

ISBN: 978-602-9030-88-4

**Revealing the Secrets of Life
Through Protein and Peptide**

PROCEEDING

INDONESIAN PROTEIN SOCIETY (IPS)
INTERNATIONAL SEMINAR AND WORKSHOP 2014

October 29-30, 2014

Jember, Indonesia

Editors

Hardian Susilo Addy

Miswar

Jayus

Tri Agus Siswoyo

Maurice Ku

Toshiharu Hase



*Membangun Generasi
Menuju Insan Berprestasi*



Center for Development of Advanced
Sciences and Technology,
University of Jember



Indonesia Protein Society (IPS)

PROCEEDING

INDONESIAN PROTEIN SOCIETY (IPS)

INTERNATIONAL SEMINAR AND WORKSHOP 2014

Revealing the Secrets of Life Through Protein and Peptide

Editor:

Hardian Susilo addy

Miswar

Jayus

Tri Agus Siswoyo

Maurice Ku

Toshiharu Hase

Bambang Sugiharto

ISBN: 978-602-9030-88-4

Published first: December 2015

By:

UPT PENERBITAN

UNIVERSITAS JEMBER

Jl. Kalimantan 37 Jember 68121



Table of Contents

WELCOME ADDRESS

Chairman of Organism Committee	1
President of IPS	2
Rector of University of Jember	3
Acknowledgement of Sponsor	5

INVITED SPEAKERS

Molecular Architecture and Performance of GS/GOGAT Cycle for Plant Nitrogen Assimilation Toshiharu Hase	6
Graded Polishing and Germination of Cereals Are Good Strategy for Hypo-Allergenicity Naofumi Morita, Tomoko Maeda and Tri Handoyo	7
The Roles of Xylanolytic System on <i>Goebacillus thermotovorans</i> IT-08 Ni Nyoman Tri Puspaningsih	8
Study: Mlinjo Seeds Protein Show Promise in Managing Hypertension Tri Agus Siswoyo	9
Discovery of a New Transcription Factor in Rice Male Sterility and Its Regulation by Protein-protein Interaction Maurice S. B. Ku	10
Nitrite Transport Activity of a Novel HPP Family Protein Conserved in Cyanobacteria and Chloroplasts Shin-ichi Maeda, Mineko Konishi, Shuichi Yanagisawa and Tatsuo Omata	11
Comparison of β -Glucanases Production by <i>Acremonium</i> sp. IMI 383068 in Batch and Continuous Culture System Jayus	14

RESEARCH ARTICLES

Unique Cellulase Enzyme of <i>Bacillus firmus</i> From Organic Waste Purkan, Sumarsih, S., Rachmadani, D.A	21
Lipoprotein Associated Phospholipase A2 Activity and It's Additive Value in Cardiovascular Risk Stratification Andi W, Miryanti C., Saifur R, Dadang H., Widodo	28
Immobilization of Lipase on Surfactant-Modified Bentonite and Its Application for Biodiesel Production from Simulated Waste Cooking Oil Ruth Chrisnasari, Angelia Yonardi, Hesti Lie, Restu Kartiko Widi and Maria Goretti Marianti Purwanto	36
Cloning coat protein gene of cbsd (cassava brown streak disease) at cassava (<i>Manihot esculentum</i>) Didik Pudji Restanto, Slameto, Budi Kriswanto, Dwi Setyati, Hardian Susilo Addy and Tri Handoyo	46



Analysis AZF Gene Deletions in Infertile Men in Indonesia Evi Hanizar, Aucky Hinting	51
Review: Phylogenetic Similarity based on amino acid sequence and Molecular characterization of 3- Phytase from <i>Klebsiella pneumoniae</i> ASR1 Sajidan and Hailu Weldekiros Hailu	59
Overexpression Sucrose Transporter Protein (Sut) and Sucrose Content In Genetically Modified Product (Gmp) Sugarcane(<i>Saccharum officinarum</i> L.) Parawita Dewanti, Purnama Okviandari, Nina Oktaria dan Bambang Sugiharto	67
Imunogenic Protein of Salivary Gland from <i>Anopheles sudaicus</i> Yunita Armiyanti, Moh. Mirza Nuryady, Sugeng Setyo Utomo, Teguh Wahyu Sardjono, Loeki Enggar Fitri, Kartika Senjarini	76
Biochemical Resistance Mechanism of Several Genotype of Soybean to Rust Diseases Moh. Setyo Poerwoko, Endang Budi Trisusilowati dan Amarullah	82
Mineral Nitrogen in Soil of Sugarcane Plantation of PG Jatiroto Ketut Anom Wijaya	89
Physical Properties of Gel and Edible Plastic from Whey and Tapioca In Various Ratio and pH Value Triana Lindriati, Herlina, Ahmad Nafi	93 ✓
Specific sequence of Plasmodium falciparum DBL domains associated with severe malaria outcome Erma Sulistyaningsih, Loeki Enggar Fitri, Thomas Loescher, Nicole Berens-Riha	102
Zinc Biofortification of Rice Using Fish Protein Hydrolysates Mixed With Zinc Sulfate. Achmad Sjaifullah	107
The Potency of Protein Extracts from <i>Candida albicans</i> Bioreceptor on Immunosensor for Diagnosis of Candidiasis Masfufatun, Noer Kumala and Afaf Baktir	111
Exploration of Lipase Enzyme from Soil through Metagenomic Approach Sri Sumarsih, Afaf Baktir, Budi Putri Ayu Andina	117
Optimization pH of Enzymatic Hydrolysis of Endo-1,4- β -Xylanase for Xylooligosaccharides Production Anak Agung Istri Ratnadewi, Andika Ade Kurniawan, Wuryanti Handayani	124
Transformation of Plasmid pET Endo-1,4- β -xylanase from <i>E. coli</i> TOP10 to <i>E. coli</i> BL21 Agung Budi Santoso, Eka Yuni Kurniawati, AA Istri Ratnadewi	130



The Influence of Supplementary Feeding (Probiotic and Azollapinnata) on Protein and Amino Acids Content in Patin Fish Ika Oktavianawati, Meirinda Hermiastuti, Novita Rahmawati, Wuriyanti Handayani, I Nyoman Adi Winata	138
Screening and Isolation of Cellulolytic Bacteria From Bagasse and Characterization of The Cellulase Produced Lanny Hartanti, Fandy Susanto, Caesilia Putri Utami, Emi Sukarti, Henry Kurnia Setiawan, Martha Ervina	146
Technical Functional Properties of Crude Water Soluble Polysaccharide From Durian Seed (<i>Durio zibethinus</i> Murr.) Herlina, Triana Lindriati, Noer Novijanto, Ayu Anggraini	154 ✓
The Efficiency of GFP Gene Transformation on Peanut Embryo somatic Using Agrobacterium and Particle Bombardment Sholeh Avivi, Ralf G. Dietzgen, Colleen M. Higgins, Sudarsono	165
Dye-Sensitized Solar Cells (Dssc) Using Natural Dyes Extracted From Red Cabbage And Counter Electrode Based TiO_2 -Graphite Composites Tanti Haryati, Tri Mulyono, Ika Oktavianawati and Wawan Badrianto	169
The inhibition of bacterial metalloenzymes and fungal protein synthesis on explants surfaces by sterilizing agents Wina Dian Savitri	177
Molecular Dynamic Simulation for Thermal Stability Properties of Endo β -Mannanase Enzyme Adi Yulandi, A A Hermosaningtyas, Sheila Sutanto, Antonius Suwanto	182
The Effect of Bitter Melon Extract (<i>Momordica charantia</i>) in Inhibition of NFkB Activation in Leptin treated HUVECS Azham Purwandhono, Candra Bumi	188



Research Article

Physical Properties of Gel and Edible Plastic from Whey and Tapioca in Various Ratio and pH Value

Triana Lindriati, Herlina, Ahmad Nafi

Department of Agricultural Product Technology, Jember University.

Corresponding email: lindriatitriana@yahoo.com

ABSTRACT

Edible plastic usually made from hydrocolloids and could be produced by solvent casting method whereas gel was made before casting. Potential of whey protein combined with tapioca as edible plastic based component was studied at this research. pH of solvent could affect interaction of whey and tapioca during gel preparation. The gel's characters affected on plastic's characters. The research objectives were to study effect of whey and tapioca ratio and pH of solvent on physical properties of gel and edible plastics. Randomized Factorial Block Design were used. The two factors were porpotion of whey protein-starch mixture (0%, 20%, 40%, 60%, 80%, 100%) and pH of solvent (4, 7 and 9). Increasing of whey porpotion could increase solubility of gel but decrease gel's lightness and texture. Increasing of pH could increase gel's solubility and decrease lightness and texture. There was significant effect ($p \leq 0,05$) of whey porpotion and pH interaction on gel's characters. Increasing of whey porpotion could increase tensile strength and decrease elongation and solubility of edible plastics but the increasing of tensile strength was not significant ($p \geq 0,05$). Increasing of pH could increase elongation and decrease tensile strength and plastic's solubility. There wasn't significant effect ($p \geq 0,05$) of whey porpotion and pH interaction on edible plastic's characters. The result showed that whey addition had decreased plastic's characters even at 100% whey ratio tensile strength and elongation value were zero, edible plastic could not be produced from 100% whey.

Keyword: texture, solubility, elongation, tensile strength, interaction

INTRODUCTION

Indonesia as agricultural country has abundant source of biological polymer that could be explored as edible plastic. Edible plastic could be made from hydrocolloid such as polysaccharide and protein. Previous study (Lindriati dkk, 2007a, 2007b; Lindriati dan Arbiantara, 2011) showed that combination of protein and carbohydrate would increase physical and mechanical character of edible plastic.

Many researches into edible films has involved the production of films from the method of solvent casting. Solvent casting method usually use water as solvent to dissolve edible plastic based component and to gelatinize by heating the mixture. After gelatinization the mixture was

casted in order flat and transparant edible plastic could be produce. Properties of edible plastics that were produce from solvent casting method depend on the gel characters.

In this research tapioca and whey protein were used as edible film based component. Tapioca already widely used in food industry as edible/biodegradable plastic because of renewable, inexpensive and could produce good physical and mechanical characters (Bourtoom, 2007). Whey protein could result in plastics that were transparent, soft, flexible, odorless, colorless and has the aroma barrier properties. Plastics from whey protein have much disulfide bond so the plastic was not easily soluble in water (Wieddyanto, 2007).

Carbohydrate-protein interactions in the gel influences quality of edible plastics. Such interactions could be influenced by the composition and pH of the solvent. According to Graham (1977) and Zayas (1997), at pH where isoelectric point occur, protein solubility is low and if the pH above isoelectric point, solubility of protein could increase. The solubility of proteins would certainly affect the interaction of the protein with carbohydrates in edible plastic.

The aims of this research were to study effect of whey protein proportion and pH of solvent on edible plastic properties. Outcomes of this research is expected to provide a preliminary understanding of protein-carbohydrate interactions in edible plastic.

MATERIAL AND METHOD

Material

Tapioca and whey protein (industrial grade) were used as edible plastic based component was obtained from local market. The composition of the whey protein powder was reported as 76,97% protein, 10% carbohydrate, 6,36% fat and 6,67% ash. The composition of tapioca was reported as 4% protein, 93% carbohydrate, 2% fat and 1% ash. Glycerol was used as plasticizer that was p.a grade (merck). NaOH 0,2 M and HCl 0,2 M were used to control pH of solvent.

Gel and edible plastic preparation

Gel was made by solubilizing 10 grams mixture of whey protein and tapioca into 100 ml aquadest with variation of whey protein proportion (0%, 20%, 40%, 60%, 80% and 100). pH of aquadest was adjusted 4, 7 and 9 by using NaOH 0,2 M and HCl 0,2 M. 2.5 g of glycerol was added in to the mixture and then stirred for 10 minutes. The mixture was heated with steam (100°C) for 20 minutes to gelatinize. 10 gr gel was casted into 10 x 10 cm² plat porcelain and then dried in regulating oven at 50°C for 20 hours. Plastics were produce by peeling from plat porcelain and stored in desicator filled with silica gel for 24 hours. For gel observation, the gel was pour in to a cup (50 ml) and than stored in a refrigerator (4°C) for 24 hours and gel's parameters was observed.

Color Measurement (Colour Reader Minota CR-10)

Determination of color was performed by touching the lens of color reader as close as possible to the surface of material whereas white paper was lied under the plastic. The data obtained was Lightness value (L). Measurements were performed on each sample gel from 5 different point.

Texture Measurement (RheoTex, Type SD-700, Ogawa Seiki)

Depth which needle penetrate sample were set 12 mm and the load required to puncture was value of texture (gr/mm.detik). Measurements were made at 5 points on each sample. The scale on the monitor was proportional to the force required to penetrate the material and it was proportional to the hardness of the material.

Degree of solubility (Dahle, 1971)

Measurement of solubility of gel was done by measuring the absorbance by spectrofotometry, the value of absorbance depend on amount of ingredients that were dissolved. The measurement procedure begins by weighing 0.5 grams of samples put in centrifuge tubes and 15 ml of distilled water was added. Centrifuge was operated in 1200 rpm for 5 minutes. The absorbance of resulting supernatant was observed by spectrofotometry at 650 nm wave length.

Measurement of Water Holding Capacity (WHC) (AACC, 1991, modified)

WHC measurements was done by weighing the centrifuge bottle as a gram, then added \pm 0.5 gram sample. Sample and bottle was weighed as b gram. Distilled water was added 7 times of the material weight (3.5 g). The mixture was homogenized by vorteks at room temperature for 5 minutes, then centrifuged at 2000 rpm for 5 minutes. The supernatant was discarded slowly and precipitate was weighed as c gram.

$$WHC = \frac{(c-a) - (b-a)}{(b-a)} \times 100\%$$

Tensile strength and elongation (according to ASTM standards D638-94 in Chang et al, 2000)

Pieces of edible plastic with a width of 10 mm and a length of 80 mm may be stored in a jar containing silica gel for one day. Then the tensile strength was measured using a Universal Testing Machine (Shimadzu). The size of the specimen can be seen in Figure 1.

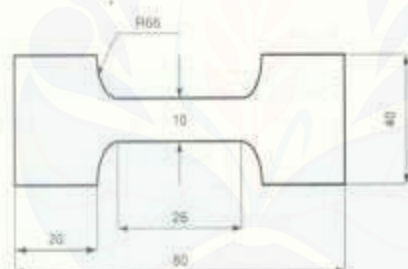


Figure 1. Tensile Test Specimens (according to ASTM D638-94 in Chang et al, 2000)

The tensile strength can be calculated with this formula:

$$\sigma = \frac{F}{A}$$

σ = tensile strength (N / mm²); F = tensile force (N); A = area of work (mm²).

In accordance with tensile strength procedure, strain measurement was done using Universal Testing Machine. The strain can be formulated:

$$\epsilon = \frac{\Delta l}{l_0}$$

ϵ = Strain (%); Δl = length addition (mm); l_0 = Initial length (mm)

Solubility of edible plastic (Gontard et al., 1992)

Measurement of solubility of edible film in water based on percentage of initial dry material that dissolves. Pieces of edible film with a size of 2.5 x 5 cm were dried in an oven 105°C for 24 hours, then weighed (a gram). After drying edible film was soaked in 30 ml of distilled water and

placed in a container that was covered with plastic and then stored at room temperature for 24 hours. Pick up the resembled pieces and then dried in an oven at 105°C for 24 (b gram). Solubility value is g/g calculated by $(a-b)/a$ or $((a-b)/a) \times 100\%$ if the units was %.

RESULT AND DISCUSSION

Properties of edible plastic's gel before casting

The results of lightness measurements were from 25.30 to 51.10. Completely Radomized Design showed that lightness was significantly ($\alpha = 5\%$) affected by pH of solvent, proportion of whey protein and interaction of these two parameters. The results of measurements of lightness (L) gel as a variation of solvent composition and pH are presented in Figure 2.

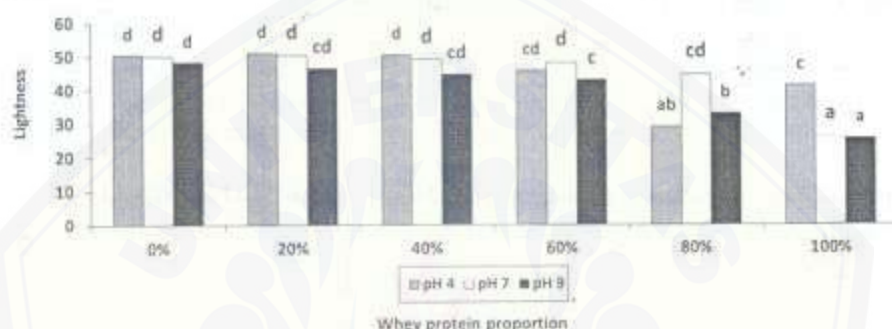


Figure 2. Result of lightness measurement as variation of whey protein proportion and pH of solvent.

Figure 2 showed that increasing in whey addition would decrease lightness because whey protein resulted in opaque color gel that affected the value of lightness. Decreasing in tapioca proportion would decrease the clear-colored of starch gel.

On porportion of whey 0%, 20%, 40% lightness value was not affected by pH of solvent. Increasing of pH at proportion of whey 60% and 100% would decrease lightness value, it was due to an increase in pH increased protein solubility resulted in formation of a homogeneous gel so the color of whey would dominate the gel's color. Whey protein proportion at 80% increased pH from 4 to 7 would increase lightness, while when pH increased from 7 to 9 would decreased lightness. This was probably because a unique carbohydrate protein interaction during gel preparation at pH 7.

Results of texture measurements ranged from 0.00 to 0.41 g / mm. Variation of solvent's pH, proportion of whey and interaction of these two parameters had significant effect on texture of gel ($\alpha < 5\%$). Figure 3 showed result of texture measurement in variation of pH and whey proportion.

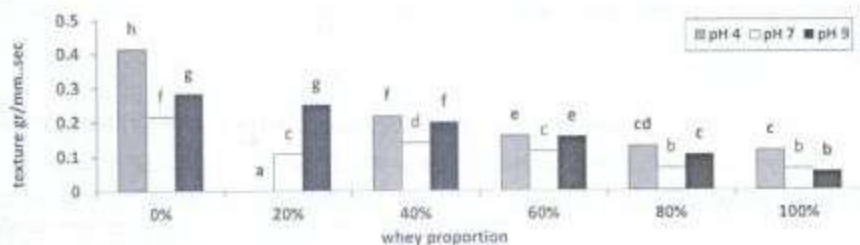


Figure 3. Result of texture measurement as variation of whey protein proportion and pH of solvent.

Figure 3 showed that increasing of whey proportion would decrease gel texture. Whey would soften the gel and gel of starch when cooled will turn into a hard and rigid. Increasing of pH generally would decrease texture, especially when pH increase from 4 to 7. The pH of 4 is isoelectric point, where protein has lowest solubility therefore protein matrix could reinforces the gel matrix of starch-protein resulting in increasing texture value. While at pH 9 the highest protein solubility allowing co-solubility interaction between starch and protein molecules that was why increasing pH from 7 to 9 would increase texture value. Proportion of whey 100%, increasing pH from 4 to 9 would decrease texture, increasing pH would increase protein solubility, without starch there are no carbohydrate protein interaction could be involved. The results of absorbance measurements to measure the solubility ranged from 0.01 to 1.08. Variation of solvent's pH, proportion of whey and interaction of these two parameters had significant effect on texture of gel ($\alpha = 5\%$). Figure 4 showed result of texture measurement in variation of pH and whey proportion.

Based on figure 4 showed that increasing of whey protein proportion would increase the mean absorbance value of more soluble gel. That is because the protein gel would softened and more easily to solubly. Generally increasing pH would increase solubility presumably because of increasing protein solubility.

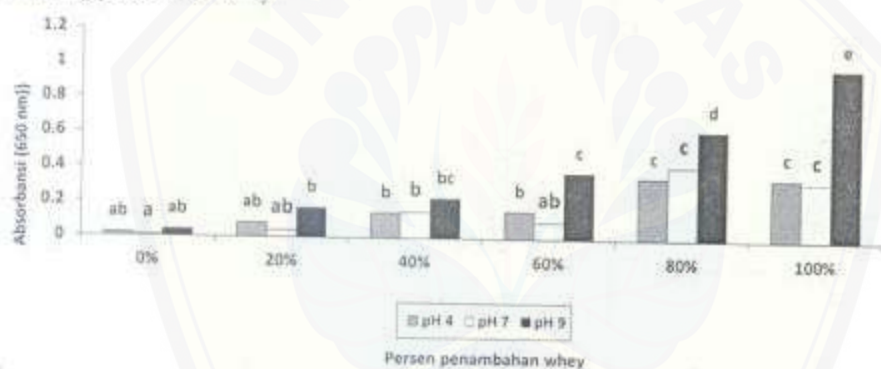


Figure 4. Result of texture measurement as variation of whey protein proportion and pH of solvent.

Properties of edible plastic

Results of strain measurements in this study was 0.00% to 142.68%. Results of analysis of variance showed that variation of whey proportion and pH of solvent had significant effect on strain value ($\alpha < 5\%$). Figure 5 showed result of strain measurements as a function of whey proportion and pH of solvent.

Based on the data in figure 5 increasing of whey protein proportion would decrease strain of edible plastic. Plastic-based on proteins have a smaller strain than films made from tapioca other words edible film made from tapioca will result in a more elastic film. Lindriati et al (2007b) showed similar result where addition of protein from *Cannavalia Ensiformis* to the matrix of edible film from maizena decreased elongation. Research of Poeloengasih and Marseno (2003), also showed a similar trend, where the addition of protein fractions decreased elongation of edible film from tapioca starch.

Generally, figure 5 showed that the pH does not affect the value of the strain. The results of tensile strength measurements ranged between 0.00 MPa to 2.68 MPa. Results of analysis of variance showed that whey protein proportion and pH of solvent had significant effect on tensile strength but interaction between this two parameters did not. Figure 6 showed result of strain measurements as a function of whey proportion.

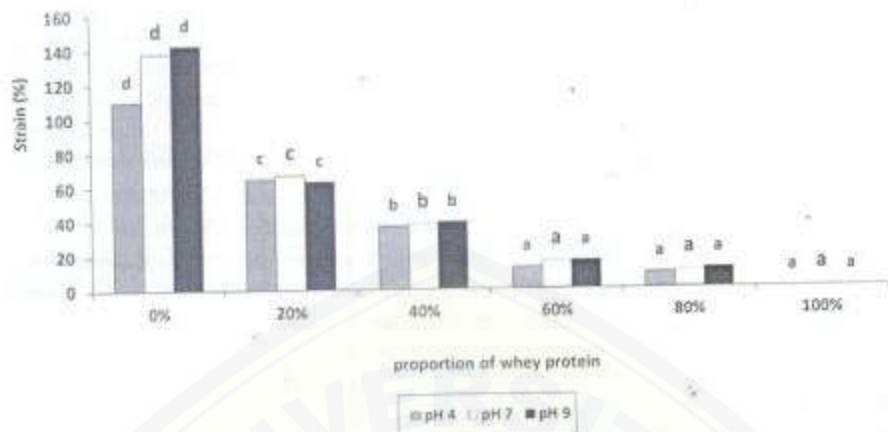


Figure 5. Result of strain measurement as variation of whey protein proportion and pH of solvent.

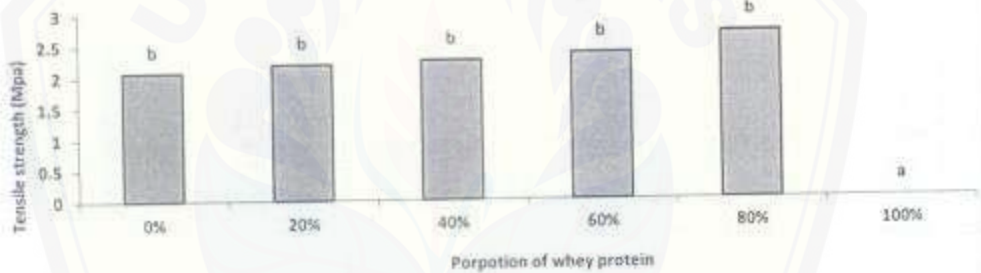


Figure 6. Result of tensile strength measurement as variation of whey protein proportion.

Based on the data in Figure 6 showed that increasing of whey proportion would increase tensile strength. According to Guilbert and Graille (1994), generally, mechanical properties of edible films from protein better than the edible film of starch. This is because starch is a homopolymer, whereas the protein has a specific structure composed of at least 20 hydrogen bonds. Tensile strength measurement on 100 % whey was zero because plastic produce from 100% whey was brittle so the tensile test could not be performed.

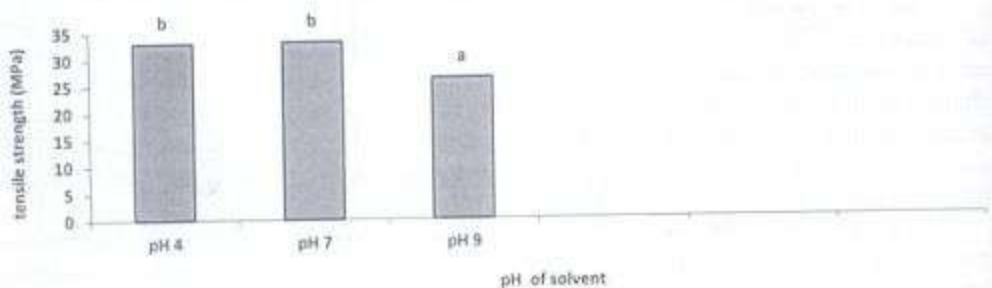


Figure 7. Result of tensile strength measurement as variation of pH of solvent.

The results of tensile strength measurements (Figure 7) showed that increasing of pH would decrease tensile strength of edible plastic, it was thought to relate with protein solubility. Increasing of pH would increase protein solubility. According to Mawarti et al. (2001), by decreasing pH value, effect of glycerol as a plasticizer was getting smaller, that cause of increase in tensile strength and decrease in extension.

The results of the solubility measurements ranged between 22.90% to 34.69%. Results of analysis of variance showed that whey protein proportion and pH of solvent had significant effect on solubility but interaction between this two parameters did not. Figure 8 showed result of strain measurements as a function of whey proportion.

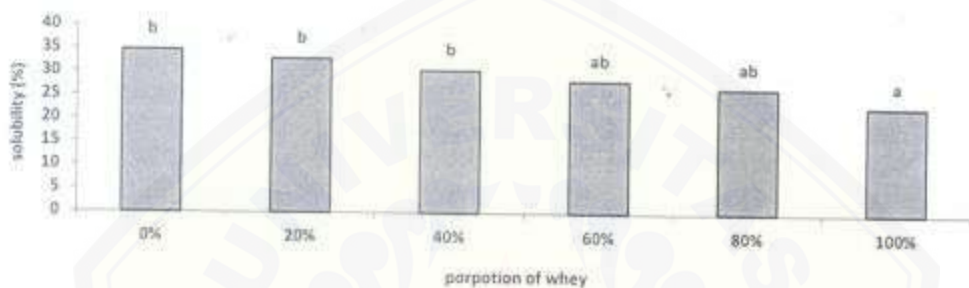


Figure 8. Result of solubility measurement as variation of whey protein proportion.

Figure 8 shows that increasing in whey proportion would decrease of solubility. High solubility of edible plastic showed that it was easily soluble in water. During gel preparation, whey protein would be denatured when heated, whereas hydrophobic group was located outside and a hydrophilic group would be folded and more hydrophobic plastic could be produce.

The results of the solubility measurements as a function of pH is shown in Figure 9. The data showed increasing of solubility with increasing of pH. At pH 4 edible plastic had solubility in water less then plastic at pH 7 and pH 9. It was presumable there was plastic solubility depend on protein solubility whereas at pH 4 near the isoelectric point protein had lowest solubility.

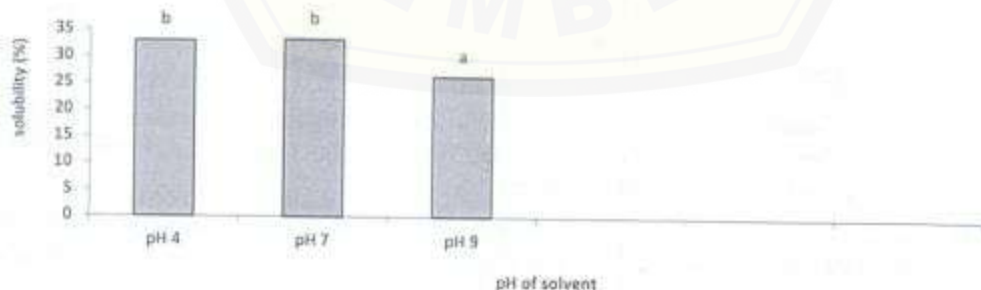


Figure 9. Result of solubility measurement as variation of pH of solvent.

CONCLUSION

Whey protein proportion and pH of solvent had significant effect on gel's characters (lightness, texture and solubility) and edible plastic's characters (strain, tensile strength and solubility). Interaction of whey protein proportion and pH had significant affect on lightness, texture and solubility of gel and strain of edible plastic but had no significant effect on tensile strength and solubility. In this research edible plastic could not be produce from 100% whey protein.

REFERENCES

- American Association of Cereal Chemist (AACC) Technical Committee. 1981. Water Holding Capacity. *Cereal Foods World*, 26: 291
- Bourtoom, T. 2007. Effect of Some Process Parameters on the Properties of Edible Film Prepared from Starches. Department of Material Product Technology, Prince of Songkla University, Hat Yai, Songkhla, 90112. *Maisstaerken und Ermittlung von strukturgeignungsbeziehungen* (PhD dissertation). Berlin: Technischen Univ. Berlin
- Chang, Y.P., P.B. Cheah and C.C. Seow. 2000. Plasticising-Antiplasticising Effect of Water on Physical Properties of Tapioca Starch Films in The Glassy State. *J. Food Sci.*, 65 (3):445-451
- Dahle, L.K. 1971. Wheat Protein-Starch Interaction. I. Some Starch Binding Effect of Wheat Flour. *Cereal Chem.*, 48: 706 - 715
- Gontard, N., S. Guilbert and J.L. Cuq. 1992. Edible Wheat Gluten Films: Influence of The Main Process Variables on Film Properties Using Response Surface Methodology. *J. Food Sci.*, 57: 190 - 195
- Graham, H.D. 1977. *Food Colloids*. The Avi Publishing Co. Inc. Westport Connecticut
- Guilbert, S. and J. Graile. 1994. *Biomate Riaux Et Mole'cules Fonctionnelles*. Paper Presented at 1st Colloque National Sur les Valorisations Non Alimentaires Des Grandes Productions Agriles, Nantes.
- Lindriati, T. dan H. Arbiantara. 2009. Pengembangan Teknologi Edible Film Aktif dari Tepung Koro Pedang. *Proceeding Seminar Nasional Persatuan Ahli Teknologi Pangan Indonesia*, Jakarta, 3-4 November
- Lindriati, T., D.A. Wulandari, Y. Praptiningsih dan Maryanto, 2007a. Pengaruh Penambahan Carboxy Methyl Cellulose (CMC) terhadap Sifat Fisik dan Mekanik Edible Film dari Pati Jagung. *Jurnal Teknik Pertanian IV*, (1): 54-61
- Lindriati, T., I. Setiawan, Maryanto dan Tamtarini. 2007b. Pengaruh Penambahan Isolat Protein Koro Pedang (*Camavalia ensiformis*) terhadap Sifat Fisik dan Mekanik Edible Film dari Pati Jagung. *Jurnal Agroteknologi*, 1 (1): 47 -54
- Mawarwati, S., S. B. Widjanarko, dan T. Susanto. 2001. Mempelajari Karakteristik Edible Film Berantioksidan dari Gandum (*Triticum aestivum* L.) dan Pengaruhnya dalam Pengendalian Pencoklatan Pada Irisan Apel (*Malus sylvestris*). *Biosain*, 1 (1): 61-76
- Poeloengasih, C.D. dan D.W. Marseno, 2003. Karakterisasi Edible Film Komposit Protein Biji Kecapir dan Tapioka. *J. Teknologi & Industri Pangan*, 14(3): 224 - 232
- Wieddyanto, E. 2007. Karakteristik Edible Film Protein Whey dengan Berbagai Tingkat Pemanasan. Skripsi. Fakultas Peternakan. Universitas Brawijaya Malang