 1.34 ±0.19 MPN/ml, 1.62 ± 1.02 log CFU/ml, and 1.31 ± 0.40 log CFU/ml, respectively with the 1.5% crude extracts, 10 ml. and 20 minutes for soaking times. While, the 0.5 and 1 % crude extracts, 3 and 5 ml, and 10 minutes for soaking times are not significant different.

For the commercial brand used 6 ml and 20 minutes for soaking times of Veggi brand. It gave the highest bacterial efficiency statistically. The MPN number, the total plate count, and the *L. monocytogenes* count were 1.42 ± 0.46 MPN/ml, 1.40 ± 0.64 log CFU/ml, and 1.15 ± 0 log CFU/ml, respectively.

When compare between Veggi brand; 20 min soaking time, and treatment 18; 10 ml of formula 3 with 20 min soaking time. As the result shows that The MPN number, the total plate count, and the *L. monocytogenes* count were 1.42 ± 0.46 j, 1.34 ±0.19 j MPN/ml, 1.40 ± 0.64 j, 1.62 ± 1.02 j log CFU/ml, and 1.15 ± 0 j, 1.31 ± 0.40 j log CFU/ml, respectively. The bacterial efficiency statistically are not significant different between Veggi brand and treatment 18. In general gram positive bacteria demonstrated more sensitivity to the spice. [12] Therefore, in treatment 18 use the formula contain chilli (*C. annuum*) and Chrysanthemum *(C. indicum L.*), it produce promising antibacterial substances against *B. cereus* and *L. monocytogenes* too. [19] So the *L. monocytogenes* (gram positive) are sensitive in this treatment 18.

In this experiment, the *Salmonella* sp. from table 5 was not found in the lettuce (*L. sataiva)*. So, we found that the crude extracts of herbs have effect in bacterial efficiency in this experiment.

However the using treatment 18; 10 ml of formula 3 with 20 min soaking time, gave the highest bacterial than the commercial brand because the activity of four herbs crude extracted (Lemongrass, chilli, kaffir lime, and Chrysanthemum) against the growth of MPN number, the total plate count, the *L. monocytogenes* count and the *Salmonella* sp. count. And also volume of sanitizer has effect in bacterial efficiency because the results show that when we use 10ml of sanitizer it better result compare with commercial brand. As the result, soaking time is the most important for this experiment. The better soaking is about 20 minutes has effect in bacterial efficiency.

In The Centers for Disease Control and Prevention (CDC) has been reported at least 7 outbreaks from fresh produce in the past 3 years; for example, the coliform bacteria as *Escherichia coli* O121 H7 in ready-to-eat salads, *Salmonella* Saintpaul in cucumbers, *Salmonella* Typhimurium in cantaloupe and *Listeria monocytogenes* in farm. In order to prevent foodborne outbreak in fresh produces so we need to reduce the microorganism in fresh vegetables and fruits from the natural herbs.

For the first herbs was use in this experiment, lemongrass (*C. citratus*), it was among the oils that exhibited antibacterial activity against all the *Listeria* strains tested and correlate to in research and found out that not only the oil form of lemongrass that showed activity against *L. monocytogenes* but also the ethanolic extraction form.[13]

Next, chilli (*C. annuum*) contains capsaicin which is reported as antimicrobial agent. [14] And also adding 1% w/v of dried chili in BHI can slightly inhibited the growth of *L. monocytogenes*. So increasing the amount of chilli might increase the inhibition activity. [15] Spices also rich in phenolic compounds and besides exerting antimicrobial effect they

may preserve the foods by reducing lipid oxidation as they are reported to have significant antioxidant activity. [16]

And, Kaffir lime (*C. hystrix*) peels also contain antimicrobial compounds. Kaffir lime (*C. hystrix*) peel crude ethanolic extract has antibacterial activity against of *Salmonella* sp. and other enterobacteria. Thus, not only kaffir lime peel has activity against *Sallmonella* sp. but also against *L. monocytogenes*.[17] The study of Kaffir lime show that the highest activity measured by using agar disc diffusion are 95% ethanolic extraction of kaffir lime peel. The inhibition effects of the extractions were 10.17 ± 1.96. Kaffir lime *C. hystrix*) peel crude ethanolic extract showed antibacterial activity against 20 serotypes of *Salmonella* sp. and 5 species of other enterobacteria. [18]

Chrysanthemum *(C. indicum L.*) it's always used in traditional drug formula for the treatment of several infectious disease such as pneumonia, colitis stomatitis, cancer, fever and sore and used to treat vertigo, pertussis and hypertensive symptom.[19] In Chrysanthemum *(C. indicum L.*) has antibacterial activity 1.17 ± 0.85 mm in vitro antibacterial screening results of crude 95% ethanolic extracted under normal stress so it contain antimicrobial compounds.[20]

Finally, Som poi (*A. concinna* (Willd.) D.C) is an important medicinal plant in Thailand and throughout Asian countries. This investigation was performed in order to demonstrate the antimicrobial potential against the fungal causative agents of ringworm and opportunistic infections. The study showed that the crude extract of *A. concinna* pod consisted of alkaloids, flavonoids, saponin and tannin but none of antraquinone and cyanotic glycosides. [21]

4.CONCLUSION

As the results showed that treatment 18 (using 10 ml of 1.5% crude extracts with 20 min soaking time) gave the highest bacterial efficiency statistically by vegetable sanitizer comparing with the commercial brand. The MPN number, the total plate count, and the *L. monocytogenes* count were 1.34 ±0.19 MPN/ml, 1.62 ± 1.02 log CFU/ml, and 1.31 ± 0.40 log CFU/ml, respectively, (P≤0.05). The *Salmonella* sp. was not found. And also, it was found that the difference in the activity was found when using different volume of vegetable sanitizer and soaking time.

REFERENCES

1. The Centers for Disease Control and Prevention (CDC) (2014), Investigation Announcement: Multistate Outbreak of Shiga toxin-producing *Escherichia coli* O121 Infections Linked to Raw Clover Sprouts.

2. The Centers for Disease Control and Prevention (CDC) (2013), Investigation Announcement: Multistate Outbreak of Shiga toxin-producing *Escherichia coli* O157:H7 Infections Linked to Ready-to-Eat Salads

3. The Centers for Disease Control and Prevention (CDC) (2013), Investigation Announcement: Multistate Outbreak of *Salmonella* Saintpaul Infections Linked to Imported Cucumbers

4. The Centers for Disease Control and Prevention (CDC) (2012), Investigation Announcement: Multistate Outbreak of *Salmonella* Typhimurium and Salmonella Newport Infections Linked to Cantaloupe

5. The Centers for Disease Control and Prevention (CDC) (2012), Investigation Announcement:

Outbreak of *E. coli* O157:H7 in spinach and spring mix salad

6. Pitinidhipat N, and Yasurin P, 2012, Antibacterial Activity of *Chrysanthemum indicum*, *Centella asiatica* and *Andrographis paniculata against Bacillus cereus* and *Listeria monocytogenes* under Osmotic Stress, AU.J.T.,15(4), p.239-245

7. Saenghiruna T and Yasurin P, 2012, Antibacterial activity of Thai red curry paste's ingredients between using Kang-Pa and Kang-Kati model on *Salmonella Enterica* 4,5,12:i:- (human) US clone, The 1st KMITL Agro-Industry Conference proceeding, The Emerald Hotel, Bangkok

8. Vania Utami C, Pitinidhipat N, and Yasurin P, 2012, The Antibacterial Activity Study of *Chrysanthemum indicum*, *Centella asiatica* and *Andrographis paniculata* on *Bacillus cereus* and *Listeria monocytogenes* under Low pH Stress, KMITL Sci. Tech J., 12(1),p.49-54 Thailand, p.141-148

9. Lazuardi I, Saenghiruna T, and Yasurin P, 2012, Natural Antimicrobial Activity of Thai Red Curry's Herbs on *Salmonella Typhimurium* DT104b, AU.J.T.,16(1), p.1-6

10. Dung Thi Ho N, Saenghiruna T, and Yasurin P. (2014), The individual antibacterial activity of Thai red curry paste's dry ingredients against *Listeria monocytogenes* using different ethanolic extraction. International Conference on Sustainable Global Agriculture and Food Security 2014, 16 – 18 July 2014, The Emerald Hotel, Bangkok, Thailand, p.113-116.

11. Negi, P. S. (2012). Plant extract for control of bacterial growth: Efficacy, stability and safety issue for food application. Int J. Food Microbial 156:-17

12. SHELEF, L.A., NAGL K, O.A. and BOGEN, B.W. 1980. Sensitivity of some common food borne bacteria to the spices sage, rosemary and allspice. J. Food Sci. 45, 1042–1044.

13. Lis-Balchin, M.; and Deans, S. G. (1997). Bioactivity of selected plant essential oils against *Listeria monocytogenes*. J. Appl. Microbiol. 82: 759-762.

14. Cichewicz, R. H. and Thorpe, P. A. 1996, The antimicrobial properties of chile peppers (Capsicum species) and their uses in Mayan medicine Journal of Ethnopharmacology. 52: 61-70

15. Khuwijitjaru, P., Chalooddong, K and Adachi, S. 2008. Phenolic content and radical scavenging capacity of kaffir lime fruit peel extracts obtained by pressurized hot water extraction. Food Sciences Technology Res. 14: 1-4

16. Schwarz, K.; Bertelsen, G.; Nissen, L. R.; Gardner, P. T.; Heinonen, M. I.; Hopia, A.; Hyun-ba, T.; Lamberet, P.; McPhail, D.; Skibsted, L. H.; and Tijburg, L. (2001). Investigation of plant extracts for the protection of processed food against lipid oxidation. Comparison of antioxidant assays based on radical scavenging, lipid oxidation and analysis of the principal antioxidant compounds. European Food Research and Technology. 212: 319-328.

17. Nanasombat, S.; and Lohasupthawee, P. (2005). Antibacterial activity of crude ethanolic extracts and essential oils of spices against *salmonella* and other enterobacteria. KMITL Sci. Tech. 5(3): 527-538.

18. Dung Thi Ho N, Saenghiruna T, and Yasurin P. (2014) The individual antibacterial activity of Thai red curry paste's dry ingredients against *Listeria monocytogenes* using different ethanolic extraction. International Conference on Sustainable Global Agriculture and Food Security 2014, 16 – 18 July 2014, The Emerald Hotel, Bangkok, Thailand, p.113-116.

19. Eum-Kyung Jung: Chemical Composition and Antimicrobial Activity of the Essential Oil of *Chrysanthemum indicum* Against Oral/bacteria. Joural of Bacteriology and Virology 2009, 30(2): 260-264

20. Pitinidhipat N, and Yasurin P, (2012), Antibacterial Activity of *Chrysanthemum indicum*, *Centella asiatica* and *Andrographis paniculata against Bacillus cereus* and *Listeria monocytogenes* under Osmotic Stress, AU.J.T.,15(4), p.239-245

21. Wuthi-udomlertl M. and Vallisuta , (2011), In vitro Effectiveness of Acacia concinna Extract against Dermatomycotic Pathogens. Journal, 2011; 3(19):69-74

CHANGES IN SENSORY CHARACTERISTICS, MOISTURE CONTENT AND WATER ACTIVITY OF SEVERAL FISH SPECIES CAUSED BY SMOKING PROCESS

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ABSTRACT

Smoked fish is one of seafood product which usually done by smoking process. Smoking process usually used because it can give a specific odour, unique taste, and also it can extend the self life of the product. Since it can make a new diversification product, smoking process also give effects to the smoked products. This paper was made to observe quality changes caused by smoking process based on direct observation at UD Pengasapan Indah, Demak, Central Java; in terms of sensory characteristics, moisture content, and water activity of several fish species;marine catfish (*Arius thalassinus)*(A), baby tuna (*Euthynnusaffinis)* (B), sardines (*Sardinella longiceps)*(C) and semar fish (*Mene maculata)*(D). The results showed that smoking process changed the physical characteristics of fish, after smoked for 30 minutes, fish turned to brown, had a med-compact texture and gave a nearly specific smoked fish taste and smell. The moisture contentand water activity of A; B; C; and D samples were found 72,82% and 0,947;69,50% and 0,936; 64,91% and 0,932; and 68,15% and 0,943 respectively.Generally,smokingprocess affected smoked fish products in terms of moisture content, water activity, and changes in sensory characteristics.

Keywords: *sensory characteristics, moisture content, water activity, smoking process*

1.INTRODUCTION

Smoking is a traditional method used to preserve fish in the world, although today, its acceptance in developed countries is primarily based upon the sensory characteristics it imparts to the product (Doe, 1998). Smoking preserves fish by drying, cooking and depositing natural wood–smoke chemicals like tars,phenols and aldehydes all of which have powerful bactericidalaction and prevent the growth of other microorganisms on the flesh of the fish (Aremu *et al*, 2013). An additional preservative effect is owed to salting which comprises the first step of the fish smoking process. However, smoking is not an absolute preserving method. For this reason, the quality of raw material, the concentration of salt, water activity of the fish, heat through the smoking process, the quantity of smoke, the way of packaging, hygienic circumstances and heat of storage have the most important effects to reduce the risk of deterioration (Kaya and Erkoyuncu, 1999).

Smoked fish usually processed traditionally especiallyin Indonesia, this process has already being implemented for generations. Many Indonesia's countrysides developing this method as one of the best fish processing method because the end products of this processing method as reported by Abolagba and Igbinevbo (2010), Bower et al. (2009) and Kumolu-Johnson et al. (2010) characterized by specific taste and aroma, had a long shelf life due to antibacterial activity and reduced enzymatic activity.

Direct observation had been done at UD Pengasapan Indah, Demak, Central Java, June 13th, 2014, which is the biggest centre of fish smoking processing in Central Java. Many peasants around its location used to working there. According to that, fish that processed as the main material of smoking process there, are; marine catfish (Arius thalassinus), baby tuna (Euthynnus affinis),sardines (Sardinella longiceps), and semar fish (Mene maculata). The process usually took 30 minutes untill an hour according to the fish size, but it is possible to take more time.

This paper was made to observethe effects of smoking process in case of; sensory characteristics changes, water content, and water activity pointof thus four fish species, and find out what causes it.

2. MATERIALS AND METHODS

2.1. Materials

Marine catfish (*Arius thalassinus*) (head only) about 25 kg/day the average weight of the fish head was 1,063±134.58 g and average length was 12.71±2.24 cm, baby tuna (*Euthynnus affinis*) about 25 kg/day the average weight of the whole fish was 132.88±15.58 g and average length was 16.10±2.32 cm, sardines (*Sardinella longiceps*) about 25 kg/day the average

weight of the whole fish was 102.05±9.79 g and average length was 13.95±3.36 cm, and semar fish (*Mene maculata*) about 25 kg/day the average weight of the fish head was 94.65±3.45 g and average length was 10.80±2.25 cm; imported from Indramayu, West Java; Rembang; and Semarang, Central Java. Dried Cocconut shells 1 sack/day, and dried Corncob 2 sacks/day.A moisture analyzer and an A_w meter in the Laboratory of Fish Processing Technology Department, Faculty of Fisheries and Marine Science, University of Diponegoro, Semarang.

2.2. Methods

2.2.1. Sample Preparation and Smoking Process

First fish arrived, 100 kg fish were divided into 4 smoking houses, 25 kg fisheach for the same species. Fish then washed and dripped in the salt solution for several minutes. Then fish were stabbed using arrow shaped bamboo from the fish mouth to the opposite part, or usually to the fish tail, vertically. After it done, wrap the fish stomach with a piece of thin paperto prevent breaking of the stomach contents at a time when it smoked. Meanwhile, the kiln was prepared, burn the corncob and cocconut shells fuels and wait until no flame, only smoke come out. After everything was set, then fish were smoking on the kiln for about 30 minutes for sardines (*Sardinella longiceps*) and semar fish (*Mene maculata*); and about 45 minutes for marine catfish (*Arius thalassinus*) and baby tuna (*Euthynnus affinis*).

2.2.2. Laboratory Analysis

Each of smoked fish were brought to the laboratory of fish processing, Fish Processing Technology Department, University of Diponegoro, Semarang to determining the moisture content and water activity of the products. Moisture content was determined by oven drying of 5 g of fish muscle at 105°C until a constant weight was obtained (AOAC, 1995, Method 985.14). Results are expressed as g water/100 g muscle or can be written in percent (%).Water activity (Aw) of samples were measured following the method as described by Fuentes *et al*. (2010) using Aw meter.

3. RESULTS AND DISCUSSIONS

3.1 Sensory Characteristics Changes during Smoking Process

From fig. 1 below, we know thatsmoked marine catfish (*Arius thalassinus*) has a better score than the other smoked fish, and it was acceptable by the panelists. Smoked marine catfish (*Arius thalassinus*) hasshiny yellowish brown color on its surface, but there are some parts that dark-colored (over burning).This smoked fish also smell and taste better than the other smoked fish, it has a nearly perfectly specific smoked fish for its odour and taste, even though the taste and odor still are not too strong. But it gave a better results than the other three do. Baby

tuna (*Euthynnus affinis*), sardines (*Sardinella longiceps*), and semar fish (Mene maculata) did as well as accepted by the panelists, but the appearance was not as specific as what marine catfish gave, they also had a unique odour and taste but not as strong as what marine catfish had, and it was actually still in the below average range.

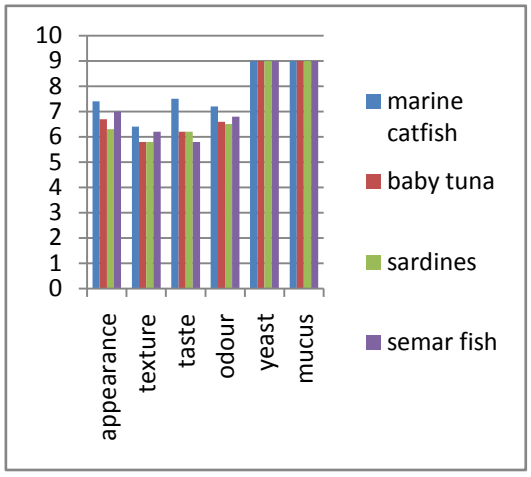

Fig 1. Sensory score of several smoked fish from UD Pengasapan Indah, Demak, Central Java.

However, the texture and odour of all samples were almost the same, and there were no yeast nor mucus on the surface, so the products were possible to consumed.

Appearance, the smell or odour, color and texture of smoked fish formed as a result of certain cluster carbonyl contained in smoke reacted to the protein and fat in fish. Smoke plays an important role in the formation of color, texture, taste and odour. The main components carbonyl smoke that played an important is phenol. These components, can play a role as antioxidants. Brown color, is produced from a reaction phenol with oxygen in the air, phenol components that have a role in smell and taste is guaiakol, 4---methyl guaiakol, 2.6---dimetoksi phenol. Smoke role in this case have an impact on the organoleptic, was caused by the reaction of amino acids, proteins and carbohydrates (Cardinal et al. , 2006; Swastawati, 2008; Swastawati., et al. , 2007).

According to Simko (2005), some factors such as physico-chemical quality of fresh fish, age, sex and season variation, and smoking process itself (smoke resources, smoke components, smoking temperature, humidity, time of smoking process and smoke density) could affect the organoleptic properties of end products as well.

3.2. Laboratory Analysis

Water is the largest componen that contained in a fish flesh. It usually used by microorganisms to grow and multiply. So that, smoking process applied to reduce the content of water in fish flesh, and it is hoped to extend the self life of the product (Swastawati, 2013). According to the laboratory analysis results, given that the moisture content and water activity of A; B; C; and D samples were found 72,82% and 0,947; 69,50% and 0,936; 64,91% and 0,932; and 68,15% and 0,943 respectively. It is believed that the differences were due to quality of fresh fish and species differentiation. However, according to

Indonesia's National Standard the moisture content suggested for smoked fish should be less than 60%. Winarno (2010) said that the water content reduced due to evaporation of the product because of the influence of heating temperature and environment humidity.

Water activity (Aw-point) becoming one of parametersin the analysis of food stability (Lupin, 1986). Measuring water activity is very important to predict and knowing the existence of microorganisms that usually cause spoilage on food products. Generally,fresh food have Aw point from 0,95 - 1. At 0,6 of Aw point microorganisms can be blocked. Otherwhile, according to Soedarto's (2008) journal, at Aw of 0,5 microorganisms are not possible to grow. Aw point from 0,91 – 0,92 can have a short self life; about 2-3 days, which mean that all of smoked fish that were brought from UD Pengasapan Indah, Demak, may can be keep in storage for about 40-55 hours.

4. CONCLUSION

Smoking process affected fish in case of sensory characteristics changes, moisture content, and Aw point**.** The results of smoked fish from UD Pengasapan Indah, Demak, Central Java need to be improved, because of some several tests showing an average – below average results. It was predicted that, those are because of improper smoking method applied and lower aspect of hygenetic and sanitazion. Therefore it is recommended to use different smoking method, such as liquid smoking method to improve the quality characteristics and the organoleptic characteristics of end products.

REFERENCES

Abolagba O. J., and Igbinevbo E. E. 2010. Microbial Load of Fresh and SmokedFish marketed in Benin Metropolis Nigeria. J. Fish. Hydrobiol. 5(2):99-104.

AOAC. 1995. Official methods of analysis. Association ofOfficial Analytical Chemists. Official methods,985.14. Gaithersburg, MD.

Aremu, M. O., S. B. Namo., R. B. Salau., C. O. Agbo., and H. Ibrahim. 2013. Smoking Methods and Their Effects on Nutritional Value of African Catfish (*Clarias gariepinus*). The Open Nutraceuticals Journal, 2013, 6, 105-112.

Bower C. K., Hietala K. A., Oliveira A. C. M., and Wu T. H. 2009. Stabilizing Oils from Smoked Pink Salmon (*Oncorhynchus gorbuscha*). J. Food Sci. 74(3):248-257.

Cardinal M., Cornet J., Serot T., and Baron R. 2006. Effects of the Smoking Process on Odour Characteristics of Smoked Herring (*Clupea harengus*) and Relationships with Phenolic Compound Content. Food Chem. 96:137-146.

Doe, P.E. 1998. Fish Drying and Smoking Production andQuality., Technomic Publishing, Lancaster, PA.: 89–115.

Fuentes, A., Fernandez S. I., Barat J. M., Serra J. A. 2010. Physicochemical Characterization of Some Smoked and Marinated Fish Product. J. Food Process. Preserv. 34:83-103.

Kaya, Y. and Erkoyuncu, İ. 1999. Değişik DumanlamaMetotlarının Balık Türlerinin Kaliteleri ÜzerineEtkisi. O.M.Ü. Ziraat Fakültesi Dergisi, 14: 93-105.

Kumolu-Johnson C. A., Aladetohun N. F., Ndimele P. E. 2010. The Effect of Smoking on the Nutritional Qualities and Shelf-life of *Clarias gariepinus* (Burchell 1822). Afr. J. Biotechnol. 9(1):073-076.

Lupin, H.M. 1986. Water Activity and Preserved Fish Product. In Cured FishProduction In The Tropics Ed. Reilly A.and L.E Berile. Pub. Coll. Fish Univ.Philippine in Vijaya-GTZ.

Simko, P. 2005. Factors Affecting Elimination of Polycyclic Aromatic Hydrocarbons from Smoked Meat Foods and Liquid Smoke Flavourings. –A Review. Mol. Nutr. Food Res. 49:637-647.

Soedarto and Hario P. S. 2013. Response of Hot Smoke Milk Fish (*Chanos chanos*) Quality with Length of Drainage. Berkala Ilmiah Perikanan Vol. 3 No. 1, April 2008.

Swastawati,Fronthea.,TriWinarniAgustini.,Y. S.Darmanto.,EkoNurcahyaDewi.2007. LiquidSmokePerformanceofLamtoro WoodandCornCob.JournalofCoastalDevelopment,Vol.10,Number3,June2007: 189---196.

Swastawati,Fronthea.2008.QualityandSafety ofSmokedCatfish(*Ariustalassinus*)UsingPaddyChaffandCoconutShellLiquidSmoke.JournalofCoastalDevelopmentVol.12No.1,October2008:47---55.

SwastawatiFronthea.,TitiSurti.,TriWinarniAgustini., and PututHarRiyadi. 2013. Quality Characteristics of Smoked Fish Processed with Different Method on Different Fish Species. Journal of Food Application Technology. Vol. 2No.3,Th.2013.

Winarno, F. G. 2010. *Kimia Pangan dan Gizi*. PT Gramedia Pustaka Utama. Jakarta.

UTILIZATION OF CORNCOB WASTE AS THE RAW MATERIAL OF GLUCOSE PRODUCTION

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ABSTRACT

Corncob, a kind of waste produced by agriculture and food industry, is abundant and less utilized. Corncob contains a high amount of cellulose, a kind of carbohydrate polymer, so it is potential to be utilized as the raw material to produce glucose. This study is aimed to figure out a method to convert cellulose in corncob into glucose and its potential to be comercially produced, therefore a literature review was conducted. Glucose can be produced by hydrolysis of cellulose either by acid or cellulase enzyme. However, the use of acid result in a low quality and poor yield of glucose, while the use of cellulase enzyme will produce a better quality and higher yield of glucose. Cellulase enzyme can be produced by mold *Aspergillus niger* and *Trichoderma reesei* in ratio 2:1. Overall, the method to use corncob to produce glucose following : raw material preparation, delignification, growing *A. niger* and *T. Reesei* culture, production of cellulase enzyme, extraction of enzyme, and hydrolysis of raw material. The yield of glucose produced by enzyme hydrolysis might reach 51% that make it possible to be comercially produced, however there is still a need for further study to find out an appropriate method to purify the glucose produced and the expert's role to build the plant.

Keywords: *Corncob, cellulose, enzyme, glucose, hydrolysis, waste*

1.INTRODUCTION

Corncob, a kind of waste produced by agriculture and food industry, is abundant and less utilized. The number of corncob waste generated from corn production is about 30%, so if it is calculated with the number of corn produced in Indonesia that is about 19.380.000 tons per year, the number of corncob waste generated is about 5.814.000 tons per year. Up to now corncob utilization is still limited, mostly for animal feed and slightly for energy source (Irawadi, 1990). The chemical composition of corncob is shown in Table 1.

Table 1. Chemical Composition of Corncob

Compound	Percentage (%)
Water	9,4
Cellulose	41
Hemicellulose	36
Xilan	30
Lignin	6
Pectin	3
Starch	0,014
Protein	2,5
Fat	0,5
Fiber	32
Ash	1,5
Free Nitrogen Extract	53,5
Neutral detergen fiber	83
Digestable nutrient	42

(Johnson,1991)

Table 1 shows that corncob contains high amount of dietary fiber, predominantly cellulose. The number of cellulose contained in corncob could reach 41% that make it potential to be utilized.

Cellulose, the most abundant polymer on earth, is a carbohydrate polymer in form of linear chain linked by β-1,4-glycosidic bond, hence cellulose is highly unsoluble. Cellulose cannot be easily degradate either by chemical or mechanical, however it still can be broken down into sugar or ethanol. Cellulose can be converted into glucose by hidrolysis either by enzyme or acid (Ariestaningtyas, 1991). This study is aimed to figure out a method to convert cellulose in corncob into glucose and its potential to be comercially produced, thus the corncob waste shall be optimally utilized.

2.MATERIAL & METHOD

Literature review was conducted to collect information about corncob, cellulose and hydrolysis methods. The literature used are scientific journals, papers and articles. All the information gained were analyzed to draw a conclusion about possibility and the most appropriate method to utilize corncob for glucose production.

3.RESULT & DISCUSSION

Glucose can be produced by hydrolysis of cellulose either by acid or cellulase enzyme. Acid hydrolysis of cellulose usually involving strong acid such as sulfuric acid, percloric acid and mostly HCl (hydrochloric acid). The hydrolysis process is done by heating the substrate (cellulose) in acid solution for 1-3 hours at 121^{o}C. The harsh condition (high temperature, high acid concentration) will liberate glucose from associated chains. Then, glucose can be obtained by adding $Ca(OH)_2$ into the solution (Ahring, Klinke dan Thomsen, 2004).

Acid hydrolysis method is quite simple compared to the enzyme hydrolysis, however, the harsh condition of process results in low quality of glucose. The glucose obtained from acid hydrolysis has low DE (Dextrose Equivalent) value and yellowish color due to the formation of hydroxymethyl furfural (HMF) as the result of the harsh condition, especially high temperature (Sastrodipuro, 1985). Moreover, during acid hydrolysis the degradation of cellulose chain is going randomly, so the glucose obtained is less stable and uniform (Howling, 1979).

The other method to hydrolyze cellulose is using cellulase enzyme. Cellulase enzyme is a complex enzyme, consist of several enzymes. Cellulase enzyme can be obtained from fungi, *Aspergillus niger* and *Trichoderma reesei*, each produces different characteristic of enzyme that is shown in Table 2.

Table 2. Characteristic of Cellulase Enzyme Produced by A. Niger and T. Reesei

Aspergillus niger	***Trichoderma reesei***
High β-glucosidase	Low β-glucosidase
Low endo-β-1,4-glucanase	High endo-β-1,4-glucanase
Low exo-β-1,4-glucanase	High exo-β-1,4-glucanase

Table 2 shows that *A.niger* produce high β-glucosidase and low β-glucanase, on the contary *T.reesei* produce high β-glucanase and low β-glucosidase. During cellulase hydrolysis, β-glucanase enzyme degradate cellulose into cellobiose and β-glucanase convert cellobiose into glucose. Therefore the cellulase enzyme must be produced by both *A.niger* and *T.reesei* in ratio 2:1 (Sutarno, R.J., Zaharah,T.A. dan Idiawati,N., 2013). Overall the method to produce glucose by cellulase hydrolysis following :

a. Raw Material Preparation
   Corncob should be washed, dried and grinded before it is hydrolyzed.
b. Delignification
   Delignification is aimed to remove lignin and extracting hemicellulose so that hydrolysis process might goes effectively. Delignification is done by immersing the raw material in NaOH solution. After delignification, raw material should be re-dried.
c. Growing *A.niger* and *T.reesei* Culture
   PDA media can be used to grow *A.niger* and *T.reesei* culture. The incubation time might vary from 3 days to 1 week.
d. Production of Cellulase Enzyme
   To produce cellulase enzyme, both *A.niger* and *T.reesei* are inoculated in prepared corncob. During their growth, both mold will produce extracellular enzyme (cellulase) to digest cellulose.
e. Extraction of Enzyme
   Cellulase enzyme is extracted using Tween 80 solution.
f. Raw Material Hydrolysis

The enzyme obtain from *A.niger* and *T.reesei* is applied onto prepared corncob in ratio 2:1 (*A.niger* : *T.reesei*). To obtain optimum condition, the mixture shoud be heated at 40^{o}C and added by HCl until the pH reach 5. The incubation time takes 8 hours (Sutarno, Rika Julfana, *et al.*, 2013).

The yield of glucose obtained from cellulase hydrolysis might reach 51%. Overall, glucose obtained from cellulase hydrolysis has better quality than acid hydrolysis, which comparison is shown in Table 3.

Tabel 3. Comparison of Acid Hydrolysis and Cellulase Hydrolysis Method in Glucose Production

Criteria	Method	
	Acid Hydrolysis	Cellulase Hydrolysis
Yield	Low	High
Raw Material	Cellulose, Starch (amylose)	Cellulose
Production Cost	High	Low
Production Time	1-3 hours	8 hours
Glucose Characteristic	Low value DE, yellowish color	High value DE, clear and transparent color

(Kosaric, Wieczorec, Cosentino, Magee dan Presonil, 1983; Lynd *et al.*, 2002)

Based on Table 3, cellulase hydrolysis method is better than acid hydrolysis method. The low production cost, high yield and good quality of glucose produced indicate that it is possible to commercially produce glucose from corncob.

4.CONCLUSION

Corncob, an abundant waste, is very potential to be utilized due to its high amount of cellulose. Cellulose can be converted into glucose either by acid hydrolysis or cellulase hydrolysis. Nevertheless cellusase hydrolysis is preferable since its produce better quality of glucose. Cellulase enzyme can be obtained from *Aspergillus niger* and *Trichoderma reesei*. The low production cost, high yield and good quality of glucose produced indicate that it is possible to commercially produce glucose from corncob. However there is still a need for further study to find out an appropriate method to purify the glucose produced and the expert's role to build the plant.

REFERENCES

Aguirar, C.L. (2001). Biodegradation of cellulose from sugar cane bagasse by fungal cellulose. *Science Technology Alignment*, 3(2), 117-121.

Alam, M. Z., Manchulur M. A., Anwar M. N. (2004). Isolation purification, characterization of cellulolytic enzyme produced by the isolate streptomyces omiyaensis. Pakist J Biol Sci 7(10):16471653.

Anindyawati, Trisanti. (2009). Prospek enzim dan limbah lignoselulosa untuk produksi bioetanol. Pusat Penelitian Bioteknologi-LIPI, Cibinong.

Anonim[a]. (2008). http://safan.wordpress.com. Bioenergi alternatif dipublikasikan pada 21 Agustus 2008.

Ariestaningtyas, Y. (1991). Pemanfaaatan tongkol jagung untuk produksi enzim selulase oleh *Trichoderma viride*. Skripsi. Fateta IPB. Bogor.

Fridayani. (2006). Produksi sirup glukosa dari pati sagu yang berasal dari beberapa wilayah di Indonesia. Institutut Pertanian Bogor, Bogor.

Irawadi, T.T. (1990). Selulase. PAU Biotek. Institut Pertanian Bogor, Bogor.

Kosaric, H., A. Wieczorec, G, P. Cosentino, R. J. Magee dan J. E. Presonil. (1983). Ethanol fermentation. Di dalam H. Dellweg (ed). Biotechnology VolumeIII. Verlag Cheme, Weinheim.

Lynd, L.R., et al. (2002). Microbial cellulose utilization: fundamentals and biotechnology. Microbiol. Mol. Biol. Rev. 66(3):506-577.

Shofiyanto, M. Edy. (2008). Hidrolisa tongkol jagung oleh bakteri selulolitik untuk produksi bioetanol dalam kultur campuran. Fakultas Teknologi Pertanian IPB. Bogor.

Suprapto, H.S. dan Rasyid, M.S. (2002). Bertanam jagung. Penebar Swadaya, Jakarta.

Sutarno R.J., Zaharah,T.A. dan Idiawati,N. (2013). Hidrolisis enzimatik selulosa dari ampas sagu menggunakan campuran selulase dari *Trichoderma reesei* dan *Aspergillus niger*. JKK, volume 2(1), halaman 52-57.

FOOD NUTRITION AND TECHNOLOGY

THE USE OF BLACK SOYBEAN MILK AND BLACK RICE FLOUR EXTRUDATES AS A SOURCE OF ANTIOXIDANT IN FORMULATIONS OF VEGETARIAN ICE CREAM BASED ON SENSORY ANALYSIS

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ABSTRACT

Black soybeans and black rice widely known contain anthocyanins. Anthocyanins have high antioxidant activity. Antioxidant has capability in delaying, slowing or inhibiting the oxidation reaction. Ice cream is a food product that is favored by many people from all ages because of its sweet tastes and its textures which melt in the mouth. Unfortunately, most of the ice cream products do not contain a functional compounds e.g. natural antioxidant compounds. Therefore, this researchhas been done to find ice cream formulation which has functional benefit. The purpose of this study was to determine the physicochemical and sensory characteristics of vegetarian ice cream by using black soybean milk and black rice extrudates flour as the source of antioxidants. In making this ice cream, black soybean milk and black rice were used as the source of the antioxidants, Ryoto Sugar SP as synthetic emulsifier and sucralose as the low-calorie sweetener. Different concentration of emulsifier added, i.e. 10%, 15%, 20% and 25% of the total weight while the other ingredients used the same concentration. Methods that being used including: the making of black soybean milk, black rice extrudates flour, ice cream, and the sensory analysis of ice cream. It was found that the sensory color of ice cream with the addition of an emulsifier 25% was the most preferred by the panelists with a score of 3.9 $\pm$ 0.7, the flavor of ice cream with the addition of emulsifier 15% was the most preferred by the panelists with a score of 4.1 $\pm$ 0.8, the texture of the ice cream with the addition of emulsifier 20% was the most preferred by the panelists with a score of 4.0 $\pm$ 0.6 and the overalls were ice cream with the addition of emulsifier concentration 15% which was acceptable by the panelists.

Keywords: *Ice cream, antioxidants, anthocyanins, black soybeans, black rice, emulsifier*

1. INTRODUCTION

Black soybeans widely known contain of anthocyanins with high antioxidant activity (Purwanti, 2004). Black rice also contain of purple pigments that act as antioxidants. Antioxidant is anthocyanins which able to prevent the cancer cells expansion. Antioxidant is a compound which has capability in delaying, slowing or inhibiting the oxidation reaction. The total content of phenolic compounds in pigmented rice is four times greater than the white rice (non-pigmented). Anthocyanin in the black soybean and black rice is an antioxidant which potentially prevents premature oxidation process and degenerative diseases. Anthocyanin is a type of flavonoid components commonly found in nature. Flavonoid is a subgroup of the phenolic components. These pigment is water soluble and supply a wide range of colors in plants, such as blue, purple, violet, magenta, red and orange (Shinta, 2010).

Ice cream is a food product that is favored by many people from all ages because of its sweet tastes and its textures which melt in the mouth. Unfortunately, most of the ice cream products do not contain a functional compounds e.g. natural antioxidant compounds. In addition, the value of commercial ice cream calories is high enough. As time goes by, the level of public awareness about health is getting higher. Therefore, this researchhas been done to find ice cream formulation which has functional benefitso that the ice cream was not only popular by its tastes but also having functional benefit.

This study was conducted to upgrade an existing product of ice cream by adding nutritional value in it. By mixing the black rice extrudates flour with black soybean milk in the ice cream formulation would enhance the functional value of ice cream and produce ice cream with high antioxidants, safe to consume and acceptable by the people either from its tastes or it's functional benefits.

The purpose of this study was to determine the physicochemical and sensory characteristics of vegetarian ice cream by using black soybean milk and extrudates flour of black rice as the source of antioxidants. The physical characteristics which being measured were: overrun, hardness, viscosity and time to melt. The chemical characteristics which being measured were: total solids, antioxidant activity and anthocyanin content. And the sensory characteristics which being measured were: color, flavor, texture and overall.

2. METHODOLOGY

This research was conducted in the Laboratory of Food Processing Engineering, Laboratory of Food Science and Laboratory of Sensory of Food Technology Study Program, Faculty of Agricultural

Technology, Catholic University Soegijapranata Semarang.

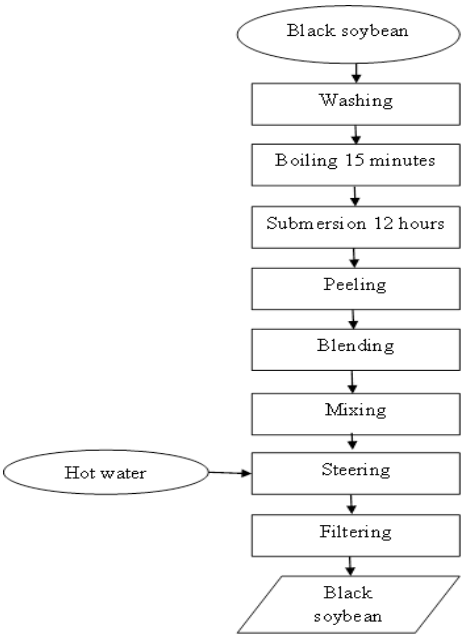
2.1. Equipments

The tools used in the creation of vegetarian ice cream include: analytical balance, single screw extruder, blender brand "Maspion", mixer brand "Philips", a sieve with a mesh size of 625, a freezer and a measuring cup. The tools used for sensory analysis were the ice cream cup sizes 100 ml and a plastic spoon.

2.2. Ingredients

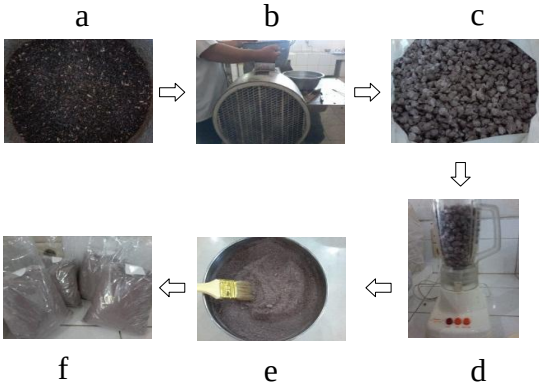
The materials used to make this ice cream include: black rice, black soy beans, emulsifier Ryoto Sugar SP, and sucralose

The making of Black Soybean Milk (BSM)



Picture 1. Making Black Soybean Milk

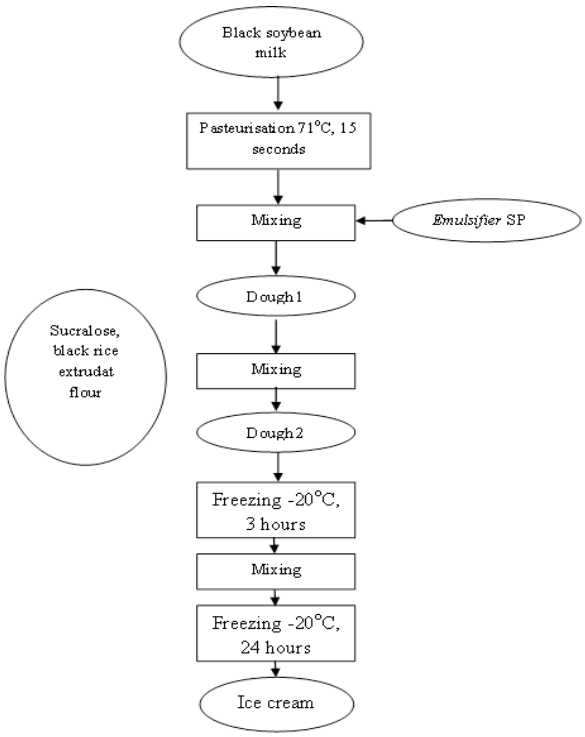
The Making of Black Rice Extrudates Flour (BREF)



Picture2.Making Black Rice Flour Extrudates

Caption :
(a) Black Rice
(b) Extrusion
(c) Black Rice Extrudates
(d) Grinding black rice extrudates
(e) Sieving
(f) Black Rice Extrudates Flour

The making of Vegetarian Ice Cream



Picture 3. Making Ice Cream

Table 1.Formulation Ingredients Ice Cream

Ingredients	Samples				
	K	A	B	C	D
BSM (ml)	500	500	500	500	500
SP (%)	20	10	15	20	25
Sucralose (g)	2	2	2	2	2
BRFE(g)	-	50	50	50	50

Description:
K: Icecreamwithout the addition ofblack rice
A: Icecreamwith the use ofan emulsifier 10%
B: Icecreamwith the use ofan emulsifier15%
C: Icecreamwith the use ofan emulsifier20%
D: Icecreamwith the use ofan emulsifier 25%

2.3.Sensory Analysis

This sensory analysis aims was to determine the public acceptance of the vegetarian ice cream with the use of black soy milk and black rice extrudates flour. The sensory analysis was performed using sensory parameters include: assessment of the color, flavor, texture, and overall. The method used for sensory analysis in this study was rating test. Panelists were asked to give a score from one to five, where 1 represents the lowest value (least preferred) and 5 was the highest score (highly preferred). Panelists involved were all church people of Ebenhaezer Nazarene Christian Church as many as 50 people.

Data Analysis aims was to process data obtained from the testing that has been done. The result data of sensory analysis were analyzed using variance in one direction (One Way ANOVA) to test the different. Computing statistics for this data analysis was using SPSS for Windows software (version 13) and Microsoft Office Excel (Santoso, 2004).

3. RESULTS AND DISCUSSION

Two kinds of research which conducted in this research werePreliminary Research (sensory analysis) to determine the consumer acceptance of color, texture and sweet taste of vegetarian rice ice cream and the Main Studies (sensory analysis of vegetarian ice cream.)

3.1. Preliminary Research

Table 2.*Sensory Characteristics of the Texture and Color*

BRFE	AcceptanceScorePanelists	
	Color	Texture
60 grams	3,6 ± 0,5	3,4± 0,5
50 grams	4,7 ± 0,5	4,6 ± 0,5
40 grams	4,3 ± 0,5	4,4± 0,5

Description:
Rating Assessment of SensoryAnalysis:
1= Do not like
2=Less like
3=Quite like
4= Like
5 = Very like

Based on Table 2 above, it can be seen that with the addition of black rice extrudates flour by 50 grams was the most preferred concentration by panelists either in terms of color or texture with a score of 4.7 ± 0.5 on color and 4.6 ± 0.5 on texture.

Table3.*SensoryCharacteristicsof theTaste*

Sucralose (grams)	AcceptanceScorePanelists Taste
1,5	3,4± 0,5
2	4,6 ± 0,5
2,5	3,7± 0,5
3	3,6 ± 0,7

Description:
RatingAssessmentof SensoryAnalysis:
1= Do not like
2=Less like
3=Quite like
4= Like
5 = Very like

Based on Table 3 above, it can be seen that with the addition of 2 grams of sucralose was the most preferred concentration by panelists in terms of the tastes with acceptance scores of 4.6 ± 0.5.

3.2.Main Research

No.	Treatment	Color	Taste	Texture	Overall
1.	Kontrol	3,9± 0,9^{a}	4,0± 0,8^{a}	4,1± 0,7^{a}	4,2± 0,7^{a}
2.	*Emulsifier* 10%	2,7± 0,9^{b}	3,1± 0,8^{b}	2,8± 0,7^{b}	2,0± 0,7^{b}
3.	*Emulsifier* 15%	3,2± 0,9^{c}	4,1± 0,8^{a}	3,5± 0,6^{c}	4,2 ± 0,7^{a}
4.	*Emulsifier* 20%	3,6± 0,9^{c}	3,3± 0,9^{b}	4,0± 0,6^{a}	3,8 ± 0,7^{c}
5.	*Emulsifier* 25%	3,9± 0,7^{a}	2,6± 0,7^{c}	2,3± 0,8^{d}	3,3± 0,8^{d}

Description:
•All valuesweremean ±SDvalues
•Valueswithdifferentsuperscriptina columnindicatesignificant differencesbetween treatmentsat the95% confidence level(p <0.05) byone way ANOVAusingDuncan's multipletestareasasdifferent test

A preliminary sensory test conducted wasto determine the formulation of how much flour extrudate of black rice and sucralose were used in the making of ice cream. Consumer acceptance of color, texture and sweet taste of vegetarianice cream and the primary research which conducted to determine the physical condition, antioxidants, and sensory vegetarian ice cream. From Tables 2 and 3, it can be seen that the preliminary test resultsof sensory analysis which used for theformulation of ice cream with the addition of 2 grams of sucralose and 50 grams of black rice extrudates flour. Preliminary test carried out based on the theory of Alvarez, et al., (2005) who argue that consumer acceptance depends on the quality of the ice cream texture and taste. The physical properties of the ice cream is made up of small globula fat, has a certain viscosity, have a melting rate, can be formed in accordance with the container, and the taste which sensory acceptable.

Colour is a quite important sensory aspect because it relates to the visual aspect of the consumer. Consumers will be interested to taste the food when visually the color is interisting so that the color of the ice cream is worth noting. Ice cream with the addition of emulsifier 25% had bright colors compared with the three other ice cream so the color of it was acceptable similar with the control ice cream. While the color of ice cream with the addition of emulsifier 10% was not preferred by the panelists due to the dark color. The ice

cream flavor with the addition of emulsifier 15% was the most preferred while flavors of ice cream with the addition of emulsifier 25% least preferred by the panelists due to the bitter taste caused. The texture of the control ice cream was the most preferred by the panelists which didnot have a noticeable difference on the ice cream with the addition of emulsifier 20% while the texture of ice cream with the addition of emulsifier 25% was not preferred by the panelists due to the soft texture so that unpopular amongs the panelists. Overall the controls ice cream was the most preferred by the panelists which was not significantly different from the ice cream with the addition of emulsifier 15% so that it could be said that the ice cream with the addition of emulsifier 15% became the ice cream formulation that can be used as the panelists like it.

CONCLUSION

- The best ice cream formulation was the ice cream with the addition of emulsifier 15% which did not have a noticeable difference on the sensory characteristics of the control

REFERENCES

Adapa, S., H. Dingeldein, K. A. Schmidt and T. J. Herald. (2000). Rheological Properties of Ice Cream Mixes and Frozen Ice Creams Containing Fat and Fat Replacers.*Journal of dairy science 83: 2223-2229.*

Akoh, C. C. and D. B. Min. (2002). Food Lipid : Chemistry, Nutrition and Biotechnology. 2nd edition, revised and expanded. New York : Marcel Dekker Inc.

Alvarez, V. B, C. L. Wolters, Y. Vodovotz; and T. Ji.(2005). Physical Properties of Ice Cream Containing Milk Protein Concentrates. *Journal of Dairy Science Vol. 88, No. 3, 2005, Page 862 – 871.*

Arbuckle, W. S. (1996). Ice Cream. The Avi Publishing Company, Inc. London

Baer, R. J, N. Krishnaswamy and K. M. Kaperson. (1999). Effect of Emulsifiers and Food Gum On Nonfat Ice Cream. *Journal of Dairy Science 82:1416-1424.*

Codex Food and Agriculture. (2008). *Joint FAO/WHO Food Standards Programmed CODEX Committee on Food Additives, Fortieth Session*. FAO/WHO

Decker, Eric A., Kathleen Warner, Mark P. Richards, AndFereidoonShahidi. (2005). Measuring Antioxidant Effectiveness in Food. *Journal Agricultural Food Chemistry 53:4303-4310*

Edwards, W. P. (2000). *The Science of Sugar Confectionery*.RSC Paperback. UK.

Farrow, J. & S. Lewis. (2000). Ice Cream And Iced Desserts Lorenz Bookz. Annes Publishing. Columbia.

MacDougall, B. (2002). Color in Food. Improving Quality Boca Raton: CRC Press. ISBN 0849315425) (393s)

McClements, D. J. (2005).Food Emulsion Principle: Principles, Practices and Techniques Second Edition. Boca Raton: CRC Press.

McClements, D. J. and J. Weiss. (2005). *Lipid Emulsions*. Dalam*Bailey's Industrial Oil and Fat Products 6th Edition (Ed. F. Shahidi)*.John Wiley &

Sons, Inc. United States of America.457-502.

Meilgaard, M., G. V. Civille and B.T. Carr.(1997). Sensory Evaluation Tecnique Third Edition.CRC Press. ASA

Mitsubitshi – Kagaku Foods Corporation. (2003). Ryoto Sugar Ester.Technical Information.

Muse, M.R & R.W. Hartel.(2004). Ice Cream Structural Elements That Affect Melting Rate And Hardness.American Dairiy Science Association.*Journal of Dairy Science 87:1-10.*

Potter, N.N and J.H Hotckiss.(1996). Food Science 5th edition.CBS Publisher & Distributors. New Delhi.

Purwanti, S. (2004).KajianSuhuRuangSimpanTerhadapKualitasBenihKedelaiHitamdanKedelaiKuning.JurnalIlmuPertanian (II) (I):22-31.

Saldivar, S. & S. R. Othon. (2010). Cereal Grains: Properties, Processing and Nutritional Attributes. CRC Press. USA.

Shinta. (2010). BerasBerpigmen: SebuahPeluang Usaha PengembanganPanganFungsional. *Food Review*Ind.vol.V/No.9/September 2010:34-38.

EVALUATING IN VITRO CYTOTOXICITY OF UNMODIFIED AND PEGYLATED PAMAM (G2) DENDRIMERS ON MOUSE BLASTOCYSTS

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ABSTRACT

Biological properties of dendrimers are highly important because the increased interest in using them for biomedical applications. Cationic dendrimers, like PAMAM, are generally haemolytic and cytotoxic. Their toxicity is generation-dependent and increases with the number of surface groups, suggesting that low generation dendrimers may be promising agents for targeted drug delivery. Conjugation with poly(ethylene glycol) (PEG) chains is also considered as a method of reducing the toxicity and increasing the biocompatibility of dendrimers. PEG is chosen because it is nontoxic, nonimmunogenic, and has favorable pharmacokinetics as well as tissue distribution. In this study to ensure its safety in biomedical usage, the low generation of unmodified and PEGylated PAMAM dendrimer are evaluated for their cytotoxicity with respect to its apoptotic effects in mouse blastocysts. Female IRC mice (6-8 weeks) are intraperitoneally injected with 100 µl PMSG and 47-49 hours later with 100 µl hCG. One day after mating, the female mice with vaginal plug are separated from males and three days later, the embryos in blastocyst stage are collected and exposed to various concentrations of unmodified and PAMAM-PEG (G2) denderimer (50 µg/ml, 100 µg/ml, 200 µg/ml, 300 µg/ml, and 400 µg/ml) and evaluated via TUNEL assay, Hoechst staining, and blastocysts outgrowth assay. The results showed that mouse blastocysts that were treated with PAMAM-PEG (G2) dendrimer showed a little apoptotic lesions. It can be concluded that unmodified PAMAM (G2) dendrimer still has a little cytotoxic effect on mouse blastocysts and PEGylation of PAMAM dendrimer can reduce the cytotoxic effect with respect to the apoptotic effect, cell proliferation, and blastocysts outgrowth.

Keywords：*PAMAM (G2) dendrimer, PEGylation, mouse embryo, blastocyst*

1. INTRODUCTION

The study of dendrimers is still in the beginning stage, but the interest in using dendrimers as a targeted drug delivery vehicle, which shows great promise for the treatment of disease, significantly increases (Duncan and Izzo, 2005). Dendrimers are composed of individual dendrons that radiate from a central core, where each layer of branch constitutes one complete generation (G) in the dendrimer series and is identified with a specific generation number (Medina and El-Sayed, 2009). The surface reactive groups may also be used to covalently attach drug molecules via labile chemical linkages. Using these approaches, dendrimers can be constructed with precise control over the number of generations and surface functionalities, producing structures with typically high monodispersity when compared to the synthesis of traditional polymers which typically produce statistical mixtures of products (Kaminskas *et al.*, 2012).

PAMAM (polyamidoamine) dendrimers, which contain a high surface density with an empty inner space, can be applied in drug/gene delivery (Chauhan et al., 2010). Their radial structure contains a 2carbon ethylenediamine core and primary amino groups on the surface. Successive generations (referred to as G0-G10) have increasing diameter and double the surface functional amino groups than the preceding generation. The systematically variable structural architecture and the large internal free volume make these dendrimers an attractive candidate for biomedical applications (Mukherjee *et al.*, 2010)

However, limited studies have been performed to investigate how the chemistry of dendrimer and its molecular architecture affect biodistribution and toxicity. Despite the extensive interest in pharmaceutical application of dendrimers, very little is known about their cytotoxicity, and how it causes cell death. There is conflicting evidence regarding their biological safety (Winnicka et al., 2009). These materials, particularly the higher generations (≥G7) of the cationic amino-terminated dendrimers, have been shown to be toxic in vitro. Nevertheless, a few toxicity studies on PAMAM dendrimers have been performed, suggesting that low generations of PAMAM dendrimers (below G5.0) are more biocompatible (Duncan and Izzo, 2005).

Biological properties of dendrimers are highly important because the interest in using them for biomedical applications becomes higher. Cationic dendrimers, like PAMAM, are generally haemolytic and cytotoxic. Their toxicity is generation-dependent and increases with the number of surface groups (Klajnert and Bryszewska, 2001). Roberts *et al.* (1996) have shown that G3, G4 and G5 dendrimers are not toxic to male Swiss-Webster mice. However, G7 dendrimers may

pose potential biological complications, since 20% of the rats treated with G7 dendrimers died, and all survived rats showed some liver vacuolorization. These studies support the results of cell culture studies in which the toxicity of dendrimers appears to be generation-dependent. Therefore, these studies suggest that low generation dendrimers may be promising agents for targeted drug delivery.

Conjugation with poly(ethylene glycol) (PEG) chains is also considered as a method of reducing the toxicity and increasing the biocompatibility of dendrimers. PEG is chosen because it is nontoxic, nonimmunogenic, and has favorable pharmacokinetics as well as tissue distribution (Wang *et al*., 2009). In general, attachment of PEG (i.e., PEGylation) improves water solubility, reduces toxicity, decreases enzymatic degradation, and increases the in vivo half-lives of small-molecule drugs. A possible reduction in drug potency due to the sterics imparted by a long flexible PEG chain can be compensated by a reduced renal elimination rate (Kim *et al*., 2008).In this study to ensure its safety in biomedical usage, the low generation of unmodified and PEGylated PAMAM dendrimer are evaluated for their cytotoxicity with respect to its apoptotic effects, cell proliferation, and blastocysts outgrowth in mouse blastocysts.

2. EXPERIMENTAL PROCEDURES

2.1. Materials

The materials used in this research are ICR (Imprinting Contol Region) mice, pregnant mares serum gonadotropine [PMSG] (China Chemical and Pharmaceutical Taiwan), human chorionic gonadotropine [hCG] (China Chemical and Pharmaceutical Taiwan), ethanol, Earle's balanced salt solution (EBSS), L-glutamine (Gibco, USA), NAC [N-acetyl-L-cysteine] (Sigma, USA), sodium pyruvate (Sigma, USA), triton-100 (Merck, USA), Tween 20 (Sigma, USA), polyamidoamine (PAMAM) G2 Dendrimer (50 µg/ml, 100 µg/ml, 200 µg/ml, 300 µg/ml, and 400 µg/ml), PAMAM-PEG G2 Dendrimer (50 µg/ml, 100 µg/ml, 200 µg/ml, 300 µg/ml, and 400 µg/ml), pronase (Roche, USA), PBS as the wash buffer, paraformaldehyde, 3% H_2O_2, 0.1% Triton-citrate, In Situ Cell Death Detection Kit (Roche, USA), peroxidase (POD), and diaminobenzidine (DAB), Hoechst / Bisbenzimidine H33342 (Calbiochem, USA), FBS (Gibco, USA), and CMRL-1066 (Sigma, USA).

2.2. Mouse Superovulation

The female ICR mice that had reached their sexual maturity (6-8 weeks) are intraperitoneally injected with 100 µl PMSG between 2.00 p.m – 4.00 p.m. As many as 100 µl hCG is injected intraperitoneally into female mice at 47 to 49 hours after the PMSG injection. Female mice are manually

restrained for the intraperitoneal injection. Immediately after hCG injection, each female mouse is allowed to mate with only one male mouse of the same strain. Male mice for breeding are maintained in individual cages and ranged in age from 3 to 8 months. The vaginal plug is checked the next day between 9.00 am – 12.00 p.m. The female mice with vaginal plug are separated from males (Luo *et al.*, 2011).

2.3. Blastocysts Stage Embryo Collection

Approximately 3.5 days after mating, the embryos of the mice are in blastocyst stage and ready to be collected. Mice are killed by neck-breaking. The mouse is lied on its back and sprayed with alcohol. A cut is made across the belly and the skin is cut away to expose the gut. The uterus is dissect out with sterile forceps and scissors. Then, the embryos are flushed into the 4-well dishes with 0.3% EBSS into the uterus. Blastocyst-stage embryos are then transferred to the well containing 0.3% EBSS with different concentrations of PAMAM (G2) Dendrimer and PAMAM-PEG (G2) Dendrimer (50 µg/ml, 100 µg/ml, 200 µg/ml, 300 µg/ml, and 400 µg/ml). After that, they are incubated in the presence of dendrimers for 24 hours before TUNEL Assay, Hoechst Staining, and blastocysts outgrowth assay (Merck Millipore Protocol, 2007).

2.4. TUNEL Assay

Embryos were moved to the well containing pronase for 10 minutes, rinsed with the wash buffer PBS, and fixed with the paraformaldehyde solution for 30 minutes. After washed with PBS, embryos were treated with 3% H_2O_2 for 5 minutes. After washed with PBS, embryos were treated with 0.1% Triton-citrate for 2 minutes. After washed with PBS, embryos were stained into TUNEL solution for 30 minutes, POD for 30 minutes, and DAB for 1 minute on the slides with rinsing them in the wash buffer among the moving to different solution. After final wash with PBS, embryos were placed on the new slides and observed by fluorescene microscope for their apoptotic reaction at certain concentration of unmodified and PEGylated PAMAM (G2) Dendrimer.

2.5. Hoechst Staining

Mouse blastocysts that have been incubated for 24 hours in the culture medium with the presence of unmodified and PAMAM-PEG (G2) Dendrimer (50 µg/ml, 100 µg/ml, 200 µg/ml, 300 µg/ml, and 400 µg/ml) are treated with 0.4% pronase for 10 minutes to remove the zona pellucida. After that they are fixed in paraformaldehyde for 15 minutes, then treated with 1% citrate-triton and stained with 20 µg/ml bisbenzimide and 10 µg/ml propidium iodide (PI) for 30 minutes at 37^{o}C, with rinsing them in the wash buffer among the moving to different solution. The stained blastocysts are then transferred to slides and

protected from light before observation. They are observed in blue color under UV light excitation. The number of nuclei are considered to represent an accurate cell number because multinucleated cells are not common in preimplementation embryos.

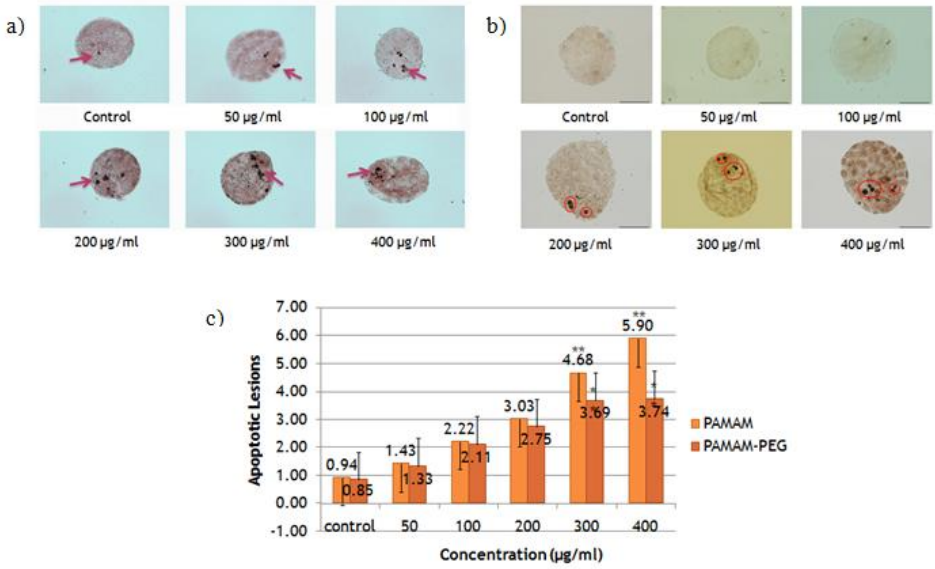
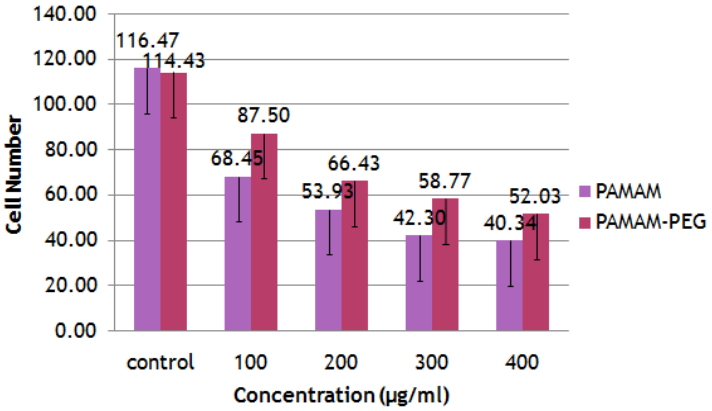


Figure 1. Apoptotic Cell Number per Embryo in Various Concentrations of **a)** PAMAM (G2) dendrimer and **b)** PAMAM-PEG (G2) Dendrimers on Mouse Blastocysts, c) Apoptotic Cell Number per Embryo in Various Concentrations of PAMAM and PAMAM-PEG (G2) Dendrimer.
** significant different compared to control, one way ANOVA ($p<0.01$)

2.6. Blastocysts Outgrowth Assay

Mouse blastocysts are treated with unmodified and PAMAM-PEG (G2) Dendrimer (50 µg/ml, 100 µg/ml, 200 µg/ml, 300 µg/ml, and 400 µg/ml) and transferred separately to 96-well plate coated with fibronectin. They are implanted onto fibronectin in CMRL-1066 with 10% FBS at 37°C, 5% CO_2 for 72 hours. The outgrowing embryo morphology is observed under light microscope. The blastocyst outgrowth is classified into 3 grades according to their expansion degree and hatching status; 1) Grade I: The mouse blastocysts still contain the zona pellucida, thereby losing the ability to be hatched, and not allow outgrowth process onto fibronectin; 2) Grade II: The expansion of trophectoderm (TE) cell is



below part and inner cell mass (ICM) itself exhibited an altered morphological structure; 3) Grade III: The expansion of outgrowth TE

cells covers ICM. The good development of blastocyst is represented by the Grade III outgrowth.

2.7. Statistical Analysis

All of the data are analyzed using T-test independent or one-way ANOVA and presented as mean ± SEM and p-value < 0.05 is considered as significant different.

3. RESULTS

3.1. TUNEL Assay

Figure 2. Cell Proliferation Number via Hoechst Staining of PAMAM and PAMAM-PEG (G2)

The cells of a multicellular organism are members of a highly organized community. The number of cells in this community is tightly regulated—not simply by controlling the rate of cell division, but also by controlling the rate of cell death. If cells are no longer needed, they commit suicide by activating an intracellular death program. This process is therefore called programmed cell death, although it is more commonly called apoptosis (Albert *et al.*, 2002).

Apoptotic cells with degraded DNA were represented with black spots on the moue blastocysts embryo which have been exposed to various concentration in PAMAM (G2) and PAMAM-PEG (G2) dendrimer for 24 hours (Figure 1a and 1b). There were 5 different concentration of dendrimer used in the study (50 µg/ml, 100 µg/ml, 200 µg/ml, 300 µg/ml, and 400 µg/ml). The control embryo without any exposure to dendrimer showed a very low apoptotic lesions with just 0.94 and 0.85 lesion in average, and these number were gradually increase with the increase of dendrimer concentration, both in PAMAM (G2) and PAMAM-PEG (G2). The highest apoptotic cell number was shown in 400 µg/ml PAMAM (G2) dendrimer, which reached 5.90 black dots in average. Compared with the unmodified ones, the apoptotic lesions in PEGylated PAMAM were significantly decreased for all concentration tested. Significant difference of the results were also shown on the two highest concentration (300 µg/ml, and 400 µg/ml) of both unmodified and modified PAMAM (G2) dendrimer (Figure 1c).

3.2. Hoechst Staining

Hoechst staining is a cell-permeable DNA stain that is excited by ultraviolet light and emits blue fluorescence at 460-490 nm. Hoechst 33342 binds preferentially to adenine-thymine (A-T) regions of DNA. This stain binds into the minor groove of DNA and exhibits distinct fluorescence emission spectra that are dependent on dye:base pair ratios. Hoechst 33342 is used for specifically staining the nuclei of living or fixed cells and tissues. This stain is commonly used incombination with 5-bromo-2'-deoxyuridine (BrdU) labeling to distinguish the compact chromatin of apoptotic nuclei, to identify replicating cells and to sort cells based on

their DNA content. A combination of Hoechst 33342 and propidium iodide have been extensively used for simultaneous flow cytometric and fluorescence imaging analysis of the stages of apoptosis and cell-cycle distribution.

As shown in Figure 2, exposure of cells to both kinds of dendrimer resulted in the decrease of cell proliferation. However, the cell number in PAMAM-PEG (G2) dendrimers were higher in all concentration. A normal blastocyst cell without any exposure on dendrimer had an average number of cell more than 100 cells. The cell proliferation were gradually decrease in the higher concentration of dendrimer for both kinds. All of the concentrations showed significant difference of cell number compared with the control. However, the proliferation induced by PEGylated dendrimers were better than the unmodified ones, which indicated that conjugating with PEG arms protected against dendrimer-induced cytotoxicity.

3.3. Blastocysts Outgrowth Assay

Successful implantation of the blastocyst is essential for reproduction. he process of mammalian implantation has been investigated using an in vitro model system wherein the trophoblast cells of mouse blastocysts attach to and outgrowth on tissue culture plates containing a complex medium. Blastocysts that adhered to the culture plate were designated as adhesion blastocysts. When trophoblast cells had grown outward from the adhered blastocysts and the primary trophoblast cells became visible, these embryos were designated as outgrowth blastocysts. The proportions of blastocysts undergoing adhesion and outgrowth were estimated at 72 h after growth factor treatment.

The proportions of hatched blastocysts showing adhesion or outgrowth were used to estimate the implantation capacity of blastocysts in vitro. The blastocyst outgrowth is classified into 3 grades according to their expansion degree and hatching status; 1) Grade I: The mouse blastocysts still contain the zona pellucida, thereby losing the ability to be hatched, and not allow outgrowth process onto fibronectin; 2) Grade II: The expansion of trophectoderm (TE) cell is below part and inner cell mass (ICM) itself exhibited an altered morphological structure; 3) Grade III: The expansion of outgrowth TE cells covers ICM.

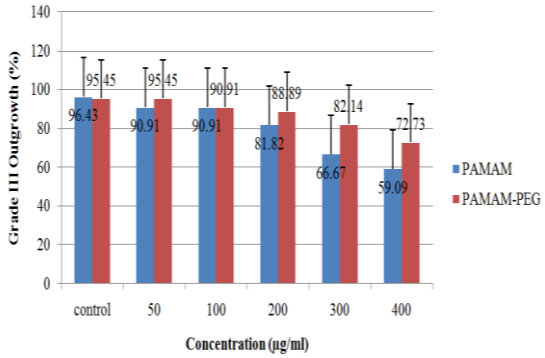


Figure 3. Grade III Blastocysts Outgrowth Percentage of PAMAM and PAMAM-PEG (G2)

As the grade III of outgrowth process indicates the good development of embryo, Figure 3 showed the percentage of grade III blastocysts after 72 hours exposure of both PAMAM (G2) and PAMAM-PEG (G2) dendrimers in various concentration. It can be seen that the control embryo without any addition of dendrimers showed the highest percentage of grade III development, which were 96.43% and 95.45%. As the concentration increase, the percentage of embryo which underwent grade III outgrowth gradually decrease. However in PAMAM-PEG group, the grade III blastocysts percentage was higher than the unmodified one for all of concentrations. It showed that the PEG conjugation on PAMAM (G2) dendrimer resulted in better outgrowth process.

4. DISCUSSION

Dendrimers are the few types of synthetic polymers that can be precisely controlled in size and structure. The well-defined structure renders dendritic macromolecules unique properties for versatile applications, especially in the biomedical field. The outer layer possesses a defined number of reactive functional groups that may be modified to influence the valency, solubility, tissue binding, pharmacokinetics or biodistribution properties of the dendrimer. In addition, the surface reactive groups may also be used to covalently attach drug molecules via labile chemical linkages. Using these approaches, dendrimers can be constructed with precise control over the number of generations and surface functionalities, producing structures with typically high monodispersity when compared to the synthesis of traditional polymers which typically produce statistical mixtures of products (Kaminskas *et al.*, 2012).

In recent years, there has been an explosion in the use of dendrimer- based nanocarriers for drug delivery. Conventionally, drugs are attached directly via linkers or spacers to dendrimer terminal groups and, in some instances, in combination with targeting moieties. The hydrophobic interior or the reactive periphery of the dendrimers can be used as host for various drugs or imaging agents. Also, they have been shown to be rapidly internalized into cells through endocytosis due to their nanometer-scale dimensions. These unique properties make dendrimers attractive for biological and drug-delivery applications.

Polyamidoamine (PAMAM) dendrimers were the first complete dendrimer family to be synthesized, characterized and commercialized (Esfand and Tomalia, 2001). PAMAM with an ellipsoidal or spheroidal shape is one of the most-studied starburst macromolecules. Due to specific synthesis PAMAM dendrimers have some interesting properties, which distinguish them from classical linear polymers, e.g. PAMAM has a

much higher amino group density comparing with conventional macromolecules. Also, PAMAM Dendrimers possess empty internal cavities and many functional end groups which are responsible for high solubility and reactivity. These specific properties make dendrimers suitable for drug delivery systems.

PAMAM dendrimers are arguably the most extensively studied dendrimers for biomedical applications, especially as carriers of biologically active agents. Considering the use of dendrimers for drug delivery, it is necessary that they are nontoxic and biocompatible. As has been widely documented, dendrimers bearing NH_2 termini display concentration and generation-dependent cytotoxicity. Although negatively charged carboxyl terminated dendrimers are less toxic, they are less suited for drug delivery as they are unlikely to interact or bind to the negative surface of cells. It is thought that the cytotoxicity of cationic PAMAM dendrimers resulted from the interactions between positively charged dendrimers and negatively charged molecules located on the cell surface. Noteworthy, the modification of cationic dendrimers with other molecules is likely to decrease the positive charge on the dendrimer surface, and leads to a gradual decrease in cytotoxicity.
Conjugation with PEG chains has been considered as a method of reducing the toxicity and increasing the biocompatibility of dendrimers because PEG is nontoxic, nonimmunogenic, and has favorable pharmacokinetics as well as tissue distribution. Earlier, PAMAM dendrimers with PEG grafts on the surface exhibited improved biocompatibility and efficiency of carrying genes or drugs. Although cationic amine-terminated dendrimers were conjugated with PEG to reduce their cytotoxicity, the modification of these novel delivery vehicles should not disturb their wide applications. Endocytic internalization is a prerequisite for effective performance. The rate of cellular uptake of dendrimers might be essential in the context of their proposed use as gene/drug delivery systems. PAMAM dendrimers had been proven to be easily endocytically captured. The uptake rate of PEGylated dendrimers should be quantified to ensure optimal design for applications (Wang *et al.*, 2009).

Based on Figure 1, mouse embryos still showed some apoptosis when they were exposed to various concentrations of PAMAM-PEG (G2) dendrimers (ranging from 50 µg/ml, 100 µg/ml, 200 µg/ml, 300 µg/ml, (and) to 400 µg/ml). Apoptosis in mouse embryos results in defective cells, manifesting as black spots (apoptotic lesions). The number of black spots is in proportion to increased dendrimer concentrations. Cationic PAMAM dendrimers generations 1–4 had been reported to have low cytotoxicity (Duncan &

Izzo, 2005), since the apoptotic lesions were low in mouse embryos.

The conjugation of PAMAM (G2) dendrimer with PEG chain made the apoptotic lesions on mouse blastocysts become lower than the ones exposed with the unmodified PAMAM (G2) dendrimer. This conjugation also caused less damage in the embryo, proven by more number of cell proliferation compared with the unmodified one in Figure 2. Morover as shown in Figure 3, the blastocyst outgrowth exposed to PAMAM-PEG (G2) dendrimer also resulted in more grade III outgrowth development. In conclusion, unmodified (G2) PAMAM Dendrimer still has a little cytotoxic effect on mouse blastocysts and PAMAM-PEG (G2) Dendrimer can reduce the cytotoxic effect with respect to the apoptotic effect, cell proliferation, and blastocysts outgrowth. PEGylated dendrimers were more suitable and valuable for practical applications in which they presented as safer nanoparticle for human consumption in the future research.

5. ACKNOWLEDGEMENT

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REFERENCES

Albert, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. (2002). Molecular Biology of the Cell, 4th edition. New York: *Garland Science*

Chauhan, A.S., Jain, N.K., & Diwan, P.V. (2010). Pre-clinical and Behavioural Toxicity Profile of PAMAM Dendrimers in Mice. *Proc. R. Soc. A* 466, 1535–1550

Duncan, R. & Izzo, L. (2005). Dendrimer Biocompatibility and Toxicity. *Advanced Drug Delivery Reviews* 57, 2215– 2237.

Esfand, R. And Tomalia, D.A. (2001). Poly(amidoamine) (PAMAM) Dendrimers: From Biomimicry to Drug Delivery and Biomedical Applications. *DDT* Vol. 6, No. 8 April

Kaminskas, L.M, McLeod, V.M., Porter, C.J., and Boyd, B.J. (2012). Association of Chemotherapeutic Drugs with Dendrimer Nanocarriers: An Assessment of the Merits of Covalent Conjugation Compared to Noncovalent Encapsulation. *Mol. Pharmaceutics*, 9, 355−373

Kim, Y.K., Klutz, A.M., and Jacobson, K.A. (2008). Systematic Investigation of Polyamidoamine Dendrimers Surface-Modified with Poly(ethylene glycol) for Drug Delivery Applications: Synthesis, Characterization, and Evaluation of Cytotoxicity. *Bioconjugate Chem*. 19, 1660–1672

Klajnert, B. and Bryszewska M. (2001). Dendrimers: Properties and Applications. *Acta Biochimica Polonica*. Vol 48 No 1/2001: 199-208

Luo, C., Zuniga, J., Edison, E., Palla, S., Dong, W., and Parker-Thornburg, J. (2011). Superovulation Strategies for 6 Commonly Used Mouse Strains. J Am Assoc Lab Anim Sci. 50(4): 471–478

Medina S.H. & El-Sayed, E.H. (2009). Dendrimers as Carriers for Delivery of Chemotherapeutic Agents. *Chem. Rev*., 109, 3141–3157

Merk Millipore Protocol. (2007). Isolation of Primary Mouse Embryonic Fibroblasts (PMEFs. Millipore Technical Publication.

Mukherjee, S.P., M. Davoren, and H.J. Byrne. 2010. In Vitro Mammalian Cytotoxicological Study of PAMAM Dendrimers – Towards Quantitative Structure Activity Relationships. *Toxicology in Vitro* 24: 169–177.

Roberts, J.C., Bhalgat, M.K., Zera, R.T. (1996). Preliminary Biological Evaluation of Polyamidoamine (PAMAM) Starburst TM Dendrimers. *J. Biomed. Mater. Res.* 30, 53–65.

Wang, Wei, Xiong, W., Wan, J.L., Sun, X.H., Xu, H.B., and Yang X.L. (2009). The Decrease of PAMAM Dendrimer-Induced Cytotoxicity by PEGylation via Attenuation of Oxidative Stress. *Nanotechnology* (20) 105103 (7pp).

Winnicka, K., Bielawski K., Ruzak, M., & Bielawski A. (2009). The Effect of Generation 2 and 3 Poly(amidoamine) Dendrimers on Viability of Human Breast Cancer Cells. *Journal of Health Science*, 55(2) 169-177

THE EFFECTIVENESS INCREASMENT ANTIOXIDANT AND REDUCTION HARDNESS OF CATFISH MEATBALL UNDER PURPLE SWEET POTATO (*IPOMEA BATATAS L.*) FLOUR

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ABSTRACT

Catfish meatball is one of children favored foods. The food is good because low colestrol and high protein. However the meatball is normally hard to bite for the children because they use a tapioca wich contain amylopectin high cocentration. Sense the meatball favored by children, this food is a good agents to delivered antioxidant. Substitution with flour of purple sweet potato (*Ipomea batatas L.)*, will increase antioxidant concentration, and at the same time would reduce the hardness of the meatball produced. Because purple sweet potato (*Ipomea batatas L.)* contain low amylopectin. To meet these goals two stages of research were conducted 1 (first) add the identity of most appropriate levels of purple sweet potato (*Ipomea batatas L.)* concentration wich was accepted by panelist to this five levels of flour cocentation where the five levels is 0% (control); 10%; 20%; 30% and 40%. Produced meatballs where evaluated by sensorically intern of color, smell (aroma), and overall. For 20% concentration flour purple sweet potato (*Ipomea batatas L.)* was preferer by panelist in term of its texture, taste and taste. However more than 20% of concentration flour purple sweet potato, make the hardness too soft and was not aceepted and the taste was dominantly by flour purple sweet potato. The research was concentrate use of two concentration flour purple sweet potato (*Ipomea batatas L.)*. The results showed that the protein wasn't significantly different from both concentration purple sweet potato (*Ipomea batatas L.)* between 10%; 20% and control. Where are the antioxidant will increased by substitution of both 10% concentration of flour sweet potato (*Ipomea batatas L.)* and 20% concentration flour purple sweet potato (*Ipomea batatas L.)*. The higher antioxidant is in 20% concentration of purple sweet potato (*Ipomea batatas L.)* flour. The hardness of produced meatballs treated with 10% and 20% of flour were lower than the unsubstitution (control). This analysis uses a non-parametric test for first stage. Then using test one way anova to test the effect of texture and antioxidant treatment.

Keywords: *catfish meatballs, tapioca flour, purple sweet potato*

1.INTRODUCTION

Meatball is a processed meat product that is very well liked. Beef prices is costly. Beef meatballs can be replaced with fish meat that has a fairly high protein content. Protein in catfish 17.7%. Catfish *Clarias batrachurs* by its Latin name, it is very popular with all people because the meat is very tasty and delicious. The low fat from catfish can helping a pregnant woman to growth of the fetus either for good heart health and for growing children, (Team Agriminakultura, 2008). Catfish meatballs substituted by purple sweet potato flour (Ipomoea batatas L.) because it has several advantages, namely fiber, and high anthocyanin than other types. The function of anthocyanins in purple sweet potatoes have antioxidant effects that can destroy free radicals, as a result of nicotine, air pollution and chemicals. (Iriyati, 2012). The purpose of this study to obtain the exact composition between tapioca flour with sweet potato flour on the physicochemical and sensory of catfish meatball

According Wibowo (1995), meatballs sensory quality criteria include:

1 Texture

Texture that has good quality is a compact texture, elastic, springy but not tough, no meat fibers, not mushy, not wet watery and fragile.

2 Colors

The meatballs were good for a young brown beef and white for fish balls. The resulting color depends on the color of meat is used as the base material and the addition of tapioca flour. The more tapioca flour used color fading meatballs.

3 elasticity

Good level of resilience is the meatballs were not too chewy tapioca flour as more and more were added to the clay structure gelnya that the resulting product is less good meatballs.

4. sightings

Round-shaped fish balls should be smooth, uniformly sized, clean, and not dull.

5. Odor

Distinctive odor of fresh fish stew (depending on the type of fish used), the smell of spices is quite sharp, not fishy / stale

6 Sense

Delicious, flavor of the fish in accordance with the type of fish used, not too salty seasonings.

2.MATERIAL AND METHOD

2.1.Material and Tools

The materials used in this study is the purple potato flour, tapioca flour, meat catfish, ice water, salt, spices, such as garlic, sesame oil, egg whites. The tools used are spoons, containers, pots, stoves, knives, blender, analytical balance. Tools for test hardness is Texture Analyser the brand LLOYD. Equipment to test moisture content is porcelain bowls and oven. Equipment for protein assay include kjeldahl tube, distillation and other chemical tools.

Equipment for antioxidant test vials tube, flask, aluminum and other chemical tools.

2.2.Methode

The study was conducted two phases: the first preliminary test to determine the ratio of sensory subtitusion tapioca flour and a purple potato flour against catfish meat to be used in the main study. The second main is a study protein test, antioxidant test and texture. This analysis used non parametric test for sensory. Then using one way ANOVA test for the effect of antioxidant treatment trials base on texture and self life

3.RESULTS AND DISCUSSION

3.1.Sensory

Table 1: Results of Preliminary Test of Sensory Research on Catfish Meatballs with Various Concentrations of Tapioca Starch.

Purple sweet potato flour substitution treatment	Parameter					
	Color	Aroma	Texture	Taste	After taste	Overall
Control	4,17 ± 1,05 [a]	3,40 ± 1,61 [a]	3,70 ± 1,41 [a]	3,70 ± 1,41 [a]	3,70 ± 1,29 [a]	3,97 ± 1,29 [a]
10%	3,80 ± 1,21[a]	3,67 ± 1,21 [a]	3,27 ± 1,33 [ab]	3,70 ± 1,20 [a]	3,67 ± 1,29 [a]	3,73 ± 1,28 [a]
20%	3,07 ± 1,01 [b]	2,83 ± 1,05 [b]	3,47 ± 1,07 [a]	3,07 ± 1,38 [ab]	3,10 ± 1,39 [a]	3,03 ± 1,24 [b]
30%	1,97 ± 0,99 [c]	2,60 ± 1,22 [b]	1,97 ± 1,15 [c]	2,17 ± 1,17 [c]	2,23 ± 1,16 [b]	2,20 ± 1,18 [c]
40%	2,00 ± 1,23 [c]	2,50 ± 1,61 [b]	2,60 ± 1,40 [bc]	2,37 ± 1,18 [bc]	2,30 ± 1,26 [b]	2,07 ± 0,98 [c]

Specification:

• All values are mean ± standard deviation

• Values with different superscript in each parameter showed a significant difference at 95% confidence level ($p < 0.05$) using the Kruskal Wallis and Mann Whitney.

Based on Table 1 above it can be seen that the concentration of purple sweet potato flour substitution of 10% is most preferred by consumers in terms of color, aroma, and overall. Purple sweet potato flour substitution concentration of 20% is most preferred by consumers in terms of texture, flavor and after-taste. From the results it was found that if the starch concentration of 10% and 20% had no noticeable difference in the assessment of the panelists all parameters tested. Therefore, the main study used two concentrations of the purple sweet potato flour. The results showed that the higher the percentage the addition of flour, of which more than 20%, namely 30% and 40% giving a purple potato flour, the lower level of preference panelists. This is because the panelists prefer a scent that smells meat meatball aroma compared with flour. During ripening reaction will occur between fillers and meat, so the aroma of the meat is reduced during processing, (Montolalu, 2013). Panelists also like meatballs that color is not flashy, and not dark. Protein content in purple sweet potato caused color being brown. The more purple potato flour, make

more taste of the purple sweet potato flavor than the meat., (Montolalu, 2013). The texture of the meatballs hardness was diminished during the addition of purple sweet potato flour. Similarly, the after taste meatballs, where more and more flour is added has a sweet potato after taste purple.

3.2.Protein

Table 2. Protein Test Results on the Main Test with a concentration of 10% and 20%

Concentration purple sweet potato	**Protein levels (%)**
Control	36,59 ± 1,36 [a]
10%	34,75 ± 1,25 [a]
20%	34,81 ± 2,17 [a]

Specification:

• Data shown are mean ± standard deviation

• Values with different superscript in each parameter showed a significant difference between treatments at the 95% confidence level (p <0.05) by one-way ANOVA

From the data in Table 2, it is known that protein content catfish meatballs are higher than in the control catfish meatballs with purple sweet potato starch concentration of 20% in the amount of 34.81% and catfish meatballs with purple sweet potato starch concentration of 10% is equal to 34.75%. In control of catfish meatballs with purple sweet potato flour 10% and 20% there is no real difference. Catfish meatballs with purple sweet potato flour 10% with catfish meatballs purple potato flour 20% known no real difference. This means that the substitution of sweet potato flour for each different treatment does not give significant effect on the value of the protein content meatballs, (Yudanto. 2009).

3.3.Antioxidant

Table 3 Antioxidant Content of Catfish meatballs with potato starch concentration Various Purple

Concentration purple sweet potato	**Antioxidant**
Control	11,95 ± 7,95 [a]
10%	17,20 ± 7,62 [b]
20%	24,13 ± 7,92 [c]

Specification:

• Data shown are mean ± standard deviation

• Values with different superscript in each parameter showed a significant difference between treatments at the 95% confidence level (p <0.05) by one-way ANOVA

The highest antioxidant concentration on catfish meatballs with purple sweet potato flour 20% of 24.13% than catfish meatballs purple potato flour concentration 10% by 17.20%. Lowest antioxidant found in catfish meatballs control the amount of 11, 95%. Meatballs control of purple potato meatball starch concentration of 10% and 20% are known from the table there is a real difference. Between meatballs concentration 10% purple sweet potato flour and potato starch concentration of 20% purple had real difference. The addition of purple potato starch concentration of 20% has a higher antioxidant content than others. The presence of pigments in sweet potato can serve as a

healthy food components, such as anthocyanin compounds found in purple sweet potato, (Suprapto 2012). Anthocyanin levels in the meatballs tend to rise as more and more substitution purple sweet potato is used in making meatballs so the more the levels of anthocyanins contained in the meatballs.

3.4.Hardness

Table 4 Texture Hardness In Purple Sweet Potato Flour Concentration Different

Concentration purple sweet potato	**Hardness (gf)**
Control	1227,64± 178,67 [a]
10%	1112,07 ± 95,98 [b]
20%	1098,22 ± 133,55 [b]

Specification:

• Data shown are mean ± standard deviation

• Values with different superscript in each parameter showed a significant difference between treatments at the 95% confidence level ($p < 0.05$) by one-way ANOVA

The meatballs were given a purple potato flour texture hardness decreased. There is a real difference between the control catfish meatballs with meatballs purple potato flour treatment concentration of 10% and 20%. While the meatballs concentration of 10% purple sweet potato flour with meatballs concentration of 20% purple sweet potato flour no real difference. Meatball texture is determined by the water content, fat content and the types of carbohydrates. High water content will cause the texture becomes soft. The ability of the water holding capacity on meatballs, affects the texture of meatballs. The texture can also affect the level of fragility caused by gluten which when coupled with water and mechanical work will form a dough that is elastic, (Montolalu, 2013).

According to Santoso (2006), the ratio of amylose and amylopectin starch sweet potato with tapioca inversely related. Purple sweet potato flour had 69.82% amylose and amylopectin tapioca has 30.18% instead of 14% amylose and amylopectin 30.18%. The meatballs were generated by substitution of purple sweet potato has a higher level of tenderness of the meatballs without substitution purple sweet potato.

Based on table 3 and 4. The higher substitution of purple sweet potatoes flour will increase an antioxidant and decrease on harness. But as known as table 1. Substitution purple sweet potatoes 30% and 40% was not preferred because the color being too dark, aroma and taste would dominated by purple sweet potatoes as well as decreasing hardness.

4.CONCLUSIONS

The substitution of purple sweet potatoes flour as a filler to produce a percentage 20% of meatballs with good sensory properties and antioxidant as well as texture. The addition of purple sweet potato flour increase

antioxidants and decrease of hardness. 20% antioxidant arebest. Based hardness 10% and 20% were not significantly different. More than 20% the meatballs will be too soft, flashy color and more flavor from purple sweet potatoes

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6.REFERENCES

Iriyanti, Yuni. (2012). Substitusi Tepung Ubi Ungu Dalam Pembuatan Roti Manis, Donat dan Cake Bread. Proyek Akhir. Universitas Negeri Yogyakarta. Yogyakarta.

Montolalu, Siska; N. Lontaan; S. Sakul; A. Dp. Mirah. (2013). Sifat Fisiko-Kimia dan Mutu Organoleptik Bakso Broiler dengan Menggunakan Tepung Ubi Jalar (Ipomoea batatas L. Jurnal Zootek ("Zootek"Journal), Vol.32 No.5.:No 158–171. Fakultas Peternakan Universitas Sam Ratulangi Manado. Manado.

Santoso,U., S. Elik., dan M.N. Cahyanto. (2006). Pengaruh Pemanasan pada Aktivitas Antioksidan Ekstrak Etanol. Beberapa varietas ubi jalar *(Ipomea batatas L.)*. J. Agritech 26:194-198.

Suprapto, Hadi; Yuliani, Nur Aliffah. (2012). Pengaruh Substitusi Ubi Jalar (Ipomoea batatas L.)dan Media Penggorengan Terhadap Mutu Donat Ubi Jalar Ungu. Jurusan Teknologi Hasil Pertanian, Fakultas Pertanian Universitas Mulawarman. Jurnal Teknologi Pertanian 7 (2): 68-73.

Tim Agriminakultura. (2008). Bisnis dan Budidaya Lele Dumbo dan Lokal. PT Gramedia Pustaka Utama. Jakarta.

Wibowo, S. (1995). Pembuatan Bakso Ikan dan Bakso Daging. Penebar Swadaya.

Yudanto, Novi Arif. (2009). Kadar Protein, Warna dan Tingkat Kesukaan Bakso Sapi dengan Substitusi Tepung Ubi Ungu *(Ipomea batatas L.)*. Skripsi. Fakultas Peternakan, Universitas Diponegoro. Semarang.

APPLICATION OF ARROWROOT STARCH (*Maranta arundinacea L.*) AND SUPLEMENTATION OF ANGKAK IN PRODUCTION OF INSTANT LOW GLYCEMIC INDEX ARROWROOT PORRIDGE

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ABSTRACT

Arrowroot (*Marantha arundinaceae L.*) is one type of Marantaceae family that has high nutrition and proven to be potentially developed as a source of carbohydrates. Arrowroot tubers also have low Glycemic Index (14) thus suitable to be consumed by people with diabetes mellitus. Angkak is natural colorant that have been used widely in Asia as a natural colorant food. The pigment that produce by angkak contain antosianin as an antioxidant compound that can increase endurance and prevent free radical. Angkak can reduce insulin and blood glucose levels in diabetes patients group. The aims of this research were to produce instant arrowroot porridge with low Glycemic Index, to find the effect of angkak suplementation to antioxidant activity of instant arrowroot porridge, and to know the best drying temperature and growth location that produces the lowest Glycemic Index. Variation of drying temperatures were 80^0C, 90^0C, 100^0C and growth location of arrowroot were Demak, Boja, and Bandungan. Production of instant arrowroot porridge begins with production of arrowroot starch for further gelatinized (80˚C, 10 minutes), dried, and dry mixing with the mix of skim milk, salt, sugar, and angkak. Chemical analysis consist of analysis of amylose content, resistant starch content, starch digestion content, antioxidant content, and in vitro Glycemic Index. The hypothesis are high temperature can form higher content of amylose and resistant starch. The higher the amylose and resistant starch content, the lower starch digestion and in vitro Glycemic Index. The higher amount of angkak, the higher antioxidant activity.

Keywords *: arrowroot, angkak, gelatinization, drying, location, amylose, resistant starch, starch digestion, antioxidant, in vitro Glycemic Index*

1. INTRODUCTION

Recently, the population of Indonesia is rely to the rice consumption to satisfy the needs of carbohydrate each day. Sometimes, dependence of Indonesia society is not comparable with availability of rice in Indonesia. To solve that problem, we can substitute rice with other foodstuffs, for example : *Maranta arundinaceae L*. or *arrowroot* which has high carbohydrate (Karjono, 1998).

One of the typical characteristic from arrowroot tubers is that it can grow on various type of soil and altitude. But, arrowroot tuber grow better at heights ranging from 60-90 meters above sea level with moist soil conditions. Arrowroot tubers grow with at location that has minimum rainfall 150-2000 mm per year, temperature 22-32˚C, and humidity 50-80% (Rukmana, 2000). So, arrowroot tubers are found in various parts of Indonesia, especially in Java.

Arrowroot tubers have low Glycemic Index (14). It is because arrowroot tubers contain fiber food, resistant starch, high amylose, and the possibility that presence of phenolic components which can inhibit starch digestibility. Besides that, amylose is difficult to digest. So arrorroot tubers can be used as food source which have low digestibility values (Marsono, 2002). The Glycemic Index (GI) of arrowroot tubers is 14, so it can be consumed by diabetic patient. Diabetes melitus (DM) is a condition where the blood sugar levels is higher than the normal blood levels and generally accomanied by a variety of metabolic disorders due to hormonal disturbances in the body (Hariwijaya dan Sutanto, 2007).

Then, this research is conducted to produce instant arrowroot porridge which is supplemented with angkak. Angkak is one of the fermentation product which is from rice and produced by mold *Monascus purpureus* and resulting in red or yellow pigmen (Steinkraus, 1983). The pigmen that produced by angkak contains antosianin as an antioxidant compound that can increase endurance and prevent free radical (Timotius, 2004). There is a study in Cina said that angkak extract can decrease insulin and blood glucose levels in patient of diabetes type II (Fang and Li, 2000). Advantages of the use of angkak are easily obtained base material, relative stable color, the color substance is dissolved in water, the color can be mixed with other pigments and food ingredients safely (Steinkraus, 1983).

Starch is glucose homopolymer with α-glycosidic bond, which there is lots on plants, especially grains and tubers (Jane, 1995). Starch consists of at least by three main components. There are amylose, amylopectin and other material such as protein and fat (Greenwood *et al*., 1979). Starch digestible is the ease level of a type of starch to be hydrolized by the starch-breaking enzymes

into simple units (Nugent, 2005). Starch digestible is affected by the composition of amylose and amylopectin. Most of scientist said that amylose is digested more slowly than amlylopectin because amylose is a polymer of the simple sugar with a straight-chain dan unbranched (Behall & Hallfrisch, 2002). Foods that are low in amylopectin and high amylose content have lower IG value. While the comparative ratio of food with a higher amylose component results lower IG value, because amylose is harder to digest and gelatinize (Rusilanti, 2008). The composition ratio of amylose-amylopectin starch correlated with starch digestion in the human body. According to Palupi *et al.* (2007), there are some factors that can decrease starch digestion: the usage of excessive high temperature at the time of processing, interaction between starch with non starch component, and the amount of resistant starch in starch.

2. HYPOTHESIS

High temperature results in higher content of amylose and resistant starch. The higher the amylose and resistant starch content, the lower starch digestion and in vitro Glycemic Index. The higher amount of angkak, the higher antioxidant activity.

3. MATERIAL & METHODS

This research consist of several steps :

3.1.Production of Arrowroot Starch

Arrowroot tubers were washed under running water and peeled of the skin. Arrowroot tubers that have been cleaned then shredded, squeezed, and deposited during 12 hours. The deposite obtained was dried using dehumidifier (70˚C, 2.5 hours) until the water level reached 8%. Next, the dried starch were sieved using mesh of 625 to produce arrowroot starch powder.

3.2.Production of Gelatinized Starch

Arrowroot starch that had been produced then being gelatinized by dissolving into water with ratio of 1 : 4 (starch : water). The solution was stirred until homogen and heated in hot water (80˚C, 10 minutes) till it became gelatinized. Gelatinized arrowroot starch then dried by oven dryer at temperature 80˚C, 90˚C, and 100˚C for 1 hour.

3.3.Production of Instan Arrowroot Porridge

Gelatinized arrowroot starch that have been dried was mixed with other ingredients, such as skim milk, salt, sugar, and angkak (1,5%).

Table 1. Formulation of Instant Arrowroot Porridge

Materials	AGSP
Arrowroot Gelatinized Starch (g)	**21**
Skim Milk (g)	**31,5**
Salt (g)	**1,5**
Sugar (g)	**6**
Angkak (g)	**0,9**
Total Weight (g)	**60,9**

Caption:
AGSP = Arrowroot Gelatinized Starch Porridge.

3.4.Chemical Analysis

Chemical analysis consist of analysis of amylose content, resistant starch content, starch digestion, antioxidant, and in vitro Glycemic Index. Amylose content analysis (Apriyantono *et al*, 1989) and resistant starch content (Goni *et al*, 1996) were done for the sample: (1) arrowroot starch and (2) dried gelatinized arrowroot starch. Amylose content analysis was also being done for arrowroot tubers and arrowroot instant porridge. While antioxidant analysis (Apriyantono *et al*, 1989) was done for the sample: (1) arrowroot tubers and (2) arrowroot instant porridge. Analysis of starch digestion (Goni *et al*, 1997) and in vitro Glycemic Index value (Frei *et al*, 2003) were done for arrowroot instant porridge.

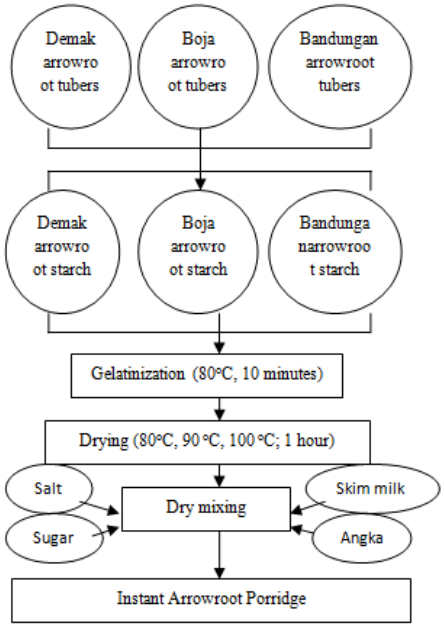

Figure 1. Flow Chart of Research Plan

REFERENCE

Behall, K.M. and J. Hallfrisch. (2002). Plasma Glucose and Insulin Reduction After Consumption of Bread Varying in Amlylose Content. Eur J Clin Nutr 56 (9): 913-920.

Fang, Y. H and Li, W. (2000). Effect of Xuezhikang on Lipid Metabolism and Islet Cell Function in Type II Diabetic Patients. J Capital Med., 7(2): 44-45.

Greenwood, C.T. dan D.N. Munro. (1979). Carbohydrates. Di dalam R.J. Priestley,ed. Effects of Heat on Foodstufs. Applied Seience Publ. Ltd., London.

Hariwijaya, M., & Sutanto. (2007). Pencegahan Dan Pengobatan Penyakit Kronis. Jakarta : Edsa Mahkota.

Jane, J., (1995). Starch Properties, Modifications, and Application, Journal of Macromolecular Science, Part A.32:4,751-757.

Karjono. (1998). Umbi-umbi Potensial Penghasil Tepung. Trubus 347-Th XXIXOktober.

Marsono, Y. (2002). Indek Glikemik Umbi-umbian. Agritech 22 (1):13-16.

Nugent, A.P. (2005). Health properties of resistant starch. Br. Nutr. Foundation Nutr.Bull. 30:27–54.

Palupi, N.S., F.R. Zakaria dan E. Prangdimurti. 2007. Pengaruh pengolahan terhadap nilai gizi pangan. Topik 8. Modul e-learning ENBP. Departemen Ilmu dan Teknologi Pangan, Fateta – IPB. Bogor.

Rukmana, R. (2000). Umbi Garut. Kanisius, Yogyakarta.

Rusilanti. 2008. *Menu Sehat untuk Pengidap Diabetes Melitus*. Kawan Pustaka, Jakarta.

Steinkraus, H. (1983). Indigenous Fermented Food. Marcel Dekker. New York.

Timotius, K.H. (2004). Produksi Pigmen Angkak Oleh Monascus. Jurnal Teknologi dan Industri Pangan, vol. XV, No. 1. Tahun 2004.

POSTER PRESENTATION

ISOLATION AND PURIFICATION OF CELLULASE FROM ALKALINE-TOLERANT BACILLUS SUBTILIS

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ABSTRACT

Lignocellulosic biomass is one of the most abundant renewable resource in the world. It has high potential to be used as material for biorefinery process because it yields fermentable sugar that can be converted to various bioproducts via microorganism functions. To make the biorefinery process success, the efficient hydrolysis process is needed to be improved to release the maximum amounts of sugars from lignocellulosic biomass. Natural environment is an important source to find an efficient cellulase producing bacteria. Previously, an alkaline-tolerant cellulase-producing bacterium, *Bacillus subtilis* strain MSB9, was screened and isolated from Botanic garden in Mahasarakham province, Thailand. In this study, we focused on the purification and characterization of cellulase enzyme produced by *B. subtilis* strain MSB9. The crude cellulase enzyme was partially purified and concentrated by ammonium sulfate precipitation and fractionated by using size exclusion chromatography using sephacryl S-100 HR column. Two of purified cellulase have relative molecular mass of 35 and 45 kDa, as determined by SDS-PAGE combined with CMC-zymogram.

Keywords: *Cellulase, Purification, Thermophilic bacteria, Bacillus, Thailand*

1. INTRODUCTION

Lignocellulosic biomass is one of the most important renewable material for biorefinery worldwide. It is the main component of plant cell walls. Mainly, it composed of three main components, cellulose (a homologous polymer of glucose molecules connected by β-1,4 linkages), hemicelluloses (a heterologous polymer of 5- and 6-carbon sugars), and lignin (a complex aromatic polymer) (1). Based on these characters, it draws economic interest to develop processes for effective conversion and utilization of lignocellulosic biomass as inexpensive sugars or carbon sources in fermentation processes.

For biorefinery, the step to convert lignocellulosic biomass to fermentable sugars is a major bottleneck (2) for the aspects of time and cost of the process. To achieve the success for lignocellulose conversion, the development of cellulase enzyme is needed with high efficiency enzyme, low cost, stress-tolerance properties.

In this study, we aimed to isolate and purify cellulase enzyme produced by an alkaline-tolerant bacterium, *Bacillus subtilis* strain MSJ9 that was screened and isolated from Botanic garden in Mahasarakham province, Thailand.

2. MATERIALS AND METHODS

2.1. Culturing condition for cellulase production

Bacillus subtilis strain MSB9 was previously screened and isolated from soil samples in Botanic garden in Mahasarakham province, Thailand (3). This bacterium is able to grow in carboxymethylcellulose (CMC) agar plates (0.5% CMC, 0.1% $NaNO_3$, 0.1% K_2HPO_4, 0.1% KCl, 0.05% $MgSO_4$, 0.05% yeast extract, 1.5% agar) (4) at pH 10.0. Therefore, it can be categorized to be an alkaline-tolerance bacterium.

The total cellulase activity was performed by transferring 100 µl of overnight culture to 10 ml of fresh CMC broth media, and cultures were incubated at 45 °C for 48 h. Then the crude cellulase enzyme from supernatant fractions of each bacterial isolates were tested for activity to digest two different cellulose substrates, filter paper and CMC using standard method (5) and the sugar products were measured by dinitrosalicylic (DNS) assay (6). Enzyme activity was defined as the amount of enzyme that released 1 µmol of glucose per min. Protein determination was done by using Bio-Rad protein assay kit with protocol described in manual. All experiments were performed with three replicates.

2.2. Enzyme preparation and purification

After incubation in CMC broth at 45°C for 48 h, the culture was centrifuged and

supernatants were used as crude cellulase enzyme. 200 ml of the crude enzyme was brought to 80% saturation with ammonium sulfate. Pellet was collected by centrifuged at 10,000 rpm for 20 min, and dissolved in 5 ml of 50 mM sodium phosphate buffer (pH 5.0) for dialysis.

Before protein dialysis, dialysis membrane (Float-A-Lyzer, Spectrum Lab, USA) with 10 kDa MWCO was prepared as described in the manual. Dissolved pellet was dialyzed against 30 mM sodium phosphate buffer (pH 5.0) at 4 ^{o}C with three changes of buffer. This solution was applied to a Sephacryl S-100 column (16 x 600 mm; HiPrep 16/60 Sephacryl S-100 HR, GE Healthcare Life Science, USA) equilibrated with 30 mM sodium phosphate buffer (pH 5.0) at a flow rate of 0.2 ml/min. The cellulase fractions were pooled and concentrated by Vivaspin-500 column (GE Healthcare Life Science, USA) with 10 kDa MWCO. Concentrated cellulase fraction was analyzed by 12% gel SDS-PAGE to determine the molecular weight of purified enzyme sample by comparing with standard protein marker (BLUeye Protein Ladder, RBC Bioscience, China). Protein samples were stained with Coomassie Brilliant Blue R-250.

To generate the zymogram pattern, concentrated cellulase fraction was applied to 12% gel SDS-PAGE (containing 1% CMC). Then, SDS was removed by soaking the gel in wash buffer (30 mM sodium phosphate buffer containing 40% isopropanol (pH 7.2)) for 1 h. The gel was soaked in equilibrated buffer (30 mM sodium phosphate buffer (pH 7.2)) for 1 h, and transferred into renaturing buffer (30 mM sodium phosphate buffer, 5 mM β-mercaptoethanol, 1 mM EDTA (pH 7.2)) at 4 ^{o}C overnight. The renatured gel was stained with 1% congo red solution for 15 min, and washed with 1 M NaCl solution. The band of cellulase was seen as a clear zone against background.

3. RESULTS AND DISCUSSION

To monitor cellulase activities of *Bacillus subtilis* strain MSB9, crude cellulase enzyme was first prepared by culturing this bacterium in CMC broth media at 45 °C for 48 h. The total cellulase activity was determined by standard method (5) using filter paper and CMC as substrates. The result was shown that FPase and CMCase activity are 2,022 and 4,297 U/L, respectively.

Next, cellulase enzyme produced by *Bacillus subtilis* strain MSB9 was prepared and purified. Crude cellulase enzyme of MSB9 was prepared from supernatant fraction of bacterial culture as described in method. After protein precipitation by adding ammonium sulfate, sample pellet was dialyzed, and fractionated by Sephacryl S-100 column. Each collected fractions were tested for cellulase activity using CMC and

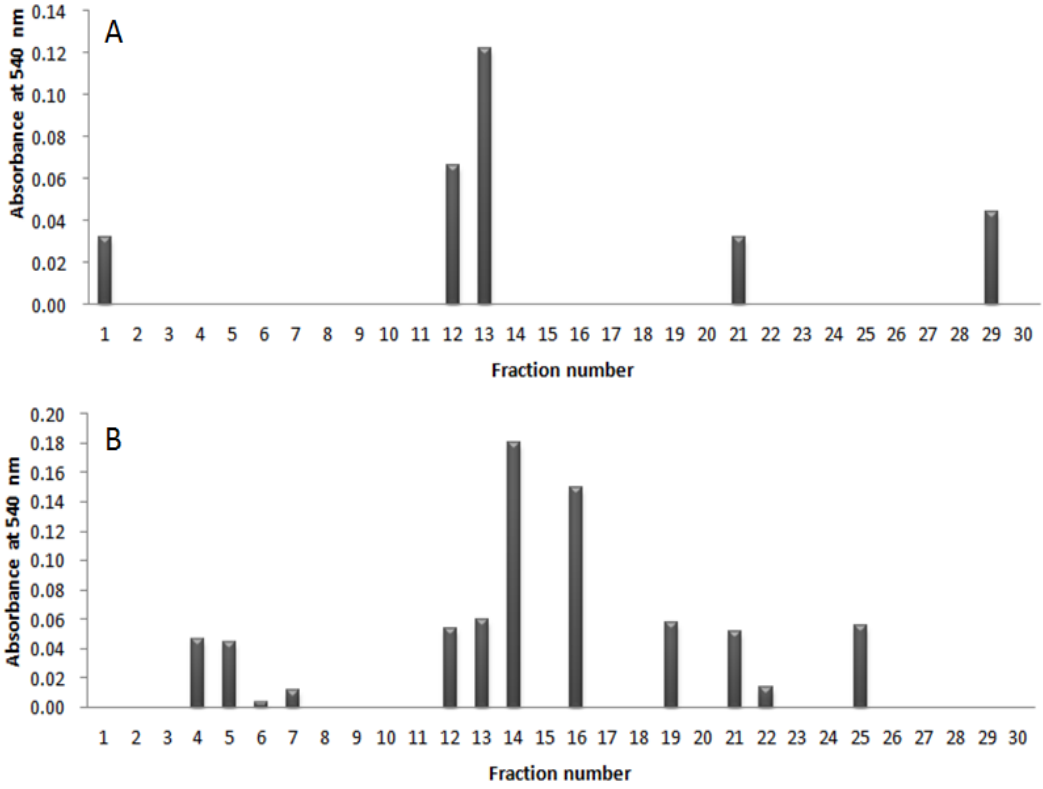

Figure 1. The enzyme activity of *B. subtilis* strain MSB9 in each fraction by using different substrate. (A) CMC and (B) Filter paper.

filter paper as substrates (Figure 1). The cellulase activities were determined based on the absorbance at 540 nm of DNS assay. The results showed that fraction number 13 and 14 have the highest CMCase and FPase activities, respectively.

To analyze with SDS-PAGE and CMC zymogram, fraction number 12 and 13 were pooled together and concentrated again, and the pooled sample then was analyzed by SDS-PAGE and CMC-zymogram as described in methods (Figure 2). SDS-PAGE analysis of pooled fraction (fraction number 12 and 13) from MSB9 protein samples revealed at least three major polypeptides of apparent size 20, 35, and 45 kDa after stained with Coomassie brilliant blue R-250. However, zymogram analysis, using CMC as substrate, revealed two polypeptides with approximately 35 and 45 kDa bands showed clear zone indicating the CMCase activity. Based on SDS-PAGE and CMC-zymogram analysis, the purified CMCase enzymes produced from MSB9 was shown to have molecular weight about 35 and 45 kDa. In this study, we did not perform zymogram using filter paper as substrate, we hypothesized that FPase enzyme should have molecular weight larger than CMCase (45 kDa) because the fraction that has the highest FPase activity is fraction number 14 and eluted out from the column later than fraction number 13.

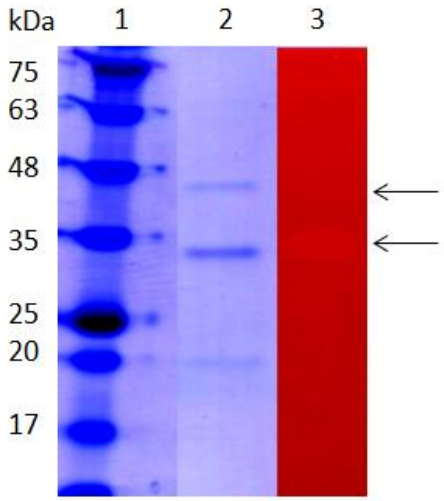

Figure 2. SDS-PAGE (lane 2) and CMC-zymogram (lane 3) analysis of pooled fraction number 12 and 13 protein samples isolated from *B. subtilis* strain MSB9.

We, next, aim to study for more details about this enzyme, including finding optimal condition for enzyme activity, and kinetic properties. The identification of polypeptide sequence should be done by using LC-MS/MS to track back to the gene encoding to this enzyme.

4. CONCLUSION

The newly bacterial isolate, *B. subtilis* strain MSB9, is a potent cellulolytic bacterium because it can tolerate at high pH condition, which makes it become a good candidate for industrial applications. Here, we identified at least two types of cellulase enzymes that have CMCase activity. We further aim for characterization of these CMCase and also FPase from fraction number 14, which helps to extend the application of these enzymes to various types of cellulose substrates.

5. ACKNOWLEDGEMENT

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6. REFERENCES

Maki M, Leung KT, & Qin W (2009) The prospect of cellulase-producing bacteria for the bioconversion of lignocellulosic biomass. *Int J Biol Sci* 5(5):500-516.

Cherubini F (2010) The biorefinery concept: Using biomass instead of oil for producing energy and chemicals. *Energy Conversion and Management* 51:1412-1421.

Sriariyanun M, *et al.* (2014) Characterization and purification of cellulase enzyme produced by Bacillus MSB12. *International Conference on Sustainable Global Agricultural and Food Security 2014* pp 77-81.

Kasana R, Salwan R, Dhar H, Dutt S, & Gulati A (2008) A rapid and easy method for the detection of microbial cellulases on agar plates using gram's iodine. *Curr Microbiol.* 57(5):503-507.

Ghose T (1987) Measurement of cellulase activities. *Pure and Appl Chem* 59(2):257-268.

Miller G (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* 31(3):426-42.

EFFECT OF VISCOSITY AGAINST OVERRUN ICE CREAM USING CORNSTRACH, MOCAF AND CANNA FLOUR AS CARBOHYDRATE - BASED FAT REPLACER

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ABSTRACT

Ice cream is a dairy product that containof high fat. The fat existence in the ice cream contributesonthe ice creambody and texture. Decreament of fat content in the ice cream product can make the ice cream melt quickly. The usage of stabilizers and carbohydrate-based fat replacer can improve the body and texture of low - fat ice cream. Cornstarch, mocaf and canna flour is severalmaterials that can be use in the ice cream products as carbohydrate - based fat replacer. The usage of each fat replacer will cause a different viscosity. The purpose of this research was to find out the characteristics of ice cream dough viscosity against the overrun. The formulationratio in this research: whipped cream against the flour (cornstarch, mocaf and canna flour) was 75% : 25%. The results showed that on mocaf flour ice cream, the value of overrun was the closest to the control ice cream rather than cornstarch ice cream and canna flour to the control ice cream. The value of control ice cream overrun was 76.25% and for the flour mocaf ice cream was 61.21%. The dough viscosity can reach valueof 66.25 d.Pa.s, and this can increase the overrun value of carbohydrate-based fat replacer ice cream.

Key words:*Ice cream, fat replacer, cornstarch, mocaf, canna flour.*

1. INTRODUCTION

Ice cream is one of dairy products whichcreated by freezing and mixing the raw materials together. Ice cream has 3-4% milk fat, 12-14% solids not fat, 29-31% total solids, 0.4% stabilizers and 13-16% sweeteners (Naresh and Shailaja, 2006). The high fat content of ice cream comes from whipped cream and milk. Whipped cream is used to help the development process and the cream, texture and shape formation of a product (Bennion and Hughes, 1975). In an ice cream product, the fat content can affect the dough viscosity and the air trapping. Arbuckle (1996) said, the viscosity is an important characteristic of a nice cream dough, which is to get a proper foaming and to restrain the air. Therefore, carbohydrate-based fat replacer was used in this research to find out the viscosity characteristics and the overrun of low-fat vegetarian ice cream. Fat replacer significantly contains less fat and calories. In general, carbohydrate-based fat replacer aims to reduce fat and calories because fat has energy value of 9 kcal / g. Carbohydrate or protein-based fat replacer has less energy i.e. 4 kcal / g.

Carbohydrate-based fat replacer which used in this research was using cornstarch, mocaf and canna flours. The reason in choosing these flours were because of their high carbohydrate content, and also the usage of these materials is one of alternative way to increasing the diversity of food in Indonesia. The usage of this carbohydrate-based fat replacer can improve the quality of low-fat vegetarian ice cream. The raw material of the flours is easily found in Indonesia because it is a local raw material. Besides it is easy to grown, this flours is also easy to processed and ended up with easy to obtained. The price of this flour is very cheap and mostly in good quality, so that people can buy and process them easily.

The starch from different sources also have different natures and characteristics due to the different type of content and molecular structures. Starch molecule consists of two fractions i.e. amylose and amylopectin. Amylose is a long-chain molecules that affect the gel characteristics during the heating and cooling.

The purpose of this research was to find out the effect of viscosity against the ice cream overrun using cornstarch, mocaf and canna flours as the alternate carbohydrate-based fat replacer.

2. MATERIALS DAN METHODS

2.1. The Research Time and Place

This research was conducted in two phase i.e. introduction research and main research. Both research was done at Food Engineering Laboratory and Food Science Laboratory of Food Technology Study Program, Faculty of Agricultural Technology Soegijapranata Catholic University.

2.2. Material

2.2.1. Materials

The materials used in this research were soybean, canna flour, cornstarch, mocaf, whipping cream, fructose, baking soda and distilled water.

2.2.2. Tools

The tools used to make ice cream were Ice Cream Maker, mixer, blender, freezer, measuring cup and a tool to do viscosity test i.e. viscometer (RION Viscotester VT - 04) (d.pas).

2.3. Methods

2.3.1. The Making of Soybean Essence

The making of soybean essence process can be seen in Figure 1.

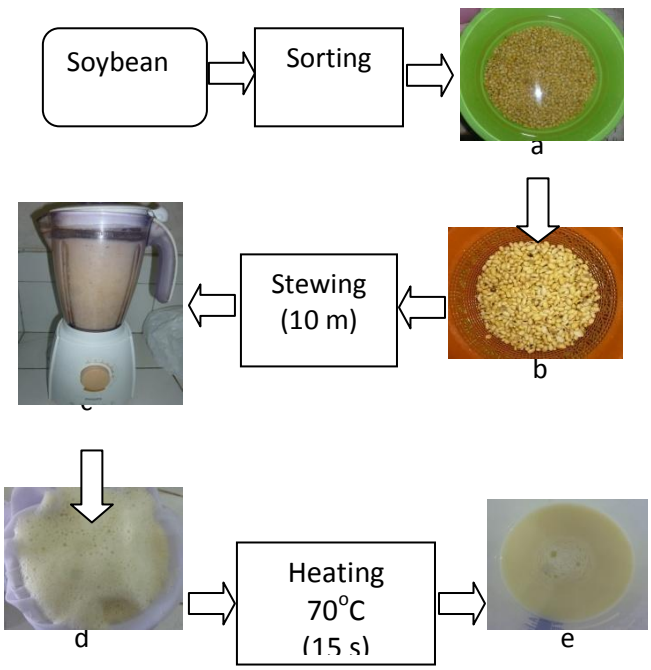

Figure 1 : Flowchart Process ofSoybean Essence(Gulo, 2006 modified)

Description:

a. The soybean being soaked with rasio of clean water:soybean (1:3) for 12 hours and 5 grams of baking soda added.
b. The soybean being washed and drained
c. The soybean being grinded by the ratio soybean:water (1:4)
d. The soybean being filtered
e. Soybean essence

2.3.2. Making the Ice Cream at Different Fat Replacer Concentrations

The process of making the ice cream can be seen in figure 2.

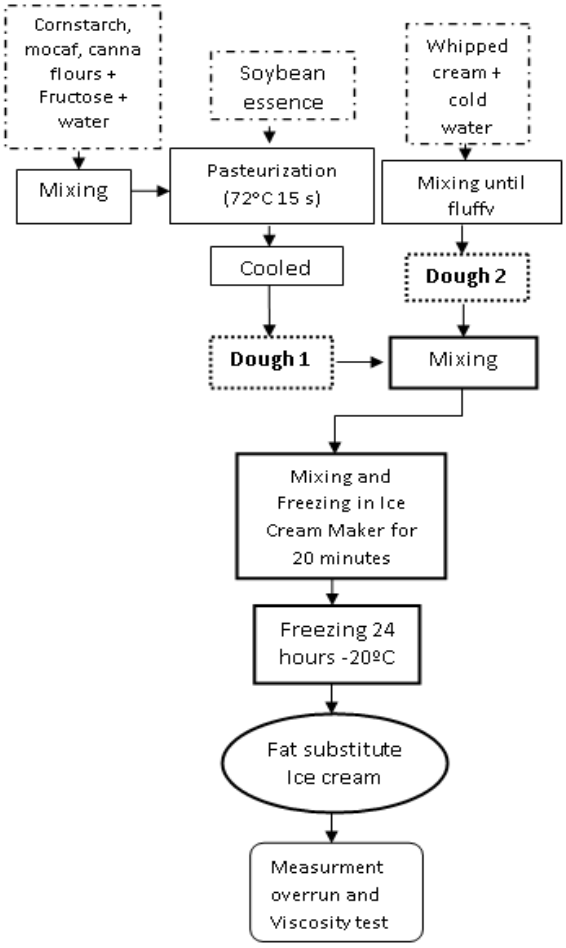

Figure 2: The process of making ice cream

Table 1. Ice cream formulations of Carbohydrate-Based Fat replacer

Materials	P0	P1	P2	P3
Soybean essence (ml)	200	200	200	200
Fructose	60	60	60	60
Whipped cream (%)	100	75	75	75
Cold water(ml)	200	150	150	150
water (ml)	-	200	200	200
Cornstarch (%)	-	25	-	-
Mocaf (%)	-	-	25	-
Canna Flour (%)	-	-	-	25

(Source : Astawan & Astawan, 1988 modified)

Description:

P0: The addition of whipped cream without adding fat replacer(control)

P1: Comparison of cornstarchand whipped creamaddition (25: 75)

P2: Comparison of mocaf and whipped cream addition (25: 75)

P3: Comparison of canna flour and whipped cream addition (25: 75)

2.3.3. Ice cream Overrun level analysis (Potter & Hotchkiss, 1996)

Overrun measurements aims to find out the increasing of ice cream volume that occurred after the freezing and mixing process. The principle of overrun measurement is the difference between the ice cream volume and the dough volume at the same time or the difference of the ice cream mass and the cought at the same volume. ice cream Overrun measurement % can be calculated with the following formula:

$$overrun = \frac{\text{volume of after mixing - volume of early dough}}{\text{volume of early dough}} \text{ x } 100\%$$

2.3.4. Ice cream viscosity measurements (Prindiville *et al*, 2000).

Ice cream viscosity measurements were performed by using a Viscotester at the room temperature. Viscosity measurements were done on ice cream that has not been and had been frozen at temperature of ±4°C. The Viscosity Measurement of each ice cream has been done for three times replications.

2.3.5. Data Analysis

The data obtained from this research were processed using SPSS for Windows version 14.0 using One Way ANOVA methods and Duncan's regiontest.

3. RESEARCH RESULTS

The physical characteristics of viscosity and ice cream overrun at various concentration of fat replacer can be seen in Table 2.

Table 2. Ice Cream Physical Characteristics in Various Fat Replacer Concentration

Sample	Characteristic		
	Overrun (%)	Viscosity1 (d.Pa.s)	Viscosity2 (d.Pa.s)
P0	**76,25 ± 6,66** [c]	53,33 ± 3,02 [a]	29,16 ± 3,41 [a]
P1	44,01 ± 7,67[a]	63,33 ± 3,02 [bc]	34,16 ± 4,67 [bc]
P2	61,21 ±	**66,25 ±**	**38,75 ±**

	6,59 [b]	**3,79 [c]**	**4,08 [c]**
P3	40,83 ± 7,54 [a]	61,25 ± 4,40 [b]	32,50 ± 3,16 [ab]

Description:

- P0: ice cream with the addition of 0% carbohydrate-based fat replacer and 100% whipped cream
- P1: ice cream with the addition of 25% cornstarch and 75% whipped cream
- P2: ice cream with the addition of 25% mocaf and 75% mocaf whipped cream
- P3: ice cream with the addition of 25% canna flour and 75% whipped cream
- Viscosity 1: viscosity before freezing.
- Viscosity 2: viscosity after freezing.
- Values with different superscript within a column indicates a significant differences between treatments at the 95% confidence level ($p < 0.05$) based on the one way ANOVA with Duncan's multiple range test using of different tests.

The research result in Table 2 shows that the control ice cream has a higher overrun value than the ice cream with flour addition. Based on one-way ANOVA test of significance using Duncan's multiple range tests, the ice cream overrun percentage significantly different from the control ice cream overrun value with the addition of cornstarch, mocaf and canna.

The real difference is shown bythe control ice cream overrun percentage which higher i.e. 76.25 ± 6.66 compared with ice cream which being added with cornstarch, mocaf and canna i.e. 44.01 ± 7.67, 61.21 ± 6.59 and 40.83 ± 7.54. Viscosity values before and after control ice cream freezing is significantly different from ice cream which being added with cornstarch, mocaf and canna because it has a lower value i.e. 53.33 ± 3.02 and 29.16 ± 3.41.

4. DISCUSSION

Overrun is the increasing of dough volume which generated from air trapping at the time of the mixing process during the freezing period (Muse and Hartel, 2004). Arbuckle (1996) said that the viscosity or flow resistance is an important characteristic of ice cream dough to get a proper foaming and for air detention. In this research, ice cream viscosity measurements had been done before and after freezing at temperature of 4 ± 1^{o}C.Viscosity measurements after freezing were done to find out the effect of the freezing process.

Table 2 shows that the overrun value of the control ice cream is higher than the ice cream with flour addition. While the viscosity of the ice cream control is lower than the ice cream with flour addition. Muse and Hartel (2004) said that the fat content in the ice cream will be more destabilizated due to mixing action so that it forming a fat globule which coat and restrain air molecule, and then linked together to form fatty tissue which will trap the air. Therefore the overrun value of the control ice cream is higher because it

contains more whipping cream. Whipping cream and milk are known as fat source within the ice cream products so that the overrun value of the control ice cream is higher because it can trap air during the mixing.

Table 2 also shows that the control ice cream has a lower viscosity value than the ice cream with flour addition. Viscosity values before ice cream freezing has a higher value than the viscosity values after freezing. According to Setianawati et al., (2002) the decreament of viscosity due to the deformation of the ice cream shape due to temperature changes (heat shock) from the freezing process to thawing process.

Overrun value of the ice cream with cornstarch, mocaf and canna flours (44.01%, 61.21% and 40.83%) addition is still below the control (76.25%) indicate that carbohydrate-based fat replacer have not been able to trap the air during the mixing. According Adapa et al., (2000), on the thick ice cream dough, the bonds between the molecules will become more tight so that it would be difficult to trap the air during the mixing process because the matrix has been become solid. Added by Akesowan (2008) that the more thick gel network can affect air entry during the freezing process, so that the overrun values is lower.

Mocaf, cornstarch and canna flours as the ice cream fat replacer has a high ability to form a water trap gel during the gelatinize process, but have a low ability to form an air trapper matrix.

Table 2 results shows that the control ice cream, cornstarch, mocaf and canna flours ice cream has viscosity values after freezing i.e. d Pa.s 29.16, 34.16 d.Pa.s, 38.75 d.Pa. s and 32.50 d Pa.s. Ice cream which treated with various types of flour as a carbohydrates-based fat replacer have a more viscous characteristics than the control ice cream. This was occur because the flours contain more starch so that it included in fat mimetic group of fat replacer because of its ability to absorb large amounts of water that can mimic some of the fat on the sensory natures of ice cream (Akoh, 1998).

Mocaf flour ice cream has a higher viscosity and overrun values compared to the cornstarch and canna flour ice cream. This relates to the relationship between overrun and viscosity. Viscosity determines the ice cream overrun. The greater viscosity value then the greater percentage of overrun untill a certain extent, and vice versa. This is occurs because at a certain viscosity the ice cream dough capable to trap the air.

If the ice cream dough is too liquid, it has less ability to trap the air. For the thick ice cream dough, there is only a few space for

the air bubble during the mixing because the composer molecule structure is too close resulting a lower overrun cough value. This is occurs because the air bubble which trapped in the ice cream has become the barrier for the fluid to flow.

5. CONCLUSIONS

- Substitution of cornstarch, mocaf and canna flours as carbohydrate-based fat replacer can change the characteristics of viscosity and ice cream overrun.
- The higher overrun value, the higher viscosity untill a certain extent.
- Ice cream with formulation which contains of 75% whipped cream and 25% mocaf flour has a higher overrun percentage (61.21 ± 6.59) and also the viscosity value before and after deep cooling (66.25 ± 3.79 and 38.75 ± 4.08) compared with cornstarch and canna flours ice cream.

6. REFERENCES

Adapa, S.; H. Dingeldein. K. A. Schmidt and T. J. Herald. (2000). *Rheological properties of ice cream mixes and frozen ice creams containing fat and Fat replacers*. Journal of Dairy Science 83: 2223-2229.

Akesowan, A. (2008). *Effect of combinated Stabilizers Containing Konjac Flour and Carrageenan on Ice cream.* http://www.journal.au_techno/2008/oct08/12(2)_Article02.pdf. 13 January 2014.

Akoh, C. C. (1998). *Fat replacer*s. Department of Food Science and Technology. The University of Georgia. Athens.

Arbuckle, W. S . (1996). *Ice cream*. The Avi Publishing Company, Inc. London.

Bennion, M and O. Hughes. (1975). *Introductory Foods*. Macmillan Publishing Co, Inc. New York.

Gulo, N. (2006). Substitusi Susu Kedelai dengan Susu Sapi pada Pembuatan Soyghurt Instan. Jurnal Penelitian Bidang Ilmu Pertanian Volume 4,Nomor 2. Unika St.Thomas Sumatera Utara.

Muse, M. R and R. W. Hartel. (2004). *Ice cream structural elements that affect melting rate and hardness*. American Dairy Science Association. Journal of Dairy Science 87:1-10.

Naresh, L; U, Shailja and Merchant. (2006). *Stabilizers Blends Their Impoertance in Ice cream Industry-A Review*. New Zealand Food Magazine. http://www.lucidgroup.com/knowledge-center/*stabilizer*,pdf. 14 Maret 2014.

Potter, N. N and J. H. Hotchkiss. (1996). *Food Science 5th Edition*. CBS Publishers and Distributors. New Delhi. India.

Prindiville, E. A.; R. T. Marshall and H. Heymann. (2000). *Effect of milk fat, cocoa butter, and whey protein Fat replacers on the sensory properties of lowfat and nonfat chocolate ice cream*. Journal of Dairy Scrience 83:2216-2223.

Setianawati, H. N.; B. Setiawan dan L. N. Yuliati. (2002). Penggunaan kombinasi bahan penstabil dalam pembuatan velva kweni (Mangifera odorata Griff) dan perubahan mutu selama penyimpanan. Media Gizi dan Keluarga.

DEGRADATION KINETICS OF RED BEET (*Beta vulgaris*) POWDER DURING THERMAL PROCESSING AND CHANGES OF pH

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ABSTRACT

Red beet (*Beta vulgaris*) is a source of antioxidants and often used as a natural colorant in the food processing. Antioxidant content in beets is called betalain, which are classified into betacyanin which is purplish red pigment and betaxhantin which is the yellow orange pigment. Antioxidants are compounds that can delay or prevent the oxidation of free radicals such as rancidity and discoloration but are very susceptible to degradation. Free radicals are highly reactive molecules because it has unpaired electrons in the outer orbital of the molecule so that it can react with the cells of the body that can cause disease. Antioxidant activity degradation is determined by calculating the activation energy. The activation energy is the minimum energy required to perform a reaction. The purpose of this study was to determine the effect of pH, temperature and heating duration on antioxidant degradation of red beet powder and to determine the degradation kinetics by calculating the value of the activation energy based on the decrease in antioxidant activity with Arrhenius model. Preparation of red beet powder is conducted by drying red beet using cabinet dryer. Methods of research conducted with three levels, i.e. pH 6, 7 and 8; with four different temperatures, i.e. 40^{o}C, 60^{o}C, 80^{o}C and 90^{o}C; and at five heating rate, i.e. 0, 5, 10, 15 and, 20 minutes. The analysis performed in this study is the analysis of antioxidant activity in red beet powder using DPPH method. The results were calculated using the Arrhenius equation. The results showed that lowest decreasing antioxidant activity is at pH 6, temperature 40^{o}C and 0 minute; is equal to 25.40% and the highest decreasing antioxidant activity is at pH 8, temperature 80^{o}C and 20 minutes is equal to 49.76%. The highest activation energy (Ea) is pH 6, at 0 minute that equal to 7303.85 J/mol. While the lowest Ea is pH 8 at 20 minutes that equal to 4230.16 J/mol.

Keywords: *red beet powder, antioxidants, activation energy, degradation kinetics*

1.INTRODUCTION

Red beet (*Beta vulgaris*) is purplish red tuber and often used as natural food colorant so the product can improve consumer attraction. Nowadays, synthetic food colorant already widely circulated in the community. However, it becomes dangerous if we consumed it for a long period because it will cause carcinogenic effects. To reduce the use of synthetic food colorant, we can use natural food colorant, i. e. red beet. The benefits of this colorant are safe use and also contain high of antioxidants. Antioxidants found in red beet called betalain. Betalain classified into two pigment, betaxhantin which has orange and yellow color and betacyanin

which has purplish red color (Pitalua *et al*., 2010).

Besides betalain, antioxidants contained in red beet namely polyphenols, vitamins, flavonoids and folic acid. Antioxidants work to prevent the oxidation reactions of free radicals which can cause rancidity and damage to food products and can inhibit cancer cells that are good for health (Windono *et al*., 2001).

Red beet powder can be used as a natural colorant for beverage products. In red beet beverage processing using heat treatment, antioxidants found in red beets will be degraded. Stability of betalain influenced by light, oxygen, water activity, pH and temperature (Gaztonyi *et al*., 2001).

Temperature affects the rate of reaction as a factor affecting the decline of the food product quality. Higher temperature will caused reaction rate become faster so the antioxidant activity became decrease. Betalain stable at temperatures below 40^{o}C and in acidic conditions with a pH range of 4-6 (Fennema, 1997)

This research use three different pH condition; pH 6 (acidic), 7 (neutral), and 8 (alkaline). Selection of the pH to determine antioxidant degradation of red beet, because not all products are produced under conditions of acidic beverages. Based on that statement, the research is conducted to determine the degradation kinetics of the antioxidant activity in red beet powder with a combination of pH, temperature and heating duration. Besides that, the Arrhenius approach is used to calculate the changes of activation energy.

2.MATERIALS AND METHODS

2.1.Chemicals and Tools.

The materials used in this study are red beet powder, methanol, distilled water, DPPH solution, 10% HCl solution and 10% NaOH solution. The tools used in this study are the cabinet dryer, blender, slicer, pH meter, a closed reaction tube, water bath, thermocouple and spectrophotometer. The data analyses are conducted with Microsoft Excel 2010 and MatLab 2010a.

2.2.Sample Preparation.

Red beet is dried with dryer cabinet to form a powder blend. The powder is dissolved, and then treated with 3 levels of pH (pH 6, pH 7, and pH 8). NaOH solution is added to make alkaline condition; meanwhile HCl solution is added to make acidic condition of sample. Next, the sample are placed in a reaction tube and heated in a water bath. Each pH level is heated with four level of temperature (40^{o}C, 60^{o}C, 80^{o}C, and 90^{o}C) for 0, 5, 10, 15 and, 20 minutes. Heating temperature of 40^{o}C betalain pigments to represent optimum degree of stabilization of pigment; and the temperature of 60^{o}C, 80^{o}C and 90^{o}C are

represent pasteurization temperature. Combination treatment performed three replications. Determination of antioxidant activity is conducted with DPPH (2, 2-diphenyl-1-picrylhydrazyl) method.

2.3.Antioxidant Activity of the Red Beet Powder (DPPH method).

0.5 gram sample was weighed and extracted with 5 ml of methanol for 2 hours in the dark room. 0,1 ml of extract were taken and treated with 3.9 ml of DPPH solution made by dissolving 2.4 mg of DPPH in 100 ml of methanol. Samples were incubated in the dark for 30 minutes and absorbance was measured at a wavelength of 515 nm. As a control, use 0, 1 ml of methanol were reacted with 3.9 DPPH solution and methanol used as a blank solution.

Antioxidant activity is expressed as % inhibition with the following equation:

$$\%inhibition = \{(A_B - A_A)/A_B\} \times 100\%$$

Description:

A_A = sample absorbance at 30 minutes

A_B= control absorbance (methanol+DPPH)

(Williams *et al*., 1995)

2.3.Logistic Equation.

The mathematics equation model (logistic equation) is used to determine degree of antioxidant activity degradation with equation:

$$A(t) = \frac{A_f}{1+Ce^{-rt}}$$

$$C = \frac{A_f}{A_o} - 1$$

Description:

A: antioxidant activity degradation

A_0: antioxidant activity degradation at t=0

A_f: antioxidant activity degradation at t time

r : constant

t: heating duration (min)

(Wolf & Venus, 1992 in Garnier *et al*., 1999; modification).

2.4.Antioxidant Degradation Kinetics of Red Beet Powder.

The determination of the Ea value using Arrhenius equation, the data obtained by analysis of linear equations where Ea is the activation energy, whose value is assumed to be constant at a certain temperature range, R is the gas constant (8.314 J/mol K), T is the temperature expressed in Kelvin (K). Arrhenius equation can be seen as follows:

$$\ln k = \ln k_0 - \frac{Ea/R}{T}$$

Where the rate constant k showed a decrease in quality, k_0 is a constant (independent of temperature), Ea is the activation energy, T is absolute temperature (K), R is the gas constant (8.314 J/mol.K) (Arpah, 2003).

3.RESULTS AND DISCUSSION

3.1.Antioxidant Activity Degradation of Red Beet Powder

Table 1. *Antioxidant Activity Degradation in Red Beet Powder with Variation of pH, Temperature, and the Heating Duration*

pH	Temperature (°C)	Antioxidant Activity Degradation (%)				
		Heating Duration				
		0 minute	5 minutes	10 minutes	15 minutes	20 minutes
6	40	25.40±0.64	28.32±1,55	32.53±1.00	35.53±0.96	36.11±0,59
	60	27.77±0.29	29.53±0.99	34.30±2.72	35.85±2.62	36.79±2.35
	80	34.28±0.90	36.02±0.35	39.32±1.50	39.40±1.13	41.42±2.54
	90	37.76±0.21	42.11±0.75	43.98±1.70	45.02±1.82	45.52±0,47
7	40	29.05±0.20	32.86±0.76	32.95±1.18	36.03±1.20	37.58±3.38
	60	30.30±0.47	33.04±0.45	35.63±1.61	37.64±1.62	40.19±2.38
	80	34.20±0.88	37.45±0.36	39.21±1.94	41.77±1,49	42.72±1.03
	90	39.86±0.61	43.90±1.52	44.46±0.76	45.13±0.79	46.51±0.94
8	40	29.71±0.98	32.59±1.56	35.38±2.06	37.89±3.29	39.71±2.61
	60	31.13±0.41	35.09±1.96	39.11±2.84	41.20±0.46	42.50±1.43
	80	36.44±0.59	36.53±0.66	43.00±1.21	45.79±0.15	47.59±0.38
	90	41.05±0.89	44.98±1.79	46.01±0.15	47.33± 0.99	49.76±2.77

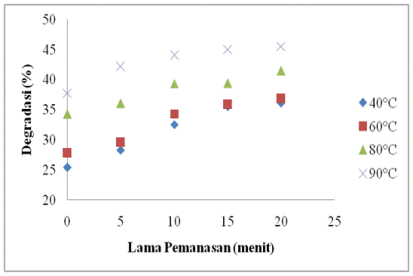
Description:

All values are mean ± SD (Standard Deviation)

From Table 1. there was gradual increase antioxidant activity degradation from red beet powder at pH 6 in temperatures ranging from 40°C to 90°C also in the heating duration is started from minute 0 to minute 20. At pH 7 and pH 8 also showed similar results, Antioxidant activity degradation will increase with higher heating temperature and longer heating duration. The higher degradation is at pH 8 with a temperature of 90°C in the 20 minutes. Fennema (1997) said that red beet has a pH optimum between pH 4 and pH 6. Therefore the antioxidant activity degradation of red beet at pH 6 is lower than at pH 7 and 8 because in pH 6 the sample is at stable pH conditions. Acidic conditions can increase antioxidant activity stability because it can prevent oxidation so as to minimize the damage of betalain pigments (Ravichandran et al., 2013). Temperature of 40°C is the optimal temperature for the pigment stability so the damage or the degradation is still relatively lower than the higher temperature (Roy et al., 2004).

Heating causes degradation in the form of betalain pigment degradation. This pigment is converted into betalamic acid and cycloDOPA 5-O-glycoside. The longer the heating process, the pigment betalain

breakdown process will be greater (Delgado-Vargas et al., 2000).



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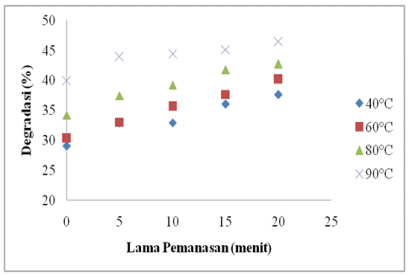

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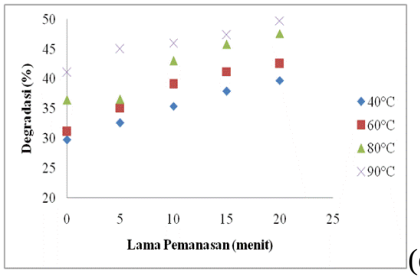
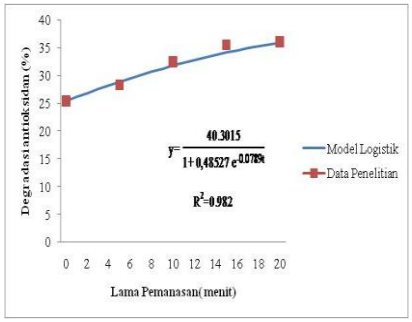


(c)

Figure 1. Decrease in antioxidant activity of red beet powder on (a) pH 6, (b) pH 7, (c) pH 8.

In Figure 1. shows that the higher pH will caused higher degradation of antioxidant activity of red beet powder. On the research from Stintzing and Carle (2004), it said that pH is a factor determines the stability of antioxidant activity. In addition to pH, temperature and heating duration can also cause antioxidant activity of red beet become unstable. Temperature affects the stability of the antioxidants in red beets. In theory of Stintzing and Carle (2004), betalain stability is affected by temperature, pH, moisture, light and oxygen. At high temperature, antioxidants in red beet powder will be damaged.

Logistic model



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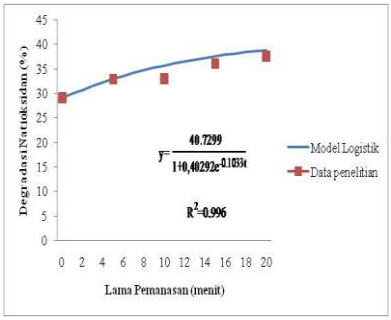

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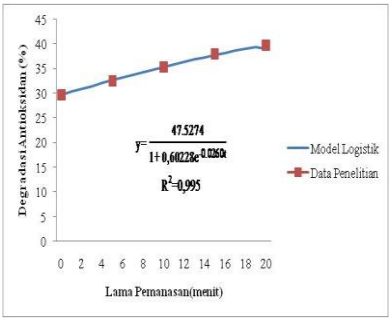

(c)

Figure 2. Decrease antioxidant activity of red beet powder to the logistic equation on (a) pH 6, (b) pH 7, (c) pH 8

In Figure 2. we can be see that the antioxidant activity degradation will increased with the longer heating duration. Highest antioxidant activity degradation is on 90^{o}C at 20 minutes and the lowest antioxidant activity degradation is on 40^{o}C at

5 minutes.

Table 2. *Correlation Coefficient of Linear Equations in Red Beet Powder*

Temperature	pH 6	pH 7	pH 8
40^0C	0.967	0.991	0,993
60^0C	0.935	0.997	0,954
80^0C	0.942	0.971	0.958
90^0C	0.953	0.846	0,946

Table 3. *Correlation Coefficient of Logistic Equations in Red Beet Powder.*

Temperature	pH 6	pH 7	pH 8
40^0C	0.982	0.996	0.995
60^0C	0.960	0.998	0.996
80^0C	0.957	0.986	0.979
90^0C	0.960	0.973	0.961

In the Table 2 and Table 3 it can be seen that the higher correlation coefficient, the relationship between x and y will be higher too (Labuza, 1978). Based on these results it can be concluded that the antioxidant activity degradation of red beet powder tends to use the principle of logistic equation, where the initial heating of antioxidant degradation begins to rise higher and then increases the longer the increase be fixed at some point. Timuneno (2008) states that the logistic equation is showed that at a certain point decrease in antioxidant activity reaches equilibrium, so that the graph will be a constant.

According to D'Amico *et al.* (2006), the temperature and time of pasteurization for red beet powder is 60^oC for 18 minutes; while according Klewicka & Czyzowska (2011) using temperature 80^oC for 10 minutes and 90^oC for 5 min. When the pasteurization temperature and time applied in the logistic equation for each pH, the value of % antioxidant activity degradation of red beet powder can be seen in Table 4. From Table 4 it can be seen that the pasteurization time and temperature are inversely related. The higher the heating temperature, the shorter the time required.

Tabel 4. *% Antioxidant Activity Degradation of Red Beet Powder Based on Temperature and Time Pasteurization*

Temperature/duration of pasteurization	% degradation		
	pH 6	pH 7	pH 8
60^oC/18 minutes	36.79%	40.19%	42.50%
80^oC/10 minutes	39.32%	39.21%	43.00%
90^oC/5 minutes	42.11%	43.90%	44.98%

3.2.Antioxidant Degradation Kinetics of Red Beet Powder

Degradation kinetics of the antioxidant of red beet powder was analyzed using the Arrhenius equation. The trick is plotting ln k (%antioxidants degradation) with 1/T (temperature heating) for each heating time and pH conditions in order to obtain a linear equation. From this linear equation obtained value gradient/slope equal to the activation energy that divided with the gas constant

values (Table 5).

Table 5. *Activation Energy Values at Each pH*

pH	t (min)	*Slope* (k)	R (J/mol.K)	Ea (J/mol)
6	0	878,5	8.314	7303,85
	5	827,7	8.314	6881,50
	10	635,4	8.314	5282,71
	15	603,2	8.314	5015,00
	20	580.4	8.314	4825,44
7	0	720,7	8.314	5991,80
	5	718,1	8.314	5970,28
	10	618,8	8.314	5144,70
	15	599,6	8.314	4985,07
	20	529,6	8.314	4403,09
8	0	681,5	8.314	5665,99
	5	626,1	8.314	5205,40
	10	579,2	8.314	4815,46
	15	511,0	8.314	4248,45
	20	508,8	8.314	4230,16

Description

T = heating duration (menit)

Slope = gradient (K)

Ea = activation energy (J/mol)

R = gas constant (J/mol.K)

From the Table 5 it can be seen that the activation energy of each sample is proportional to the value of degradation quality constant. The highest activation energy is red beet powder at pH 6 were heated for 0 minutes; while the lowest activation energy is red beet extract at pH 8 were heated for 20 minutes. According Azeredo (2009), degradation of the pigment betalain will caused the value of the activation energy decreases. This means that the greater the degree of degradation of betalain pigments, the value of the activation energy decreases.

The activation energy the minimum amount of energy supplied to a reaction (Hariyadi, 2004). From the theory of Robertson (1993), states that the smaller the activation energy, the faster the product will be severely degrades. To calculate the activation energy, make a graph of ln k with 1/T, can be seen in Figure 3. it can be seen that the obtained linear equation y=mx+b. The slope of the equation above represents the value (-Ea/R) with constant gas of 8,314 J/mol.K. In the linear equation y=-878,5x+1.433 (R^2=0.995) obtained Ea value of 7303.85 J/mol.

CONCLUSION

- Decrease in the antioxidant activity of extracts of red beet will increase when the temperature and heating time increased. Decrease in antioxidant activity in red beet powder will increase when conditioned in neutral and alkaline pH.
- The best temperature of pasteurization is at a temperature of 60^{o}C while the pasteurization temperature which causes a decrease in antioxidant activity was highest in the temperature of 90^{o}C.
- The higher the temperature of

pasteurization, the heating duration will be lower, stable antioxidant activity carried out at a temperature of 60^{o}C at 18 minutes pasteurization, pasteurization is done at a temperature of 80^{o}C at 10 minutes and at a temperature of 90^{o}C at 5 minutes.

REFERENCES

Arpah. (2003). Penentuan Kadaluarsa Pangan. Buku 2: Penuntut Praktikum. Institut Pertanian Bogor. Bogor.

Azeredo, H. M. C. (2009). Betalains: Properties, Sources, Applications, and Stability – A Review. International Journal of Food Science and Technology 44: 2365–2376.

D'Amico, D. J.; T. M. Silk; J. Wu; & M. Guo. (2006). Inactivation of Microorganisms in Milk and Apple Cider Treated with Ultrasound. J. Food Prot 69: 556-563.

Delgado-Vargas, F. & O. Paredes-López. (2003). Natural Colorants for Food and Nutraceutical Uses. CRC Press. Boca Raton.

Fennema, O. R. (1997). Food Chemistry: Second Edition, Revised and Expanded. Marcel Dekker, Inc. New York.

Gasztonyi, M. N.; H. Dadood; M. Takacs & P. Biacs. (2001). Comparison of Red Beet (*Beta vulgaris var conditiva*) Varietas on the Basic of Their Pigment Components. Journal of the Science of Food and Agriculture. 81(9): 932-933.

Hariyadi, P. (2004). Prinsip Penetapan dan Pendugaan Masa Kadaluarsa dan Upaya-Upaya Memperpanjang Masa Simpan. Institut Pertanian Bogor. Bandung.

Klewicka, E. & A. Czyżowska. (2011). Biological Stability of Lactofermented Beetroot Juice During Refrigerated Storage. Pol. J. Food Nutr. Sci. 4:251-256.

Labuza, T. P. (1982). Shelf Life Dating of Foods. Food and Nutrition Press Inc. Connecticut.

Pitalua E; M. Jimenez; E. J. Vernon-Carter; C. I. Beristain. (2010). Antioxidative Activity of Microcapsules with Beetroot Juice Using Gum Arabic As Wall Material. J. Food And Bioproducts Processing 88: 253-258

Ravichandran, K.; N. M. M. T. Saw; A. A. A. Mohdaly; A. M. M. Gabr; A. Kastell; H. Riedel; Z. Cai; D. Knorr; & I. Smetanska. (2013). Impact of Processing of Reed Beet on Betalain Content and Antioxidant Activity. Journal of Food Research International 50:670-675

Robertson, G. L. (1993). Food Packaging: Principles and Practice. Marcel Dekker Inc. New York.

Roy, K.; S. Gullapalli; U. R. Chaudhuri & R. Chakraborthy. (2004). The Use of a Natural Colorant Based on Betalain in the Manufacture of Sweet Products in India. Journal of Food science and Technology 39: 1087–1091

Stintzing, F. C. & R. Carle. (2004). Functional Properties of Anthocyanins and Betalains in Plants, Food, and in Human Nutrition. Trends in Food Science and Technology 15: 19–38

Timuneno, H. M.; R. H. S. Utomo; & Widowati. (2008). Model Pertumbuhan Logistik dengan Waktu Tunda . Jurnal Matematika 11: 43-51

Williams, W. B.; M. E. Cuveller; & C. Berset. (1995) Use of a Free Radical Method to Evaluate Antioxidant Activity. Lebensm-wiss Vol. 28. pp.25-30.

Windono, T.; S. Soediman; U. Yudawati; E. Ermawati; Srielita; T. I. Erowati. (2001). Uji Peredam Radikal Bebas terhadap 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) dari Ekstrak Kulit Buah dan Biji *Anggur (Vitis vinifera L.)* Probolinggo Biru dan Bali. Artocarpus 1: 34-43

Wolf, K. H. & J. Venus. (1992). Description of the Delayed Microbial Growth by an Extend Logistic Equation. Dalam Garnier, P. M.; R. Auria; C. Augur; & S. Revah. (1999). Cometabolic Biodegradation of Methyl T-Butyl Ether by *Pseudomonas aeruginosa* Grown on Pentane. Appl Microbiol Biotechnol 51: 498-503

PHYSICOCHEMICAL CHARACTERISTICS OF MOCAF STEAMED CAKE WITH BEET ROOT EXTRACT ADDITION AS NATURAL COLORANT

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ABSTRACT

Red beet is one of the bulbs that contain betalain pigment that gives color and has a high antioxidant activity. Pigment that gives the red color is included in the class betacyanins betalain. This makes the red beet can be used as natural dyes in food products. However, pigment betacyanins is prone to discoloration and decrease during processing. These pigments are highly influenced by the temperature during processing. The application of red beet as natural coloring agent on steamed cake products that use mocaf flour. Opportunity to experience a steamed cake browning smaller as the temperature is not too high. The use of flour can replace whole wheat mocaf to utilize local food, enriching the nutritional value of steamed cake and to reduce the community's dependence on wheat flour. The addition of red beet extract steamed cake products can provide color and antioxidants. The purpose of this study was to determine the changes in the physicochemical characteristics of steamed cake (color, volume and content of antioxidants cake) flour based mocaf supplemented with extracts of red beet during the steaming process. This study uses a liquid extract of red beet with 3 levels of concentration (the ratio of bits: water) is 1:1, 1:2, and 1:3. Physical testing a color analysis and volume development. At the time of steaming for 0, 4, 8, 12, 16, 20 and 24 minutes the color analysis. In steaming for 0 minutes (batter before steaming) and steaming for 24 minutes (steamed cake) volume measurement development. Chemical analysis includes measurement of antioxidant activity. Analysis of data using different test methods One Way ANOVA with Duncan. The results showed that the use of red beet extract will reduce the level of brightness (L* value) but will increase the red color (a* value), volume expansion, and antioxidant activity. That the longer steaming time will lower the brightness level, the value of a *, and antioxidant activity, but will increase the value of b * steamed cake products.

Keywords: *red beet, steamed cake, antioxidants, color, volume development.*

1.INTRODUCTION

Red beet contain betalain pigments which can be used as a natural coloring agent in food products and has a high content of antioxidant. Application of red beet as food colorants can be used in bakery product such as steamed cake. Steamed cake is generally use wheat flour as the raw material of manufacture. In this research, steamed cake is using flour mocaf to reduce dependence on wheat flour and enrich the nutritional value. During the steaming process, antioxidant activity, color and volume of cake can be decreased. Therefore, the aim of this research is to find out the effect of red beet extract and steaming time to physicochemical characteristics of steamed cake.

2.MATERIALS AND METHODS

2.1.Materials

The materials used in steamed cake making are mocaf flour, red beet extract, sugar, egg, vegetable oil, water, margarine, emulsifier, and salt.

2.2.Steamed cake making

First of all, 85 g sugar mixed with three eggs as much as 150 g, 4 g emulsifier and 0,1 g salt. Then the mixing process is carried out using a hand mixer on low speed (level one) for 30 seconds and then the high speed (level three) for 1.5 minutes until the mixture thickens and becomes pale. Then added 160 g of flour mocaf, 75 g of vegetable oil and 100 g of water or liquid extracts of red beet (concentration of 1:1, 1:2 or 1:3), stirred at low speed for one minute to dissolve completely. The batter is then weighed as much as 50 g and put into a baking pan that has been spread with margarine and baking paper. Furthermore, the batter was steamed for 24 minutes. Physical and chemical testing (color and antioxidant activity) of the batter before steaming (0 minutes), 4, 8, 12, 16, 20 and 24 minutes, while the physical testing (volume development) was done on the batter before steaming (0 min) and steamed cake after 24 minutes of steaming.

2.3.Volume determination

Steamed cake was determined using volume measurement pan method. This method was done by measuring the length, height, and width of batter before steamed and after steamed with calipers. Volume development is the result of subtraction the volume after and before steaming.

2.4.Color Measurement

Color measurement was done using chromameter. First of all, chromameter was calibrated. After that, chromameter can be used to measure the color of the steamed cake. The color was measured as lightness (L*), reddish-greenish (a*) and yellowish-bluish (b*).

2.5.Antioxidant activity determination

Sample was dried using freeze dryer for 24 hours. Sample was crushed and weighed as

much as 0,5 g. Subsequently the samples were extracted with 5 ml of methanol for 2 hours. Then, 0,1 ml of extract was reacted with 3,9 ml DPPH (2,2-diphenyl-1-picrylhydrazyl) for 30 minutes. The absorbance was measured using spectrophotometer at 515 nm. As a blank, 0,1 ml methanol was reacted with 3.9 ml DPPH. Antioxidant activity was measured as %inhibition and calculated using the formula:

$$\% \text{ inhibition} = 1 - \frac{At_{30}}{At_0} \times 100\%$$

At_{30}: sample absorbance at minute 30

At_0 : blank absorbance at minute 0.

3.RESULT AND DISCUSSION

3.1.Volume

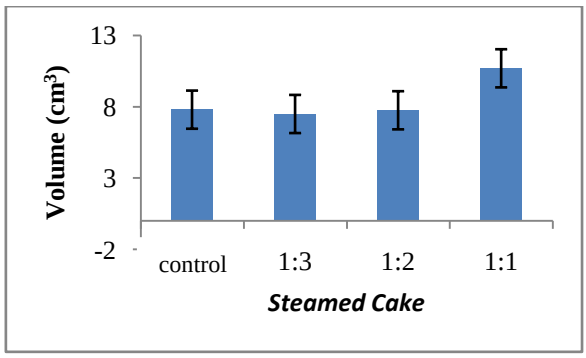
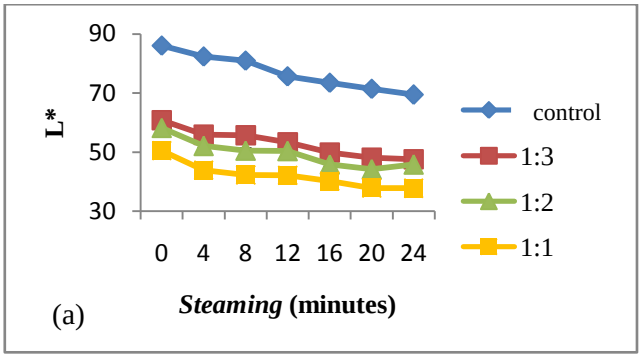


Figure 1. Volume Development of Batter with Various Red Beet Extract Concentration.

The higher concentration of red beet extract will cause an increase in cake volume after steaming. According Imeson (2010), sugar is one ingredient that can fill the space between the particles of starch that will affect the structure of starch. Additionally, the components that exist in betalain phenol red beets can interact with amylose and amylopectin structures that exist in flour mocaf through hydrogen bonds (Zhu, 2010). This can prevent collapse during the process of steaming steamed cake products in order to obtain a more fluffy cake.

3.2.Color



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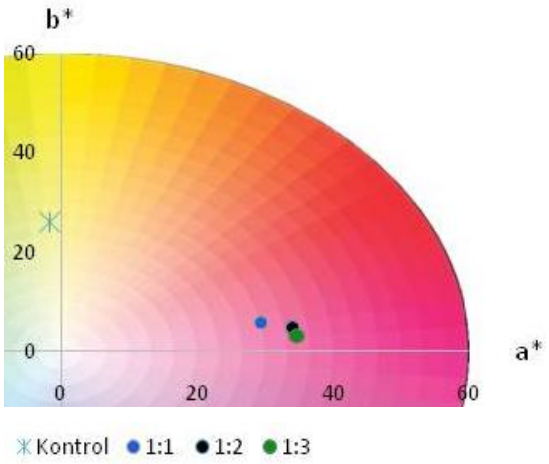

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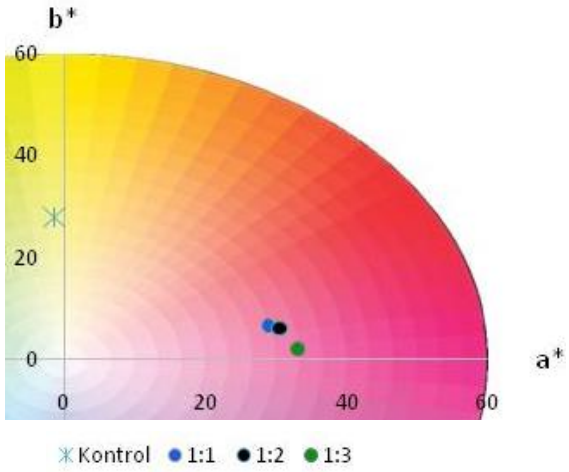

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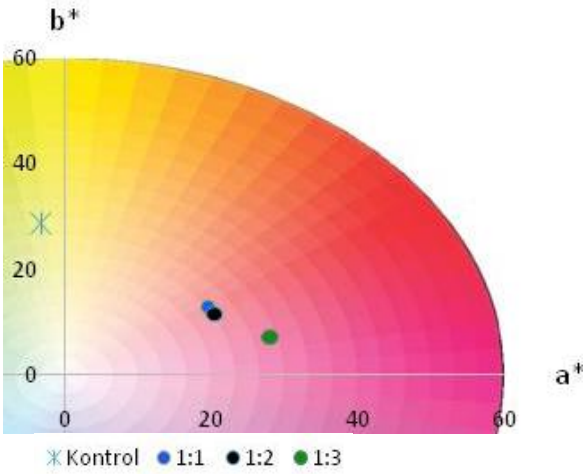

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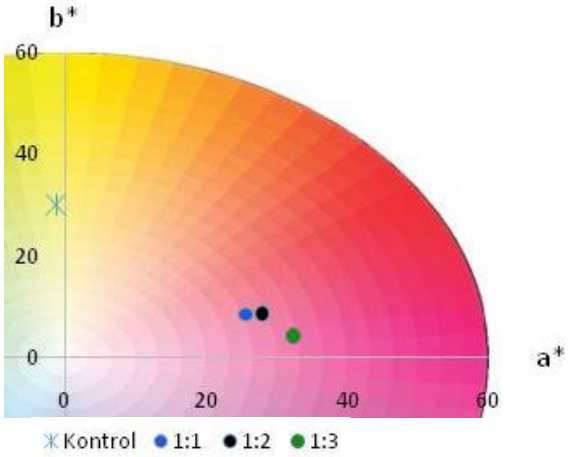

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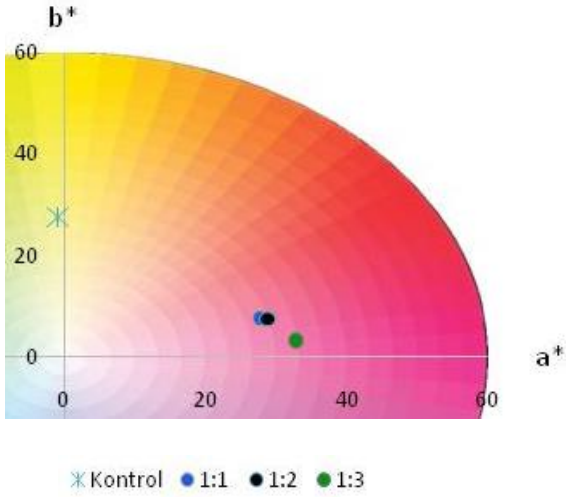

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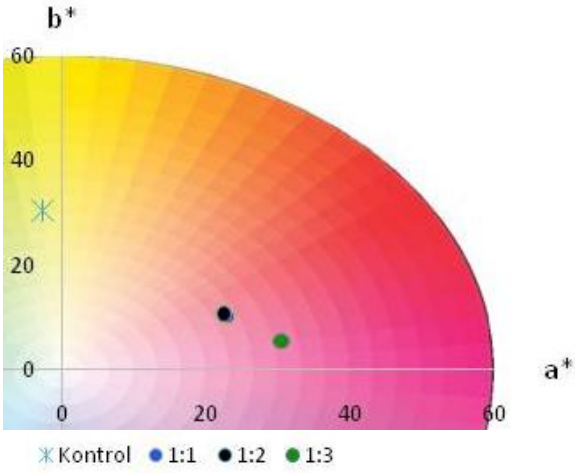

(e)

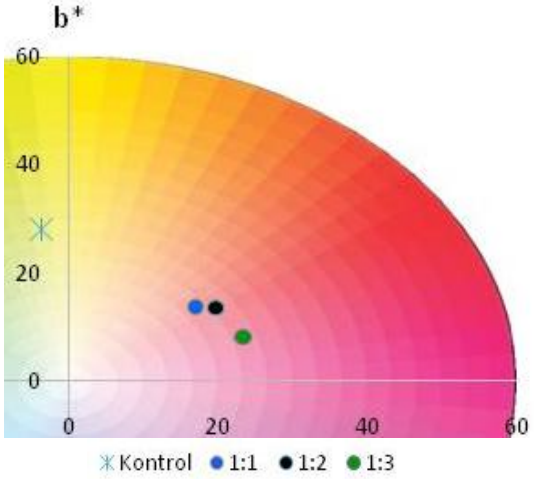

Figure 2. *(a) Lightness of Steamed Cake with Various Red Beet Extract Concentration*

(b). Color of Steamed Cake with Various Red Beet Extract Concentration at 0 minute Steaming

(c). Color of Steamed Cake with Various Red Beet Extract Concentration at 4 minutes Steaming

(d). Color of Steamed Cake with Various Red Beet Extract Concentration at 8 minutes Steaming

(e). Color of Steamed Cake with Various Red Beet Extract Concentration at 12 minutes Steaming

(f). Color of Steamed Cake with Various Red Beet Extract Concentration at 16 minutes Steaming

(g). Color of Steamed Cake with Various Red Beet Extract Concentration at 20 minutes Steaming

(h). Color of Steamed Cake with Various Red Beet Extract Concentration at 24 minutes Steaming

Increased concentrations of red beet extract will reduce the value of L* and b*, but will increase the value of a*. Decline in the value of L* (brightness) due to the darker of red color (Cai *et al*., 2005). Increase of value a* and decrease of value b* indicate that the red beet extract addition will increase the intensity of the red color and lower the intensity of the yellow color in steamed breads.

During steaming, the value of L* and a* will decrease, but will increase the value of b*. It caused by the higher temperature of steaming will make the betacyanin pigment stability decrease (Widhiana, 2000).

3.3.Antioxidant Activity

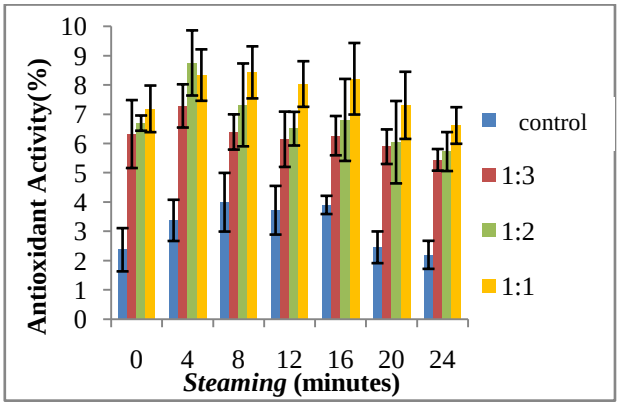

Figure 3. Antioxidant Activity of Steamed Cake with Various Red Beet Extract Concentration during Steaming

Increased concentrations of red beet extract will increase the antioxidant activity of steamed cake. During the steaming process, antioxidant activity will decrease because of betalain pigments degradation.

4.CONCLUSIONS

- The use of red beet extract will increase the volume of steamed cake.
- Addition of red beet extract concentration will increase the value of a* but lowes the value of b* and L* that affect the color of steamed cake.
- During steaming, the value of b* will increase but the value of a* and L* will decrease.
- Addition of red beet extract concentrations will increase the antioxidant activity of steamed cake product. During steaming process, antioxidant activity will decrease.

REFERENCES

Cai, Y.; Sun, M; and Corke, H. (2005). HPLC Characterization of Betalains from Plants in the Amaranthaceae. Journal of Chromatographic Science Vol. 43: 454-460.

Imeson, Alan. (2010). Food Stabilizers, Thickeners and Gelling Agents. Blackwell Publishing Ltd. Singapore.

Widhiana, E. (2000). Ekstraksi Bit (*Beta vulgaris l. var rubra l.)* sebagai Alternatif Pewarna Alami Pangan. Skripsi Fakultas Pertanian Institut Pertanian Bogor.

Zhu, Fan. (2010). Interaction of Carbohydrates with Phenolic Compounds. Ph D Thesis. Hong Kong University.

APPLICATION OF BACTERIOCINS PRODUCED BY *LACTOBACILLUS SP.* ISOLATES AS AN ANTIMICROBIAL IN ORANGE JUICE

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ABSTRACT

Lactic acid bacteria (LAB) which include *Lactobacillus sp.* commonly known as probiotics and potentially produced bacteriocins. Bacteriocin is a protein substance resulted from secondary metabolites of LAB which have very strong bactericidal properties, so it can be used as an antimicrobial in food and beverages. The method is testing the probiotics ability of *Lactobacillus sp*with bile salt testing. If *Lactobacillus sp* isolates have the ability of probiotics,it's possibilities can produce bacteriocins. Purification of bacteriocins from *Lactobacillus sp.* isolates. Determination of optimum conditions of antimicrobial bacteriocins abilities at different temperatures and pH with the addition of proteolytic enzymes and phosphate buffer pH 7 against *Escherichia coli* or *Staphylococcus aureus.* The optimum condition of antimicrobial bacteriocins was determined by formation of clear zones with well diffusion method.Bacteriocins in pH and temperature condition which have the largest clear zone will be applied to the orange juice. Addition of sodium benzoate is used as a comparator of bacteriocins antimicrobial ability. Orange juice without any addition was used as control. Inhibitory activity of Bacteriocins can be determined with counting the number of microorganism on days 0, 1, 2, 3, 4, 5, 6 and 7 by comparing the log cfu/ml of each treatment and the incubation time. The lowest value log cfu/ml is indicated as the most effective treatment as well as an antimicrobial in orange juice.

Keywords: *Lactobacillus, probiotic, bacteriocins,natrium benzoate,orange juice*

1.INTRODUCTION

Lactic acid bacteria (LAB) have been used for centuries in the fermentation of foods, not only for flavor and texture, but also due to their ability to prevent the growth of pathogenic microorganisms (Nagalakshmi*et al.*, 2013).

Based on the previous research conducted by Singgih (2014), LAB can be obtained from fermented "sawipahit" (*Brassica juncea*(L.) *Czernjaew*) that soaked in coconut water with salt concentration 5% and incubated at the temperature of 15% in a closed container for seven days. On the fermentation, 11 isolates are identified as LAB belonging to the genus *Lactobacillus*.

Lactobacillus isolates as one of LAB can produce secondary metabolite that names bakteriocin. Bacteriocins are ribosomally synthesized, extracellularly released bioactive peptide or peptide complexes which have a bactericidal or bacteriostatic effect on other species (Jeevaratnam*et al.*, 2005).But, to be classified as LAB that producing bacteriocin, the isolates should have the ability of probiotic (Noordiana*et al.*, 2013). In this case, bacteriocin can be obtain from genus *Lactobacillus* isolated from fermented "sawipahit" (Singgih,2014). The antimicrobial activity of bacteriocin will be tested with various pH and temperature on production media against *Escherichia coli* and *Staphylococcus aureus* (Nagalakshmi*et al.*, 2013).

Sweet orange (*Citrus sinensisOsbeck*) is one of the food products of the fruit juice that has many functional value but included to the perishable food. The damage often occurs in sweet orange are ropiness and off-flavors. *Lactobacillus monocytogenes*, *Bacillus cereus*, dan*Sthaphylococcusaureus* are pathogenic microorganism that are often cause of damage to the sweet orange. On the other hand, bacteria forming spores are involved in the process of damage orange juice during storage is *Alicyclobacillusacidoterrestris*.

In the orange juice, *Alicyclobacillus acidoterrestris* spores are resistant to a temperature of 80°C for 10 minutes, 90°C for 2 minutes, and 95°C for 1 minutes (Settanni&Corsetti, 2008). Thus, this research was conducted with the aim to knowing the capabilities of bacteriocin when applied as a preservative in orange juice.

2.MATERIAL AND METHOD

2.1.Probiotics ability of Lactic Acid Bacteria

One milliliter of *Lactobacillus* was inoculated into 9 ml MRS broth then incubated at 30°C for 18 hours. One milliliter of culture was added into 9 ml MRS broth containing 0,3% (b/v) bile salt, while as a control the culture into MRS broth without

any added bile salt. The growth of the bacteria was indicated by the presence of sediment at the bottom of the tube and with the levels of turbidity which measured by OD 650 nm (Charteris*et al*., 1998).

2.2.Purification and Inhibitor ability of Bacteriocin

The culture was added with ammonium sulphateas much as 70% of total culture, precipitate for 24 hours at 4^{o}C. The mixture was then centrifuged at 20.000 gram for 30 minutes at 4^{o}C. Precipitate were put into Native-PAGE, inhibitor avtivity of bacteriocin then measured by well diffusion method. Ten microliter *Escherichia coli* and *Staphylococcus aureus*were grown in 10 ml NA media. Small fraction of bacteriocins gel was implemented into hole well then keep for 3 hours at 4^{o}C, after which the fraction of bacteriocin was incubated at 37^{o}C for 24 hours. The clear zone was measured by calipers (Khunajakr*et al.*, 2008). While the remaining pieces of gel fractions was dialyzed by electroelusion process. The result of dialysis was covered with *chelophane*then running with SDS PAGE for denaturate protein, so the characteristic of purified bacteriocin was discovered (Gautam& Sharma, 2009).

2.3.pH sensitivityof Bacteriocin

Briefly, 0,5 ml crude bacteriocins were supplemented with 4,5 ml Nutrient broth in each tubes. Variation pH of crude bacteriocin in each tubes was adjusted from 3,0 – 9,0 controlled by adding HCl 1 M or NaOH 0,5 M. After which, the mixture was added with 0,15 ml commercial proteolitic enzymes and 0,15 ml phosphate buffer pH 7 then mixture with vortex. As much as 10 µl for each culture *Staphylococcus aureus*and *Escherichia coli* were put into the tube containing 10 ml NA media, then it were poured into petri dish. After which, made the hole with diameter of 5 mm on media containing bacteria and filled it with purified bacteriocin as much as 50 µl. Keep the culture at 4^{o}C for 3 hours until whole bacteriahave beendiffusedin themedia, then incubated at 37^{o}C for 24 and 48 hours. The clear zone was measured using calipers (Gautam& Sharma, 2009).

2.4.Heat stability of Bacteriocin

As much as 0,5 ml crude bacteriocins were supplemented into tube containing 4,5 ml *Nutrient broth* pH 7. Each tubes were covered with paraffin to prevent the vaporization, the samples then heated at 50^{o}C, 60^{o}C, dan 70^{o}C for 10 and 20 minutes. The samples were then added with 0,15 ml commercial proteolitic enzymes and 0,15 phosphate buffer pH 7, then mixtured with vortex. Testingthe ability ofbacteriocins using well difussion method, as much as 50 µl bacteriocin inserted into the holes diameter of 5 mm. The holes were made from 10 ml medium NA and 10 µl *Staphylococcus aureus* or 10 µl *Escherichia*

coli. After which keep them at 4°C for 3 hours until whole bacteria have been diffused in the media.After bacteriocin inserted into petri dish, then incubated at suhu 37°C for 24 and 48 hours. The clear zone was measured using calipers (Gautam& Sharma, 2009).

2.5.Application of Bacteriocin as Biopreservative in Orange Juice

Bottles were sterilized at 72°C for 2 minutes then were used as a container for 500 ml orangejuice, keep the bottle at room temperature. After which the pathogen culture (*Staphylococcus aureus* dan *Escherichia coli*) were added as much as 8,14 log cfu/ml. Purified bacteriocin as much as 2000 ppm was added into bottle A. While natrium benzoate was added as much as 600 ppm into bottle B, and as a control using 500 ml sterile orange juice without any adding in bottle C. Each treatment were incubated at 37°C, after that the amount of total bacteria was counted by Plate Count Agar at day 0, 1, 2, 3, 4, 5, 6,and7. Measurement of antimicrobial activity of each treatment performed by comparing log cfu/ml of each treatment and incubation time. Log cfu/ml with the lowest value indicates the most effective treatment as an antimicrobial in orange juice (Pratush*etal*., 2012).

3.RESULT AND DISSCUSSION

3.1.Probiotics ability of *Lactobacillus sp*.

In this research, some strains of *Lactobacillus* have the probiotics ability which resistant to human gastrointestinal tract such as the stomach acid (pH ± 2), salt concentration (less than 0.5%), bile salts, and digestive enzyme (pepsin , trypsin, and chymotrypsin). One of the *Lactobacillus* strains that have the ability of probiotics such as *Lactobacillus casei*212,3 and *Lactobacillus fermentum* KLD, has been made commercially (Charteris*et al*., 1998).

3.2.Purification of Bacteriocins

Based on the research of Gautam& Sharma (2009), *Lactobacillus brevis* produce bacteriocins with 93,4kD molecular mass, so it can be classify into class IV of bacteriocins. Class IV ofbacteriocinsconsist of complexes protein that are resistant to heat which a half of them are essential protein. Protein subunits of class IV of bacteriocins are not denatured whenNATIVE-PAGE was running.

3.3.The Ability of Bacteriocinin Various pH and Temperature

Heat treatment of bacteriocin in inhibiting the ability of the pathogenic microorganism (*Escherichia coli* and *Staphylococcus aureus*) is given at the temperature of 70°C, 80°C, 90°C, 100°C, and 121°C for 20 minutes. Should be suspected, crude bacteriocin supernatant produced by *Lactobacillus* was considered to be heat stable, as the inhibition activity remained constant after heating at 80°C for 20 min, but decreased in activity was observed when heating at 90°C from 10

to 20 minutes. Heating at 100°C for 20 minutes, the activity decreased down thereafter and when crude bacteriocin supernatant was autoclaved at 121°C for 20 minutes, its activity remained slightly. *Escherichia coli*was least sensitive to bacteriocin produced by LAB as compared to *Staphylococcus aureus*. Nagalakshmi*et al.*, (2013) described this phenomenon due to the protein degradation, protein aggregation and complex formation. The hypothesis of this study was to demonstrate that the optimum temperature for cell growth should be the optimum for bacteriocin production as well.

According to the research has been done by Chanprasert&Gasaluck (2011), the inhibition zone directed variation with bacteriocin activity. The high bacteriocin activity of initial pH 6.5 was detected at 24 and 36 h. On the other hand, bacteriocin activity of initial pH 7.0 and 7.5 was decreased at the same time. When the bacteriocin activity reached the maximum value afterward it was decreased with prolonged incubation period. The initial pH 6.5 for 36 h resulted in the maximum bacteriocin activity.

3.4.Application of Bacteriocin as the preservatives to Orange Juice

On tests carried out by Pratush, (2012), comparative study has been done to biopreservative (purified bacteriocin and sodium benzoate) for orange juice inoculated with *Staphylococcus aureus*and *Escherichia coli*. Initial log 8.14 CFU/ml of pathogen bacteria filled into orange juice with purified bacteriocin (bottle A), sodium benzoate (bottle B) and for control having no preservative (bottle C). It was found that among both the preservatives used to enhance the shelf life of orange juice, purified bacteriocin proved to be better preservative than chemical preservative (sodium benzoate).

4.CONCLUSION

Bacteriocin supernatant of *Lactobacillus* was heat stable, as the inhibition activity remained constant after heating at 80° for 20 minutes.At pH 6.5 for 36 hours resulted in the maximum bacteriocin activity. *Escherichia coli*was least sensitive to bacteriocin produced by LAB as compared to *Staphylococcus aureus*.Purified bacteriocin proved to be better preservative than chemical preservative (sodium benzoate).

REFERENCES

Chanprasert, N and P. Gasaluck.(2011). Bacteriocin Production and its Crude Characterization of Lactic Acid Bacteria Isolated from Pickled *Garciniaschomburgkiana*Pierre.*Asian Journal of Food and Agro-Industry* Vol. 4 : 54 – 64.

Charteris, W.P., P.M. Kelly, L. Morelli, dan J.K. Collins. 1998. Development and application of in vitro methodology to determine the transit tolerance of potentially probiotic *Lactobacillus* and*Bifidobacterium* species in the upper human gastrointestinal tract. *Journal of Applied Microbiology* Vol. 84: 759-768.

Gautam, N dan N. Sharma. 2009. Purification and Characterization of Bacteriocin Produced by Strain of *Lactobacillus brevis* MTCC 7539. *Indian Journal of Biochemistry and Biophysics* Vol. 46: 337 -341.

Jeevaratnam, K., M. Jamuna, dan A.S. Bawa. (2005). Biological Preservation of Foods-Bacteriocins of Lactic Acid Bacteria.*Indian Journal of Biotechnology* Vol. 4: 446 – 454.

Khunajakr, N., A. Wongwicharn, dan D. Moonmangmee. 2008. Screening and Identification of Lactic Acid Bacteria Producing Antimicrobial Compounds From Pig Gastrointestinal Tracts. *Journal of KMITL Science Technology* Vol. 8 (1): 8-17.

Nagalakshmi, P.K., R. Sumathi, K. Kanimozhi, dan T. Sivakumar. (2013). Isolation of BacteriocinNisin Producing *Lactococcuslactis* from Dairy Products.*Journal of Academy Industry* Vol. 1 (10).

Noordiana, N., A.B. Fatimah, dan A.S. Mun.(2013). Antibacterial Agents Produces by Lactic Acid Bacteria Isolated from Threadfin Salmon and Grass Shrimp. *International Food Research Journal* Vol. 20: 117-124.

Pratush, A., A. Gupta, A. Kumar, dan G. Vyas. (2012). Application of Purified Bacteriocin Produced by *Lactococcuslactis* AP2 as Food Biopreservative in Acidic Foods.*Journal of Annals Food Science and Technology* Vol 13 (1): 82 – 86.

Settanni.L dan A. Corsetti (2008).Application of Bacteriocins in Vegetable Food Biopreservation.*International Journal of Food Microbiology*Vol 121: 123 – 138.

Singgih, J.W.P. (2014). IdentifikasidanPengujianAktivitasAntimikrobapadaBakteriAsamLaktat yang DiisolasidariFermentasiSawiPahit (*Brassica juncea*(L.)*Czernjaew*) padaSuhu 15°C dalamLarutanGaram 5%.SKRIPSI.FakultasTeknologiPangan UniversitasKatolikSoegijapranata, Semarang.

BACTERIOCINS APPLICATION FROM FERMENTATION OF GEDONO'S KEFIR AS A NATURAL FOOD PRESERVATIVE

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ABSTRACT

Lactic acid bacteria have ability to form an antimicrobial component to improve the quality and safety of food by inhibit pathogenic microorganisms. Some lactic acid bacteria can be classified into probiotic bacteria. Where probiotic bacteria have ability to produce antimicrobial compounds (short-chain fatty acids, hydrogen peroxide, nitric oxide, and bacteriocins) were able to increase its ability to against other microbes and inhibit pathogenic bacteria. Bacteriocins are secondary metabolites of lactic acid bacteria which can be used as biopreservative in various food products. Bacteriocins from fermented Gedono's kefir can be obtained from the Lactobacillus.The aim of this study is determine the potential of lactic acid bacteria that isolated from Gedono's kefir to produce bacteriocins as natural preservatives. The methods of this research include gram stain, catalase test, motility test, probiotic test using bile salt, and then the growth optimization of lactic acid bacteria in various pH levels (4.4 and 9.6), temperature (10^0C, 45^0C, and 50^0C), and the levels of NaCl (6.5% and 18%), determine the Lactobacillus species using the API kit. Bacteriocins are purified by using SDS-PAGE and Native Page. Characterization of bacteriocins using pH (3-9) and temperature (50 °C, 60 °C, and 70 °C) treatments. The last step is determine the inhibitory ability against *Staphylococcus aureus* and *E. coli* using well assay method.

Keywords : *lactid acid bacteria, bacteriosin, biopreservative*.

1.INTRODUCTION

Lactic acid bacteria have been used for centuries in the fermentation of foods, not only for flavour and texture, but also due to their ability to prevent the growth of pathogenic microorganisms. They are also responsible for the fermentative processing and preservation of many food products (Nagalakshmi *et al.*, 2013). Kefir is a fermented milk product that carbonated and has low alcohol content (0.9 g/100 g) (Otles & Cagindi, 2003). Microbial activity in kefir fermentation is a symbiotic metabolic activity of a number of bacteria and yeast species, which degrade milk constituents to lactic acid, acetic acid, ethanol, carbon dioxide and other flavor compound such as acetaldehyde resulting in a distinctive flavor of the kefir beverage (Farnworth & Mainville, 2008).

Based on the previous research conducted by Sanjaya (2014), lactic acid bacteria can be obtained from fermented Gedono's kefir. On the fermentation, there were 17 isolates of lactic acid bacteria successfully isolated from Gedono's kefir. A total of 17 isolates of lactic acid bacteria were primarily identified and classified into genus as *Lactobacillus*.

Some bacteriocins of lactic acid bacteria used as biopreservatif in various food products include bacteriocins produced by *Lactobacillus sp.* LAB that producing bacteriocin, the isolates should have the ability of probiotic (Noordiana *et al.*, 2013). Some probiotic bacteria have a production capability of various antimicrobial compounds (short-chain fatty acids, hydrogen peroxide, nitric oxide, and bacteriocins) (Atassi & Servin, 2010). The antimicrobial activity of bacteriocin will be tested with various pH and temperature on production media against *Escherichia coli* and *Staphylococcus aureus* (Nagalakshmi *et al.*, 2013).

Consumers have been consistently concerned about possible adverse health effects from the presence of chemical additives in their foods. As a result, consumers are drawn to natural and "fresher" foods with no chemical preservatives added. This perception, coupled with the increasing demand for minimally processed foods with long shelflife and convenience, has stimulated research interest in finding natural but effective preservatives. Bacteriocins, produced by LAB, may be considered natural preservatives or biopreservatives that fulfill these requirements. Biopreservation refers to the use of antagonistic microorganisms or their metabolic products to inhibit or destroy undesired microorganisms in foods to enhance food safety and extend shelflife (Schillinger *et al*, 1996).

2.MATERIAL AND METHOD

2.1.Probiotics ability of Lactic Acid Bacteria

As much as, 1ml of *Lactobacillus* was inoculated into 9 ml MRS broth after that incubated at 30°C for 18 hours. 1ml of culture was added into 9 ml MRS broth containing 0,3% (b/v) bile salt, while as a control the culture into MRS broth without any added bile salt. The growth of the bacteria was indicated by the presence of sediment at the bottom of the tube and with the levels of turbidity which measured by OD 650 nm (Charteris*et al*., 1998).

2.2.Purification and Inhibitor ability of Bacteriocin

First the culture was added with ammonium sulphate as much as 70% of total culture, precipitate for 24 hours at 4^{o}C. Then centrifuged at 20.000 gram for 30 minutes at 4^{o}C. Precipitate were put into Native-PAGE, and inhibitor activity of bacteriocin measured by well diffusion method. 10 µl *Staphylococcus aureus* and *Escherichia coli* were grown in 10 ml *Nutrient agar* media.

Small fraction of bacteriocins gel was implemented into hole well after that keep for 3 hours at 4^{o}C, after which the fraction of bacteriocin was incubated at 37^{o}C for 24 hours. The clear zone was measured by calipers (Khunajakr*et al.*, 2008). While the remaining pieces of gel fractions was dialyzed by electroelusion process. The result of dialysis was covered with *chelophane* then running with SDS PAGE for denaturate protein, so the characteristic of purified bacteriocin was discovered (Gautam & Sharma, 2009).

2.3.Heat stability of Bacteriocin

0,5 milliliter crude bacteriocins were supplemented into tube containing 4,5 milliliter *Nutrient broth* pH 7. Each tubes were covered with paraffin to prevent the vaporization, the samples then heated at 50^{o}C, 60^{o}C, dan 70^{o}C for 10 and 20 minutes. After that samples were added with 0,15 milliliter commercial proteolitic enzymes and 0,15 phosphate buffer pH 7, then mixtured with vortex. For testing the ability of bacteriocins using well difussion method. 50 µl bacteriocin inserted into the holes diameter of 5 millimeter. The holes were made from 10 milliliter medium NA and 10 µl *Staphylococcus aureus* or 10 µl *Escherichia coli*. Then keep them at 4^{o}C for 3 hours until whole bacteria have been diffused in the media. After bacteriocin inserted into petri dish, then incubated at suhu 37^{o}C for 24 and 48 h. The clear zone was measured using calipers (Gautam & Sharma, 2009).

2.4.pH sensitivity of Bacteriocin

As much as 0,5 millilter purified bacteriocins were supplemented with 4,5 milliliter *Nutrient broth* in each tubes. Variation pH of crude bacteriocin in each tubes was adjusted from 3,0 – 9,0 controlled by adding NaOH 0,5 M or HCl 1 M. Then mixture was added with 0,15 milliliter commercial proteolitic

enzymes and 0,15 milliliter phosphate buffer pH 7 then mixture with vortex.

10 µl for each culture *S. aureus* and *E. coli* were put into the tube containing 10 ml NA media then poured into petri dish. After that, made the hole with diameter of 5 mm on media containing bacteria and filled it with purified bacteriocin as much as 50 µl keep at 4^{o}C for 3 h until whole bacteria have been diffused in the media. After that incubated at 37^{o}C for 24 and 48 hours. For the clear zone was measured using calipers (Gautam & Sharma, 2009).

2.5.Application of Bacteriocin as Biopreservative on food

First prepared the material, if the sample is solid, 500 gram sample is taken and if the liquid is taken as 500 ml. Then the sample is introduced into bottles that have been sterilized. Then pasteurization at 72 ° C for 2 minutes. After that the pathogen culture (*Staphylococcus aureus* dan *Escherichia coli*) were added as much as 8,14 log cfu/ml. Purified bacteriocin as much as 2000 ppm was added into bottle X compared with 500 ml/gram sterile food that used as a control (bottle Y). Each treatment were incubated at 37°C. The amount of total bacteria was counted by Plate Count Agar at day 0, 1, 2, 3, 4, 5, 6,and 7. Log cfu/ml with the lowest value indicates the most effective treatment as an antimicrobial on food (Pratush *et.al*., 2012).

3.RESULT AND DISSCUSSION

Based on the research of Dunne et al (2001), probiotics including *Lactobacillus*, *Bifidobacterium* and *Streptococcus* spp. are known to be inhibitory to the growth of a wide range of intestinal pathogens in human. Lactic acid bacteria are classified into probiotics should have antimicrobial activity against some specific microorganisms, tolerant of acid stomach, and harmless (Noordiana et al, 2013). The low pH is known to provide an effective barrier against the entry of bacteria into the intestinal tract. The pH of the stomach generally ranges from pH 2.5 to pH 3.5 (Holzapfel et al., 1998). A concentration of 0.15-0.3 % of bile salt has been recommended as a suitable concentration for selecting probiotic bacteria for human use (Goldin BR and Gorbach 1992).

Some bacteriocins of lactic acid bacteria used as biopreservatif in various food products include bacteriocins produced by Lactobacillus sp. In general, the optimum conditions of bacteriocin production is influenced by the growth phase, the pH of the media, incubation temperature, type of carbon source, type of nitrogen source, and concentration of NaCl (Kim and Ahn, 2000). The bacteriocins produced inhibited food spoilage and pathogenic bacteria such as S*taphylococcus aureus, Escherichia coli, Bacillus cereus, B. subtilis, Listeria*

monocytogenes and *Clostridium perfringens* (Bizani *et al*, 2002).

Based on the research of Chanprasert & Gasaluck (2011), the inhibition zone directed variation with bacteriocin activity. The high bacteriocin activity of initial pH 6.5 was detected at 24 and 36 h. On the other hand, bacteriocin activity of initial pH 7.0 and 7.5 was decreased at the same time. When the bacteriocin activity reached the maximum value afterward it was decreased with prolonged incubation period. The initial pH 6.5 for 36 h resulted in the maximum bacteriocin activity.

Heat treatment of bacteriocin in inhibiting the ability of the pathogenic microorganism is given at the temperature of 70°C, 80°C, 90°C, 100°C, and 121°C for 20 minutes. Should be suspected, crude bacteriocin supernatant produced by *Lactobacillus* was considered to be heat stable, as the inhibition activity remained constant after heating at 80°C for 20 min, but decreased in activity was observed when heating at 90°C from 10 to 20 minutes. Heating at 100°C for 20 minutes, the activity decreased down thereafter and when crude bacteriocin supernatant was autoclaved at 121°C for 20 minutes, its activity remained slightly. Nagalakshmi *et al.*, (2013) described this phenomenon due to the protein degradation, protein aggregation and complex formation. The hypothesis of this study was to demonstrate that the optimum temperature for cell growth should be the optimum for bacteriocin production as well. *Escherichia coli* was least sensitive to bacteriocin produced by LAB as compared to *Staphylococcus aureus*.

3.1.Application of Bacteriocin as the preservatives in Food

Now, consumers have been consistently concerned about health effects from the presence of chemical additive in their foods. Bacteriosins is one of alternative natural preservative that produced by LAB. Biopreservative is use of antagonistic microorganisms or metabolic products to inhibit undesired microorganism to enhance food safety and extend shelflife (Schillinger *et. al*., 1996). In this research we use addition of purified or semi purified bacteriocins as food preservative. Pratush *et al*, (2012) said that was found among the chemical preservatives used to enhance the shelf life of food, but purified bacteriocin proved to be better preservative than chemical preservative.

4.CONCLUSION

The bacteriocins produced inhibited food spoilage and pathogenic bacteria such as S*taphylococcus aureus, Escherichia coli, Bacillus cereus, B. subtilis, Listeria monocytogenes* and *Clostridium perfringens*. The high bacteriocin activity of initial pH 6.5 was detected at 24 and 36 h. On the other

hand, bacteriocin activity of initial pH 7.0 and 7.5 was decreased at the same time.

REFERENCES

Atassi F, Servin AL. 2010. Individual and co-operative roles of lactic acid and hydrogen peroxide in the killing activity of enteric strain **Lactobacillus johnsonii** NCC933 and vaginal strain **Lactobacillus gasseri** KS120.1 against enteric, uropathogenic and vaginosis-associated pathogens. FEMS Microbiol. Lett. 304:29 –38

Bizani, A. Brandelli. (2002) , Characterization of bacteriocin produced by a newly isolated *Bacillus* sp. strain 8A, *J. Appl. Microbiol. 93* (2002) 512–519.

Chanprasert, N and P. Gasaluck. (2011). Bacteriocin Production and its Crude Characterization of Lactic Acid Bacteria Isolated from Pickled *Garcinia schomburgkiana* Pierre. *Asian Journal of Food and Agro-Industry* Vol. 4 : 54 – 64.

Charteris, W.P., P.M. Kelly, L. Morelli, dan J.K. Collins. 1998. Development and application of in vitro methodology to determine the transit tolerance of potentially probiotic *Lactobacillus* and *Bifidobacterium* species in the upper human gastrointestinal tract. *Journal of Applied Microbiology* Vol. 84: 759-768.

Dunne C, O'Mahony L, Murphy L, Tornton, G. Morrissey, D. O'Halloran, S, Feeney M, Flynn S, Fitzgerald G, Daly C, Kiely B, O'Sullivan GC, Shanahan F, and JK. Collins. (2001). In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. *Am. J. Clin. Nutr*. **73**(2), 386-392.

Farnworth, E.R., and I. Mainville. (2008). *Kefir - A Fermented Milk Product*. CRC Press. U.S.A.

Gautam, N and N. Sharma. 2009. Purification and Characterization of Bacteriocin Produced by Strain of *Lactobacillus brevis* MTCC 7539. *Indian Journal of Biochemistry and Biophysics* Vol. 46: 337 -341.

Goldin BR, and SL. Gorbach (1992). Probiotics for humans. In: Fuller, R. (Ed.), Probiotics, the Scientific Basis. Chapman & Hall, London, pp. 355-376.

Holzapfel WH, Haberer P, Snel J, Schillinger U, and JHJ. Huis in't Veld (1998). Overview of gut flora and probiotics. Int. J. Food Microbiol. **41**, 85-101.

Khunajakr, N., A. Wongwicharn, and D. Moonmangmee. 2008. Screening and Identification of Lactic Acid Bacteria Producing Antimicrobial Compounds From Pig Gastrointestinal Tracts. *Journal of KMITL Science Technology* Vol. 8 (1): 8-17.

Kim CHGE Ji dan C Ahn. 2000. Purification and molekuler characterization of a bacteriocin from Pediococcus sp KCA 1202-10 isolated from fermented flatfish. Food Sci Biotechnology 9: 270-276.

Nagalakshmi, P.K., R. Sumathi, K. Kanimozhi, dan T. Sivakumar. (2013). Isolation of Bacteriocin Nisin Producing *Lactococcus lactis* from Dairy Products. *Journal of Academy Industry* Vol. 1 (10).

Noordiana, N., A.B. Fatimah, and A.S. Mun. (2013). Antibacterial Agents Produces by Lactic Acid Bacteria Isolated from Threadfin Salmon and Grass Shrimp. *International Food Research Journal* Vol. 20: 117-124.

Otles, S. and O. Cagindi. (2003). Kefir : A Probiotic Dairy-Composition, Nutritional and Therapeutic Aspects. *Pakistan Journal of Nutrition* Vol 2(2): 54-59.

Pratush, A., A. Gupta, A. Kumar, dan G. Vyas. (2012). Application of Purified Bacteriocin Produced by *Lactococcus lactis* AP2 as Food Biopreservative in Acidic Foods. *Journal of Annals Food Science and Technology* Vol 13 (1): 82 – 86.

Sanjaya, E. (2014). Profil Konsorsium Mikroba Dalam Kefir Produksi Lokal Gedono, Jawa Tengah. SKRIPSI. Fakultas Teknologi Pangan Universitas Katolik Soegijapranata, Semarang.

Schillinger U, Geisen R, Holzapfel WH. (1996). Potential of antagonistic microorganisms and bacteriocins for the biological preservation of foods. Trends Food Sci Technol 7:158-64.

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