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Full Length Research Paper

Functional interaction components of protein isolates and glucomannan in food bars by FTIR and SEM studies

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Functional compounds between soya, wheat, corn protein isolates, and konjac glucomannan (KGM) and these dough mixtures were investigated by FTIR studies. The results showed that fragments–$CONH_2$, were assigned to absorption bands in the region of 1634 - 1650 cm^{-1}, and fragments–carboxyl groups of protein matrix were exhibited at the region of 3235 – 3460 cm^{-1}. Whereas, the molecular structures of KGM were confirmed by fragments –CH appeared at the wave length of 808 - 875 cm^{-1}, which representated the mannose and glucose units. Fragments C=O (aceto compounds) exhibited at the region of 1636 - 1666 cm^{-1} showed the existence of β-1,4 linked glucose and mannose. Fragments C-O-C represented acetyl residue of KGM were exhibited at the absorption region of 1027 - 1244 cm^{-1}, and free –OH hydroxyl groups of KGM were strongly appeared at absorption bands of 3100 - 3391 cm^{-1}. Surprisingly, the deconvolated spectra of a dough mixtures of protein isolates with KGM was almost identical to IR spectrum of each ingredient. The presence of overlapping absorption bands at 3435.95 and 3488.99 cm^{-1} which were assigned to –OH and NH_2 stretch vibration, from protein matrix and –OH vibration from KGM intermingled to form a smooth surface of SEM image of the dough mixtures. Smooth surface plates were observed for the microstructure of food bar with konjac flour; on the contrary, that without konjac flour showed rough fracture plates. Textural studies showed increasing breaking force as the increased level of konjac flour in food bar.

Key words: Interaction components, protein isolates, glucomannan, konjac flour, food bar, FTIR, and SEM.

INTRODUCTION

The protein foods available commercially are obtained from a range of animal and plant sources and are used as functional ingredients (Periago and Vidal, 1998). Protein isolate was prepared from defatted plant materials by several researchers (Hettiarachchy et al., 1996, Nielsen et al., 1973, Hassan et al., 2010, and Karki et al., 2009).

Protein isolates are currently of special interest to processors and consumers due to low fat content and high protein content. Therefore it makes a useful ingredient for several food products, including baked foods, extruded high-protein foods, nutritional bars etc (Hassan et al., 2010). Functional properties of plant protein isolates have been reported by Ahmedna et al., (1999), Singh et al. (2008) and de-Mesa et al. (2009). The functional properties of plant protein, in general is dependent upon the structure of the molecule. The multiplicity of groups attached to the polymer chain of the protein, such as lipophilic, polar, nonpolar, negatively and positively charged groups, enables plant proteins to associate with other macromolecules, including konjac

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glucomannan (KGM).

KGM is extracted from konjac flour of *Amorphophallus konjac* C. Koch. and consists of β-1, 4-linked D-mannose and D-glucose in the ratio 1, 6:1 with being acetylated (Zhang et al., 2001). The presence of C-O groups (Xu et al., 2007), in the chemical structures of KGM open the possibility of combining KGM with other polymers, such as: protein which has -OH, $-NH_2$, $-CONH_2$ or -COOH groups in their polymer molecules as reported by Shukla and Cheryan (2001) through hydrogen bonding. The presence of acetyl residues obstruct the interaction between KGM with protein, and deacetylation process can facilitates the interaction of KGM with other polymers, such as, starch, carrageenan and xanthan (Alonso-Sande et al., 2009).

Studies in the interactions of plant proteins and starch in starch-soy protein concentrate (SPC) extrudates has been reported by Allen et al., (2007), while interactions of SPC with cassava starch-based matrix extrudates had been reported by Chang et al., (2001). However, studies on the interactions of plant proteins, especially soy, wheat and corn protein isolates with KGM in food bar seems to be limited in numbers. Therefore this study will significantly contributes to the field of interactions food components.

Studies on the chemical structure of KGM using fourier transform infrared (FTIR) spectroscopy attracts considerable attention of several researchers such as: Nishinari et al., (1992); Yu et al. (2007); and Wen et al. (2009), who studied by using fourier transform infrared (FTIR) spectroscopy. Little published informations on FTIR studies on the interaction components of protein isolates, particularly soya, wheat and corn protein isolates with KGM.

The main component of konjac flour is KGM (Kohyama et al., 1993 and Williams et al., 2000), therefore konjac flour was chosen by incorporating its with plant protein-flours in producing food bars. Emergency food relief (EFR), especially in the form of food bars, must be capable of withstanding various modes of transportation due to a lack of a delivery infrastructure. Food bars should exhibit tough and rigid texture properties, particularly, if it is distributed by air. Therefore Incorporation of konjac flour with plant protein-flours mixtures is important in enhancing the textural quality of the product.

Scanning electron microscope (SEM) is a powerfull tool for characterization of three-dimensional surface of food products (James, 2005). SEM studies on glucomannan was reported by Alonso-Sande et al. (2009) and Wen et al. (2009), but none has been done on food bars dough and food bars containing mixtures of protein-flours and konjac flour. The aim of this work is to study the functional compounds of a dough mixtures of plant protein isolates with KGM and to examine supramolecular structure of its and SEM studies of food bars containing konjac flour, as well as textural studies of food bars.

MATERIALS AND METHODS

Corn "*Bisma"* variety and soya bean "*Anjasmoro"* variety were bought from Corn and Legume Research Center, Bedali, Lawang regency; sweet potato *"Sukuh"* variety was obtained from legume and Sweet potato Research Center, Kendalpayak, Malang regency. Wheat flour "*Roda Biru*" brand was bought from Indofood sukses makmur (ISM) *Bogasari* Flour mill, company, surabaya. Sucrose, margarine and milk powder were bought from a local supermarket. *Porang* or local name for konjac bulb, native to Indonesia, with outer diameter ranged 20 – 25 cm was collected from a konjac farmer at Sumberbendo village, Saradan district, Madiun regency. All the chemicals used were of analytical grade, while the water was glass distilled.

Sample preparation

Preparation of corn flour was conducted as follows: Corn seed with 12% moisture content was sorted and separated from dirts and other impuriteis. Cleaned corn seed was milled using multi mill apparatus. Three fractions which are coarse flour, grits and dirt were collected. Grits were separated, washed and dipped in water for 3 h. Dipped grits were then dried untill 12% moisture content. Dried grits were milled using a disc mill, then sieved into 80 mesh screen.

Preparation of corn protein isolate (CPI) was illustrated as follows: Corn flour was defatted using Soxhlet apparatus. Defatted corn flour was dilluted with water, mixed and 0.05 N NaOH was added untill mixtures reached pH 8.7. The mixtures were agitated with a magnetic stirrer for 30 min, then centrifuged at 5500 rpm for 15 min. Supernatant was collected, and 0.1 N HCl solution was added, until a mixture reached pH 4.7. The mixtured was centrifugated at 5500 rpm for 15 min, then filtered, a residue was collected, while supernatan was discharged. The residue was then dried with vacuum dryer at 35°C for two h (Nielsen et al., 1973). Preparation of soya bean flour and soy protein isolate (SPI) were conducted as the above procedure, but for the preparation of soya bean flour as described by Susanto and Saneto, (1994) and for soy protein isolate preparation as mentioned by Rickert et al., (2004), respectively. While wheat protein isolate (WPI) was made similar to the preparation of CPI and SPI, but the procedure of preparing WPI as mentioned by Hettiarachchy et al. (1996).

Production of konjac flour were prepared as follows: Tuber of *Amorphophallus oncophyllus*, with outer diameter ranged 20 – 25 cm, was weighed around 1,5 kg each and sliced into 3 mm thick using a local made blade slicer. Chips were dried using a steinless steel cabinet dryer at 60°C until their moisture content reached 12% constantly. Chips were milled using a stamp mill, for 5 h. The flour was sieved using 80 mesh screen and coarse fractions were discharged. The 80 mesh konjac flour was separated from fine particles (*tobico*) which are mostly containing calcium oxalate, using a cyclone apparatus. Konjac Glucomannan (KGM) powder was obtained from the purification process of by heating konjac flour solution containing alumnium sulphate salt 10%, at 75 - 78 °C for one h and contantly stirred at 200 rpm with a hot plate stirrer (Lubinco L-32). The mixtures were filtered using fine cloth under vacuum pump. Supernatant was sedimented using isopropyl alcohol at ratio 1:1 as described by Ohashi et al. (2000).

Procedure of preparing protein isolates and KGM dough

1 g KGM powder was diluted with 100 ml aquadest and boiled at 100°C for 15 min. Dried protein isolate (soya, wheat, and corn) at

Table 1. Mean of glucomannan content from konjac flour and glucomannan extract.

Sample	Glucomannan content (%)
Konjac flour	64.36 ± 0.10
Glucomannan extract	86.24 ± 0.31

12:5:3 rasio were mixed together and added into boiled konjac glucomannan solution at the ratio of 20:1. Aquadest was then poured into the mixtures of protein isolates-KGM at 3:7 ratio (Nugroho, 2008). The mixtures were furthermore boiled at 120°C for 30 min with continuous stir. The dough protein isolates-KGM was cooled at ambient temperature (Sukamto, 2007). A similar process was conducted for control without adding KGM powder.

Procedure of making food bar

A formula containing corn flour, soya flour, wheat flour, sweet potato flour, sucrose powder, milk powder, margarine were mixed together. The amount of each ingredients involved was calculated as described by Nugroho (2008). Konjac flour at the proportion of 1% w/w was dissolved in aquadest. All ingredients were mixed with a mixer (Sanyo, Japan) at low speed for 15 min. The dough formed was baked at 150°C for 15 min and cooled for 30 min. Upon cooling the dough was transferred into a stainless steel cube (3.5 x 3 x 2 cm) and pressed using a hydrolic press at 1.2 psi. The food bar had 25 g in weight each.

Glucomannan analysis

Glucomannan assay was conducted on samples konjac flour and KGM powder as mentioned by Ohashi et al. (2000).

Determination of functional compounds

Functional compounds of each sample from a protein isolates, KGM powder and mixtures of protein isolates with KGM were determined using FTIR as noted by Instruction Manual IR Prestige-21, (2002). For functional unit determination, the Shimadzu Fourier transform Infrared Spectrophotometer- FTIR 8400 S was used.

Samples were weight-in at 0.01 g and homogenized with 0.01 g KBr anhydrous by mortar agate. The mixtures were pressed by vacuum hydraulic (Graseby Specac) at 1.2 psi to obtain transparency pellet. Scanned sample passed through infra red, where its continuing wave by detector that connected to computer and given described of tested sample spectrum. Samples were usually scanned in the absorption area of 600 to 4000 cm^{-1}. The results of analysis consisted of chemical structure, molecular binding form and certain functional groups of tested sample as basic of spectrum type.

SEM Studies

There was 5 samples for SEM studies reported which were: (1) Dough made from mixing three protein isolates (corn, soya and wheat) with KGM. (2) Dough mixtures without KGM. (3) Food bars dough with adding konjac flour. (4) Food bars without adding konjac flour and the last sample was food bars with konjac flour.

Samples were transferred into 2% glutaraldehyde solution for 2 to 3 h at 4°C. Samples were drained and washed three times for 5 min for each washing at 4°C with buffer phosphate pH 7.4. Samples were dipped into osmic acid 1% for 1 to 2 h at 4°C. Washing samples were furthermore conducted with buffer phosphate pH 7.4 three times, each of washing time took 5 min. Dehydration process of the samples were performed by dipping samples into 30, 50, 70, 80 and 90% ethanol absolute two times. The dipping process were conducted gradually, began at low up to high concentrations of ethanol absolute. Dehydration process at 30 up to 70% of ethanol was carried out at 4°C, whilst at 80% ethanol absolute were done at ambient temperature. The dipping solution was replaced by amyl acetate absolute. Samples were dehydrated by putting it's into critical point drying equipment, then fastened with a special glue to stub (samples holder). Samples were let them dried for ± 1 day. Samples were coated with pure gold or carbon for 1 h at a coating evaporator machine prior to be observed and taken its microscopic photos by scanning electron microscope (SEM) machine (JSM T-100, JEOL, Jepang).

Textural studies

To study the effect of konjac flour in food bars, protein flour mixtures were mixed with konjac flour in a food bar formula, the following experiments were conducted as follows: Complete randomized design was used in this experiment with one factor which were: the proportion of konjac flour used at 0, 1, 2, 3 and 4% w/w. Each treatment was replicated 3 times. Formula of making food bar as previously stated above and prescribed by Nugroho (2008). Breaking force of each samples were determined as described by Susanto and Yuwono (1998). Analysis of variance was calculated using excel software package followed by least significant different test.

RESULTS AND DISCUSSION

Glucomannan analysis

The level of glucomannan from sample of glucomannan extract was relatively higher than the amount of glucomannan at konjac flour (Table 1). Perdani (2009) reported glucomannan content of sample glucomannan extract was 67.04%, whereas coarse konjac flour contained only 37.78% of glucomannan. Another data reported that glucomannan content of konjac flour was around 64.98% (Arifin, 2001). Our data were relatively higher than data reported by Perdani (2009) and Arifin (2001), it might be due to a different methods of konjac flour preparation and glucomannan extraction methods.

Identification of functional compounds

Functional compounds of protein isolates

Infra red spectrum of protein isolates indicated by the presence broad bands at 3460.06 cm^{-1} for CPI (A),

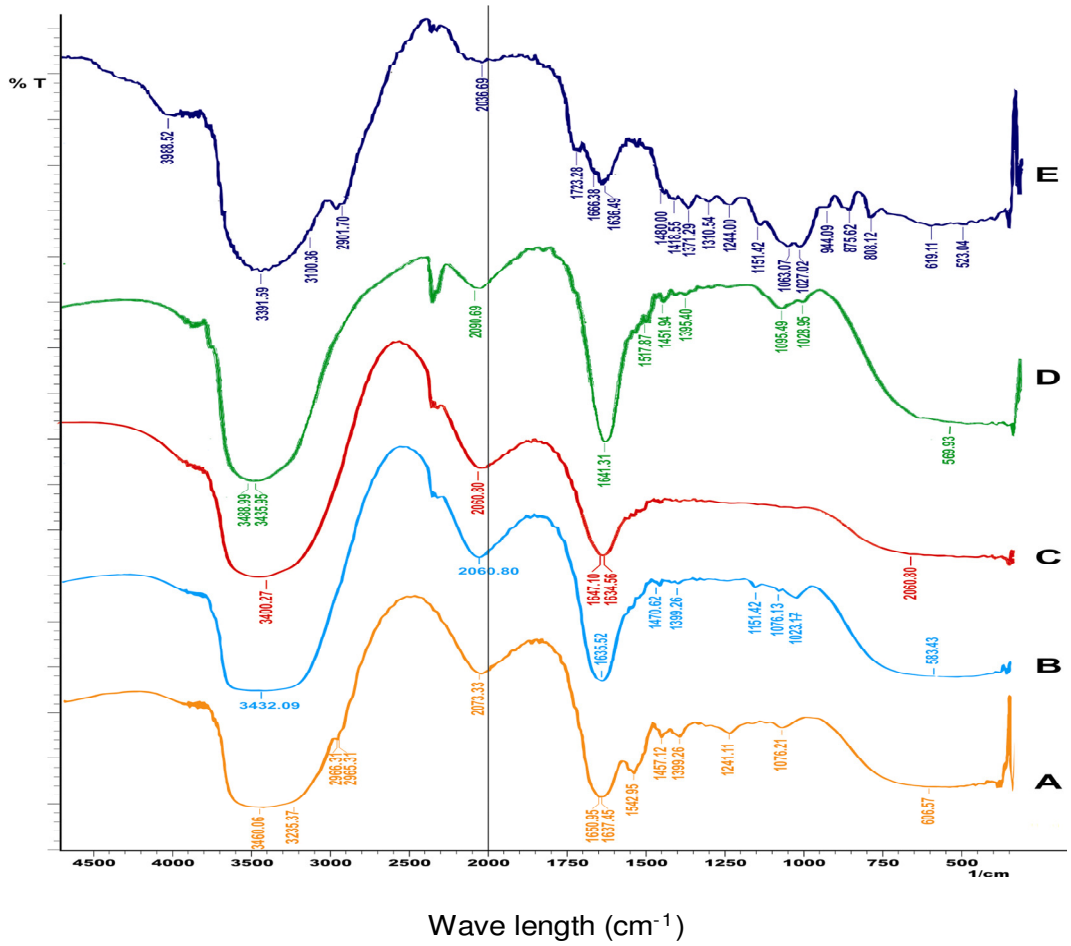

Figure 1. FTIR spectra of SPI (A), WPI (B), CPI (C), SPI+WPI+CPI and KGM mixtures (D) and KGM (E).

3432.09 cm^{-1} for WPI (B) and 3400.27 cm^{-1} for CPI (C) are attributed to –OH stretching vibrations (Figure 1). This is due to carboxylic compounds in the polymer protein isolates matrix. The presence of carboxylic and amide compounds at SPI and WPI were confirmed by the bands at 3235.37 cm^{-1} and 3432.09 cm^{-1}, respectively (Table 2). Dalgleish and Hunt (1995) stated that hydrogen bonds are formed between dipolar groups (acid and amide groups) in the protein and involved the sharing of a hydrogen atom between two such groups.

The noteworthy of the peaks bands of SPI (A) in Figure 1 were observed at 1650.95 and 1637.45 cm^{-1}, whereas for WPI (B) was assigned at 1635.52 cm^{-1} region and for CPI (C) were at 1647.1 cm^{-1} and 1634.56 cm^{-1} regions, respectively (Table 2 and Figure 1). These bands indicated the presence of functional compounds of protein amide groups –CONH– which were attributed to carbonyl (C=O) stretch vibration. Native protein gave more intense peaks in the range of 1630 - 1660 cm^{-1}, meaning that several overlapping secondary structural components of the polypeptide chains were present in the protein (Byler and Susi, 1986). Maltais et al. (2008) noted soya protein isolate component located at 1636 cm^{-1} is attributed to β-sheet structures, while the peak located at 1651 cm^{-1} is assigned to α-helical portions and can also be generated by protein unordered structure. Furthermore, functional compounds of amide primary in protein isolates matrix was supported by Jackson and Mantsch (1992) that amide primary is the major protein absorption band located at 1650 cm^{-1}, and occurs predominantly from the C=O stretching vibration of the protein amide groups.

A molecular structure of KGM is well known by the presence of hydroxyl (-OH) groups and attributed to broad bands located at 3391.59 cm^{-1} (Table 3 and Figure 1). This data was strongly confirmed by Zhang et al., (2001) that KGM spectra are dominated by a broad band assigned to the stretching vibration modes of -OH groups and water at about 3396 cm^{-1}. This hydroxyl groups were laid on characteristically by methyl groups located at 2901.7 cm^{-1} which is attributed to –CH stretch vibration (Table 3). Xiao et al. (1999) confirmed the stretching peaks of –CH of methyl at 2920 cm^{-1}.

KGM is a water-soluble non-ionic polysaccharide consists of β-D mannopyranose and β-D glucopyranose units with a low degree of acetyl groups at the side chain

Table 2. Absorption regions and vibration type of protein isolates (SPI, WPI and CPI).

SPI		WPI		CPI	
Wave length (cm^{-1})	**Vibration type**	**Wave length (cm^{-1})**	**Vibration type**	**Wave length (cm^{-1})**	**Vibration type**
1637.45	C=C *stretch*; NH_2 *bend*; *ring aromatic stretch*, C=O *stretch*	1151.42	C-OH stretch	1634.56	C=C stretch; C=O stretch; NH2 bend; ring aromatic stretch
1650.95	C=C *stretch*; C=O *stretch*	1399.26	C-N stretch; CH3 bend. sym	1647.1	C=C stretch; C=O; NH2 bend;
2966.31	CH *stretch* in C-CH_3; CH *stretch*; OH *stretch* H *bonded*	1470.62	CH3 bend. antisym; CH2 bend; ring aromatic stretch; C-N stretch	3400.27	OH stretch
3235.37	OH *stretch*; NH_2 *stretch*	1635.52	C=C stretch; C=O stretch; ring		
3460.06	OH *stretch*; H *bonded*	3432.09	OH stretch monomer; NH2 stretch		

Table 3. Absorption regions and vibration type of KGM and mixture of protein isolates with KGM.

KGM		Protein Isolates + KGM	
Wave length (cm^{-1})	**Vibration type**	**Wave length (cm^{-1})**	**Vibration type**
808.12	CH bend kel. bid	1028.95	C-OH stretch; CH kel bid. def.
875.62	CH bend kel. bid	1095.49	CH kel bid. def; H-C=O bend
1027.02	C-O-C stretch	1395.40	C-N stretch; CH3 bend sym;
1244	C-O-C stretch	1641.31	C=C; C=O stretch;
1636.49	C=O stretch	3435.95	OH stretch monomer; NH2 stretch
1666.38	C=O stretch	3488.99	OH stretch monomer; NH2 stretch
2901.7	CH stretch in C-CH3		
3391.59	OH stretch bonded		

Konjac Glukomannan and Protein Isolates with Glucomannan mixtures.

C-6 position (Kaname et al., 2003). Mannose and glucose units of KGM were assigned the β pyranose form from characteristic peaks at 808.12 and 875.62 cm^{-1}, respectively (Table 3). Hua et al. (2004) reported that mannose and glucose units were assigned from characteristic peaks at about 814 and 873 cm^{-1}, respectively, which were attributed to C-H bend vibration. The presence of β-1, 4 glucosidic and β-1,4 mannosidic linkages in Glucomannan are assigned to C-O-C stretch vibration located at 1027.02 and 1244 cm^{-1}. FTIR studies on native to China KGM revealed that characterization peaks at 1151 and 1027 cm^{-1} were assigned to fragment C-O-C (Yu et al., 2007).

KGM consists of β-1, 4 linked glucose and mannose units (Nishinari et al., 1992). The glucose: mannose ratio has been reported to be between 1:1.6 (Kato and Matsuda, 1969) and 1:1.4 (Bewley and Reid, 1985). The occurrence of β-1, 4 linked glucose and mannose of glucomannan (Figure 1, E) was proven by the carbonyl (C=O) stretch vibration located at 1666.38 and 1636.49 cm^{-1} (Table 3). The stretching peak of carbonyl groups of KGM was reported at 1730 cm^{-1} by Maeda et al., (1980). Whereas Xiao et al. (1999) noted that the absorption peak of carbonyl groups of acrylamide grafted konjac glucomannan (AKGM) were appeared at 1671 cm^{-1}.

The remarkable difference between the IR transmission curve of KGM (Figure 1E) and those the spectra of protein isolates and KGM mixtures at D, was the elimination of the peaks of KGM at the wave length 808.12 and 875.62 cm^{-1}, respectively. This indicates that the spectra of mannose and glucose residues disappeared, meaning that granules of KGM was well-

Figure 2. Microstructure of mixed dough of protein isolates with no KGM.

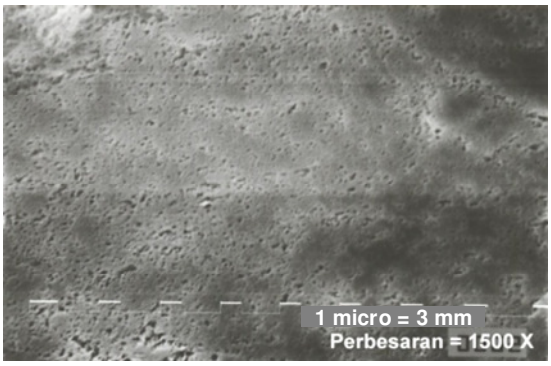

Figure 3. Microstructure of mixed dough of protein isolates with KGM.

mixed in the protein isolates-glucomannan dough mixtures. It might also be due to the proportion of the KGM is smaller than the proportion of protein isolates mixtures in the dough which was at the ratio of 1:20.

Whereas in regards to the disappearing IR spectrum at 1063.67 and 1151.42 cm^{-1} at (E), which were assigned to the fragment C-OH of KGM. It could be speculated due to the hydrogen bonding between fragment methoxy (C-O) of KGM (E) with fragment protein amide (N-H_2) of protein isolates. This was corroborated by the shift in wave length from 1063.67 and 1151.42 cm^{-1} to lower at 1028.95 cm^{-1} which were assigned to C-OH (D). Zhang et al. (2001) noted that fragment C-O, which was assigned to C-OH vibation type of KGM appeared at 1032 cm^{-1} region.

Interestingly, there is no major difference between the spectra of mixtures protein isolates and KGM (D) with the spectra of SPI (A), WPI (B) and CPI (C), at Figure 1, respectively. Indicating that molecular structure of plant protein isolates, before and after blending the dough are almost identical. The remarkable feature of the spectra D was the presence of strong absorption bands located at 1641.31 cm^{-1} which was assigned to C=O stretch vibration. This peak corresponds to the protein absorption band. Walker (2007) reported that the protein absorption bands were laid on the range regions at 1650 and 1550 cm^{-1}. This is strongly supported by Singh et al. (2008) noted that the more refined forms of soybean protein are the isolates which contain 90% of protein.

The absorption bands at 3435.95 and 3488.99 cm^{-1} (D) which were assigned to –OH and NH_2 stretch vibration, indicating the presence of overlapping –OH groups of the carboxylic groups and –NH_2 of amide groups in the mixtures of protein isolates matrix and –OH groups of KGM. Wen et al. (2009) noted the stretching of –OH groups of KGM occurs at 3422 cm^{-1}. H-bonding involved in the intra and intermolecular interactions to form gel network within the mixtures dough and water need to be observed microscopically. This was corroborated with the smooth surface of SEM pictures of the protein isolates with KGM dough compared to rough surface of SEM pictures control (protein isolates without KGM). It was therefore necessary to study supramolecular organization of mixed protein isolates with and without glucomannan.

SEM Studies

Scanning electron micoroscopy (SEM) conducted at magnification 1500x. It is conducted on samples containing protein isolates with KGM dough mixtures and food bars containing konjac flour, sweet potato flour and protein flours mixtures. The former is to observe microscopically the effect of KGM in protein isolates dough mixtures, and the latter is to investigate the effect of konjac flour, as binding agents, in food bars. As it has been mentioned at this research methodology, that protein sources for making food bars are the mixtures of protein flours and are not a protein isolates, because SPI is the primary component of soy protein flours (Wang and Johnson, 2001). The use of protein flours in food products, such as: bakery products and food bars etc, are more common and cheaper than the use of a protein isolates, if we look at a consumer purchasing capacity as it happens in Indonesia. The similar reason for the use of konjac flour and it is not KGM as a gelling agent in food bars. It is well known that KGM is the main components of konjac flour as it was reported by Lie et al., 2006 and Gao et al., 2007).

Microstructure of protein isolates dough mixtures without KGM shows rough surface with irregular sizes of pores (Figure 2). Whereas SEM picture of the dough mixtures with KGM was vice versa (Figure 3). The former may be due to the denaturation process of protein molecules during heating process of the dough mixtures at 150°C for 15 min. Protein undergoes denaturation process, by which hydrogen bonds and hydrophobic interactions are broken and the protein molecules is unfold. MacRitchie and Lafiandra (1995) noted that most of globular protein easily undergone denaturation with

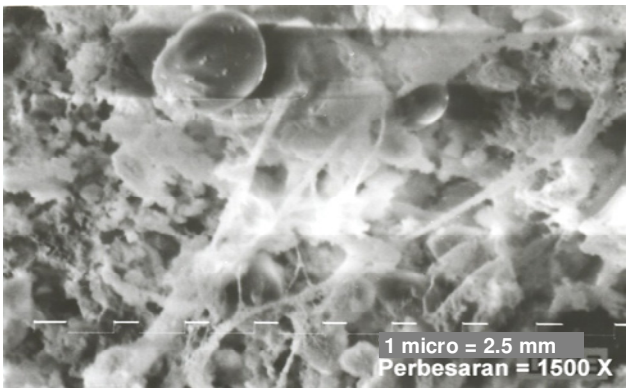

Figure 4. Microstructure of food bar dough with konjac flour.

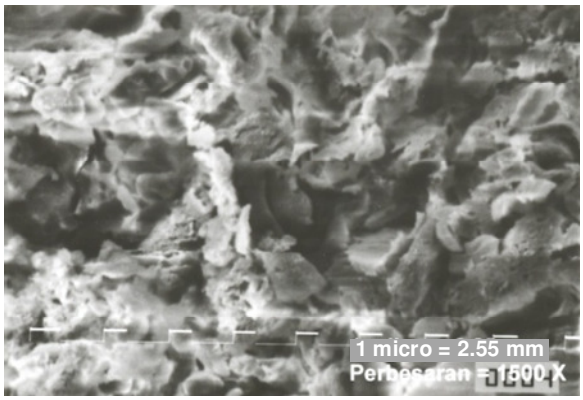

Figure 5. Microstructure of food bar without konjac flour.

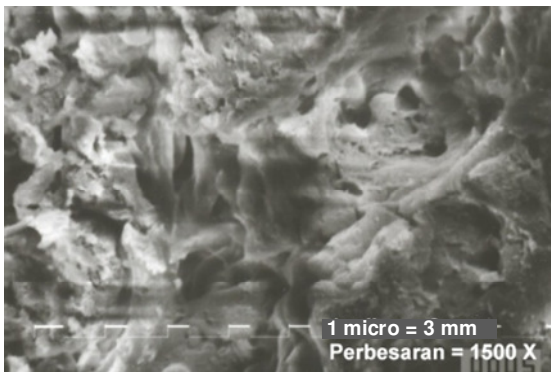

Figure 6. Microstructure of food bar with konjac flour.

opening coil structures or changing in polypeptide linkages during heating process. Ultrastructure of wheat flour-water dough at higher magnification showed rough protein molecules surfaces, compared to smooth surface of isolated gluten (Fretzdorff et al., 1982).

For the protein isolates with KGM dough mixtures, the pore size becomes smaller, uniform sizes, smooth surface (Figure 4). It is speculated due to the hydrogen bonds between fragment C-O of konjac glucomannan and fragment N-H of protein isolates. This was corroborated by the shift in wave length from 1063.67 and 1151.42 cm^{-1} to lower absorption bands at 1028.95 cm^{-1} which were assigned to C-OH (Figure 1D). Wen et al. (2009) reported that SEM pictures of KGM/PolyAcrylic Acid-Interpenetreting Polymer Network-hydrogels shown the pore size were smaller than native KGM hydrogels.

SEM picture of food bar dough with the addition of 1% konjac flour shown interconnecting smooth fibrous materials with other components within a food bar dough, and a clear round granules seem at its SEM picture. It may be speculated due to starch granules. Kusumawardhani (2007) reported that konjac flour contained 21.8% starch and 58.8% glucomannan and Lestari (1999) mentioned that dough from flour and other raw materials, starch granules look oval, although fermentation processes take place during dough ripening.

SEM image at Figure 5 shows rough all over surfaces with fracture plates, whereas smooth surfaces and smooth dough plates on SEM image at Figure 6. This may be due to hydrogen bonding between fragments C-O of glucomannan, which is the main component of konjac flour, and fragments N-H of protein flours within food bars formula. Tang et al. (2003) reported that soy protein isolate (SPI)-konjac glucomannan blend films shown better its thermostability and water vapor barrier properties of its were greatly enhanced due to strong intermolecular hydrogen bonding between SPI and konjac glucomannan. Chiffon caked prepared with a mixture of wheat flour: konjac flour: SPI exhibited a tough and compact structure due to SPI and konjac flour were functioned as water-holding capacity (Akesowan, 2007). Gomezt-Guiellen et al. (1996), using electron microscope studies, observed the formation of carrageenan network that was arranged parallel to the myofibrillar protein network of a gel made from a cephalopod muscle.

Smooth surface plate of SEM image of konjac flour added into food bars formula (Figure 6), is corroborated by the FTIR studies of protein isolates with glucomannan (D). Smooth surface of the dough may be due to the interconnection between hydrogen proton donor of –OH groups from KGM and hydrogen acceptor of –NH groups of protein isolates. Since KGM is the primary components of konjac flour in food bar formula (Zhang et al., 2001). Whereas, protein flours contained 86.71±1.74% protein isolates (data is not shown). Amino acids, peptides and proteins, display IR absorption bands in the range of 800 to 2500 cm^{-1}, such bonds include N-H and C=O (Kerese, 1984). These bonds can interconnect to –OH groups of KGM by hydrogen and electrostatic bonds. McClements (1999) stated that biopolymers that have different positive and negative charges can intermingle each others, mediated by hydrogen binding and electrostatic interactions. To support this interactions occurred, textural studies on food bars made from a formula by mixing

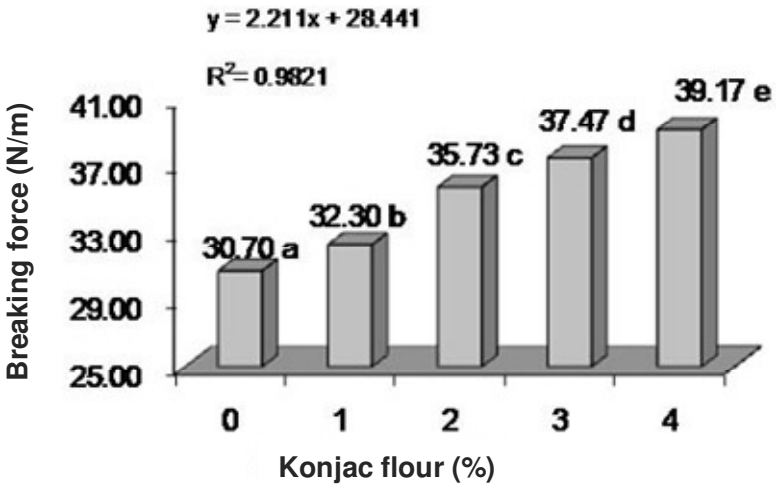

Figure 7. Breaking force of food bar at different proportion of konjac flours.

protein flours and konjac flour as described by Nugroho (2008) was conducted.

Textural studies

Figure 7 shows that there were significant differences ($p<0.05$) in the breaking force of food bars. Incorporation of konjac flour at 4% showed the highest level of breaking force, which was 39.17 N/m, significantly higher than control (30.70 N/m). The result suggested that the amount of KGM in konjac flour at about 64.36 ± 0.10% with the purity of about 86.24 ± 0.31% (Table 1) could affects the textural properties of the food bars and to increase the breaking force of the food bars. Therefore konjac flour could be used as an anti-dropping for food bar used for emergency food product (EFP). SEM image at Figure 6, showed that smooth surface of food bar with konjac flour, confirmed better interactions between KGM with protein flours in food bars formula, than food bars with no konjac flour. As with increasing the levels of konjac flours added into food bar formula, the breaking force of the food bars also increased significantly at ($p<0.05$). Xiong et al. (2009) reported that 2% KGM added into grass carp (*Ctenopharyngodon idella*) to form surimi gels showed the breaking force was the highest (422.3 ± 11.27 g), than control (no added KGM) which was 297.8 ± 5.91 g, and significantly different at $p<0.05$.

Conclusion

SEM image of food bars with the addition of 1% konjac flour indicated smooth structure surfaces and compact, compared to rough fracture surfaces of food bars prepared with no konjac flour. FTIR studies confirmed the interaction between functional compounds of protein molecules (soy, wheat and corn flours) with functional compounds of KGM presents in konjac flour in the formula of food bars. This interaction was mediated by hydrogen and electrostatic binding, which were assigned to the occurrence of –OH and NH_2 at the wave length of 3488.99 dan 3435.95 cm^{-1} of protein isolates with KGM. It was also the appearance of absorption bands at 1641.31 cm^{-1} which was assigned to C=O stretch vibration of protein isolates with KGM. The shift in wave length from 1063.67 and 1151.42 cm^{-1} to lower at 1028.95 cm^{-1} of IR spectrum of mixtures protein isolates with glucomannan, indicated the interaction hydrogen bonds between fragment C-OH of glucomannan and fragment N-H of protein isolates. As the level of konjac flour used in food bar formula increased and the breaking force of the food bars also increased. The interest of this study, for further promising results is the use of konjac flour as anti-dropping agents for EFP in conjunction to Emergency Food Relief for a distribution purposes.

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