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*by* I D Kumalasari, T Sugahara, K Nishi Immunostimulating Effect Of  
Sweet Potato Fiber Ext

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## Immunostimulating effect of sweet potato fiber extract on IgM production by HB4C5 cells

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**Abstract.** Sweet Potato (*Ipomea batatas*) is a local tuber potentially to be developed as functional food. The aims of this research is to evaluate the immunomodulatory effect of the sweet potato fibre extract (SFE) *in vitro* by animal cell culture techniques and identification the active compounds of SFE. SFE were prepared by autoclaving the sweet potato fiber powder in distilled water with room temperature 25 °C for 2 hours in distilled water and dialyzed with membrane with molecular weight cut off 14 kDa and 30 kDa. The IgM production of SFE against human hybridoma cell line HB4C5 cells was evaluated. The results of this research indicated that SFE stimulated IgM production by HB4C5 cells with heat and dialysis treatments in dose dependent manner. IgM production was increased by SFE with molecular weight more than 14 kDa and less than 30 kDa. The conclusion of this evaluation, SFE facilitated IgM production by HB4C5 cells and revealed that the SFE has positive effects on immunostimulatory activity *in vitro*.

### 1. Introduction

There are several various of Indonesian origin tubers that have dietary fiber sources. Those tubers are sweet potatoes (*Ipomoea batatas*), arrowroot (*Marantha arundinaceae*), canna (*Canna edulis*), etc. The tubers contains high amount of dietary fiber. The SFE extracted from sweet potato has potency as prebiotic source [1]. Sweet potato dietary fiber could be isolated from sweet potato pulp. Sweet potato pulp contains of 49,7% dietary fiber, which is rich in pectin (39.5%), cellulose, hemicellulose and lignin [2]. Sweet potato pectin obtained by sonication-induced modification showed lower molecular weight, strong antioxidant capacity and could induce apoptosis-like cell death in colon cancer [3, 4]

The immune function is very important for prevention and recovery from infectious diseases. There are many study about activation of the immune function by functional food. Many foodstuffs that can activate the immune system have been evaluated [5-7]. The immune response can be divided into two categories such as innate immune response and adaptive immune response. Although the adaptive immune response takes a long time after infection, it gives high specific prevention against pathogens. B cells give an important contribution to the humoral immune response by production of immunoglobulins (Igs). Our study focused on the foods that stimulate the immune function for development of functional foods [8-10].

## 2. Materials and methods

### 2.1. A preparation of SFE

Sweet potato was obtained from Karanganyar, Central Java, Indonesia. SFE was prepared as previously described [11]. Briefly, sweet potato tubers were peeled, grated, and suspended in distilled water and settled down overnight. The supernatant was collected as the sweet potato crude fiber after removing the precipitate. Then fresh of sweet potato fiber were steamed for 30 min, soaked in 80% ethanol at 60 °C for 20 min, filtrated, squeezed, oven-dried, and ground into powder. A SFE solution was prepared by suspending the sweet potato fiber powder in distilled water at 10 g/100 mL. The suspension was heated at 121 °C for 20 min or stirred at room temperatur for 2 h. Both suspensions were centrifuged at  $15,600 \times g$  for 20 min to remove insoluble materials and collect the concentrate. The supernatant was dialyzed against distilled water using a dialysis membrane with molecular weight cut off of 14 kDa and 30 kDa (Wako Pure Chemical Industries) and then sterilized by filtration.

### 2.2. HB4C5 cell and cell culture

The IgM production for immune system of SFE was evaluated using the human-human hybridoma HB4C5 cells. HB4C5 cell is a fusion product of a human B lymphocyte from a lung cancer cells with a NAT-30 [12]. HB4C5 cells producing monoclonal IgM were used for the assay of the Ig production stimulating activity [13].

Briefly, HB4C5 cells were cultured in ERDF medium (Kyokuto Pharmaceutical, Tokyo, Japan) containing 10 µg/mL of insulin (Sigma, St. Louis, MO, USA), 20 µg/mL of transferrin (Sigma), 20 µM ethanolamine (Sigma), and 25 nM sodium selenite (Sigma) (ITES-ERDF medium) at 37 °C under humidified 5% CO<sub>2</sub> -95% air for 6 hours [14]. The amount of IgM production in the culture medium was determined by enzyme-linked immunosorbent assay (ELISA)

### 2.3. Ig production stimulating effect of the sweet potato extracts on HB4C5 cells.

The stimulating activity and Ig production *in vitro* was evaluated by measuring the amount of IgM production by HB4C5 cells. The amount of IgM in culture medium 6 h after inoculation was measured by ELISA as described previously [11]. Briefly the SFE were solubilized by heat-treatment at 121 °C for 20 min and evaluated for the Ig production stimulating activity on human hybridoma HB4C5 cells. HB4C5 cells were inoculated in ITES-ERDF medium containing various concentrations of the SFE at  $5.0 \times 10^4$  cells mL<sup>-1</sup>, then performed in a 96-well culture plate. After incubation in a CO<sub>2</sub> incubator at 37°C for 6 h, the amount of IgM production in the culture medium was determined by ELISA using anti-human IgM [13]. The absorbance was measured at 415 nm after the addition of 1.5% oxalic acid to terminate the coloring reaction. The assays were carried out in triplicate

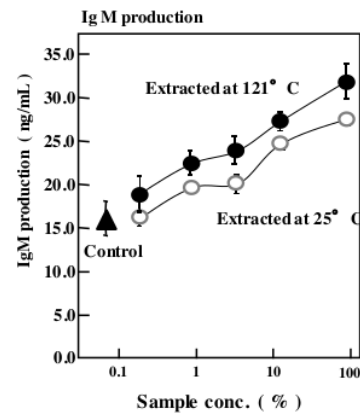
### 2.4. Statistical analysis

Each result is expressed as the mean ± standard deviation (SD). One way ANOVA followed by Tukey's test was used to assess the statistical significance of the difference

## 3. Results and discussion

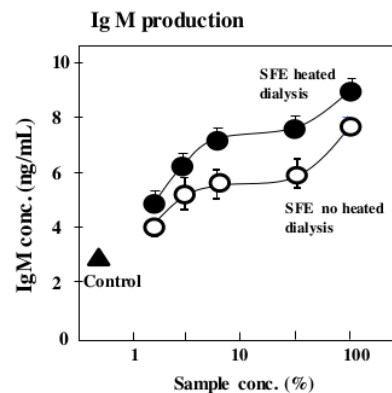
In this research we prepared two kind of SFE were obtained from different extraction methods. One SFE was prepared by heat treatment of the Sweet Potato fiber powder at 121 °C for 20 min in distilled water. The other SFE was prepared by stirring the powder at 25 °C for 2 h in distilled water and the stimulatory activity was evaluated. The IgM production-stimulatory activities of both SFE were compared to determine the suitable extraction method. The HB4C5 cells were inoculated in ITES-ERDF medium supplemented with various concentrations of either SFE and cultured for 6 h. Immunoglobulin production stimulatory effect of SFE was evaluated. As indicated in Figure 1, the IgM production by HB4C5 cells was enhanced by both SFE preparations in a dose-dependent manner. Sweet potato heat-extracts stimulated IgM production by HB4C5 cells in a dose-dependent manner. Interestingly, the sweet potato extracts prepared at room temperature slightly enhance IgM production.

These results suggest that the active substance in sweet potato tuber is insoluble in water. In addition, the active substance in the sweet potato tuber was solubilized by heat treatment. It is supposed from this result that heating process solubilized the active substance in the sweet potato tuber powder.



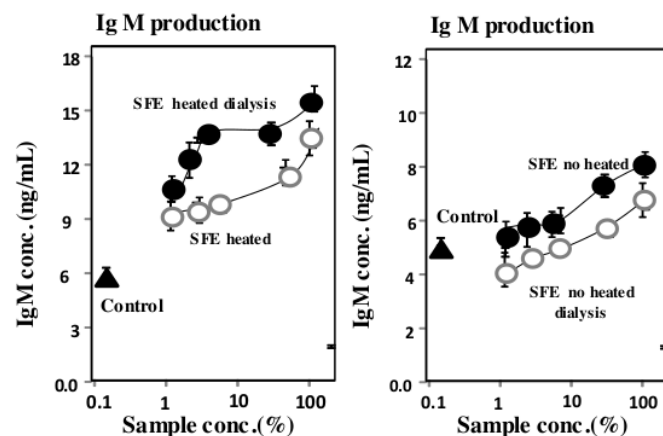
**Figure 1.** Supplementation effect of the sweet potato fibre extracts (SFE) on IgM production by HB4C5 cells. Sweet potato fibre powder was suspended in distilled water and extracted by heated at 121 °C for 20 min (black circle) and no heated at 25 oC for 2 h p (White circle). HB4C5 cells were inoculated in ITES-ERDF medium with various concentrations of each sweet potato fiber extracts and cultured for 6 h. distilled water as control (black triangle) was supplemented distilled water. Each result is represented as the mean  $\pm$  SD of three independent measurements

Then, both of the SFE were dialyzed against distilled water with a dialysis membrane that cuts off molecules under the molecular weight of 14 kDa. As shown in Figure 2 the specific activity of heat-extracted SFE was much higher than that of non-heated SFE, indicating that a heating process during extraction from the Sweet Potato fiber powder would effectively solubilize the active substance. This result also suggests that the active substance in SFE is heat-stable. Because SFE was dialyzed using a dialysis membrane with molecular weight cut off of 14 kDa, the active substance seems to be macromolecules. It is supposed from these findings that the active substance in SFE may be a complex of carbohydrates and proteins.



**Figure 2.** Supplementation effect of the sweet potato fibre extracts (SFE) on IgM production by HB4C5 cells. Sweet potato fiber powder was suspended in distilled water and extracted by heated at 121 °C for 20 min with dialysis treatment (black circle) and unheated at 25 °C for 2 h with dialysis treatment (White circle). HB4C5 cells were inoculated in ITES-ERDF medium with various concentrations of each Sweet Potato fibre extracts and cultured for 6 h. distilled water as control (black triangle) was supplemented distillate water. Each result is represented as the mean  $\pm$  SD of three independent measurements

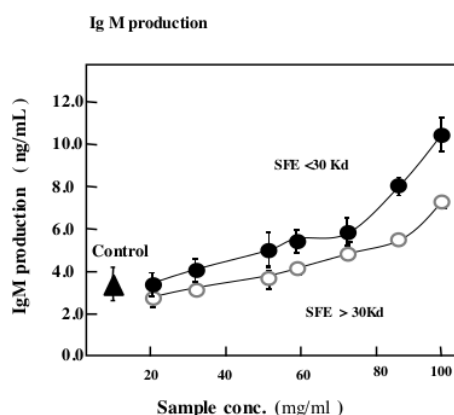
Effect of the Sweet potato extracts production on IgM production by HB4C5 cells with heat extraction and heat extraction dialysis. SFE with heat treatment extraction were dialyzed with using a dialysis membrane with molecular weight cut off of 14 kDa and compared with heat treatment but not dialyzed. In another experiment, SFE with room temperature treatments also compared between dialysis and no dialysis using a dialysis membrane with molecular weight cut off 14 kDa. The result showed, IgM production were enhanced 2.3 fold and 2 fold with both SFE prepared by heat treatment and dialysis and no dialysis compared with control (Figure 3A). Sweet potato extracts prepared at room temperature with dialysis and no dialysis did not enhance IgM production sharply (Figure 3B).



**Figure 3.** (A) Effect of the sweet potato fibre extracts (SFE) on IgM production by HB4C5 cells. Sweet potato fibre powder was suspended in distilled water and extracted by heated at 121 °C for 20 min with dialysis treatment (black circle) and without dialysis treatment (white circle). HB4C5 cells were inoculated in ITES-ERDF medium with various concentrations of each SFE and incubated for 6 h. Control (black triangle) was supplemented distilled water. (B) Effect of SFE on IgM production by HB4C5 cells. Sweet potato fibre powder was suspended in distilled water and extracted at 25 °C for 2 h without dialysis treatment (black circle) and extracted at 25 °C for 2 h with dialysis treatment (white circle). HB4C5 cells were inoculated in ITES-ERDF medium with various concentrations of each Sweet Potato fibre extracts and cultured for 6 h. distilled water as control (black triangle) was supplemented distilled water. Each result is represented as the mean  $\pm$  SD of three independent measurements.

Stimulating effect of SFE on IgM production by HB4C5 cells were analyzed. Sweet potato fiber powder was suspended in distilled water and extracted by heated at 121 °C for 20 min with filtration treatment using membrane with molecular weight cut off < 30 kDa and heated at 121 °C for 20 min with filtration treatment using membrane with molecular weight cut off > 30 kDa. The results showed that SFE were prepared with heating treatment at 121 °C for 20 min then filtrated using membrane

with molecular weight cut off > 30 kDa and < 30 kDa has stimulating activity on HB4C5 cells (Figure. 4). SFE with membrane with molecular weight cut off > 30 kDa has lower activity compared with molecular weight cut off < 30 kDa membrane. The result suggested that the dominantly active substance of SFE are substance with molecular weight less than 30 kDa.



**Figure 4.** Supplementation effect of the sweet potato fibre extracts (SFE) on IgM production by HB4C5 cells. Sweet potato fiber powder was suspended in distilled water and extracted by heated at 121 °C for 20 min with filtration treatment using membrane with molecular weight cut off < 30 kDa (black circle) and heated at 121 °C for 20 min with filtration treatment using membrane with molecular weight cut off > 30 kDa (White circle). HB4C5 cells were inoculated in ITES-ERDF medium with various concentrations of each Sweet Potato fiber extracts and cultured for 6 h. distilled water as control (black triangle) was supplemented distilled water. Each result is represented as the mean  $\pm$  SD of three independent measurements.

Because BFE was dialyzed using a dialysis membrane with molecular weight cut off of 14 kDa and under 30 kDa, the active substance seems to be macromolecules. As the previous results shown the active substance from bengkoang fiber extracts with heat treatment that has immunomodulatory effects is pectin [15]. Sweet potato also contains pectin, which has many important functions. Pectin has wide applications in both the pharmaceutical and food industries, where it acts as thickening and gelling agents, regulates the thickness and mouth-feel of fruit drink powder when the powder is dissolved in cold water [16]. It is reported that sweet potato has a good resource of pectin, contain about 15% pectin on dry matter basis [17]. In addition, pectin has proven to have beneficial effects on human health [18-20].

#### 4. Conclusions

SFE stimulated the Ig production by HB4C5 cells with heat and dialysis treatments. The active substance in SFE is heat-stable. Hence, it would be possible to consider that sweet potato fiber extracts has a potentially beneficial effect on human health and on prevention of the immune system-related diseases.

#### References

- [1] Lestari L A, Soesatyo M H N E, Iravati S and Harmayani E 2013 Characterization of Bestak sweet potato (*Ipomoea batatas*) variety from Indonesian origin as prebiotic *International Food Research Journal* vol 20(5) pp 2241-45
- [2] Takamine K, Abe J, Iwaya A, Maseda S and Hizukuri S 2000 A new manufacturing process for dietary fiber from sweet potato residue and its physical characteristics *Journal of Applied*

- Glycoscience* vol **47**(1) pp 67-72.
- [3] Ogutu F O and Mu Sonochem T H 2017 Ultrasonic degradation of sweet potato pectin and its antioxidant activity *Ultrason Sonochem* vol **38** pp 726-34.
- [4] Ogutu F O, Mu T H, H Sun and Zhang M 2018 Ultrasonic modified sweet potato pectin induces apoptosis like cell death in colon cancer (HT-29) cell line *Nutr Cancer* vol **70**(1) pp 136-45.
- [5] Miyazaki Y, Kusano S, Doi H and Aki O 2005 Effects on immune response of antidiabetic ingredients from white-skinned sweet potato (*Ipomoea batatas* L) *Nutrition* vol **21** pp 358-62
- [6] Cheng JM, Chiang W, Chiang LC 2007 Immunomodulatory activities of edible beans and related constituents from soybean *J Food Chem* vol **104** pp 613-18
- [7] Chen X, Nie W, Yu G, Li Y, Hu Y, Lu J and Jin L 2012 Antitumor and immunomodulatory activity of polysaccharides from *Sargassum fusiforme* *Food Chem Toxicol* vol **50** pp 695-700.
- [8] Nishi K, Muranaka A, Nishimoto S, Kadota A and T. Sugahara 2012 Immunostimulatory effect of b-cryptoxanthin in vitro and in vivo *J Func Food* vol **4** pp 618-25.
- [9] Morishige H, Sugahara T, Nishimoto S, Muranaka A, Ohno F, Shiraishi R and Doi M 2011 Immunostimulatory effects of collagen from jellyfish in vivo *Cytotechnology* vol **63** pp 481-92.
- [10] Daifuku M, Nishi K, Okamoto T, Nakano H, Nishimoto S, Sugahara T 2012 Immunostimulatory effects of water extract from *bulbus arteriosus* in tuna in vitro *J Func Food* vol **4** pp 263-70.
- [11] Kumalasari I D, Sugahara T, Nishi K, Harmayani E and Raharjo S 2012 Immunomodulatory activity of Bengkoang (*Pachyrhizus erosus*) fiber extract in vitro and in vivo *Cytotechnology Journal* vol **66**(1) 75-85.
- [12] Murakami H, Hashizume S, Ohashi H, Shinohara K, Yasumoto K, Nomoto K and Omura H 1985 Human-human hybridomas secreting antibodies specific to human lung carcinoma *In Vitro Cell Dev Biol* vol **21** pp 593-96.
- [13] Sugahara T, Onda H, Shinohara Y, Horii M, Akiyama K, Nakamoto K and Hara K 2005 Immunostimulation effects of proteose-peptone 3 fragment on human hybridomas and peripheral blood lymphocytes *Biochim Biophys Acta* vol **725** pp 233-40.
- [14] Murakami H, Masui H, Sato GH, Sueoka N, Chow TP and Kono-Sueoka T 1982 Growth of hybridoma cells in serum-free medium: Ethanolamine is an essential component *Proc Natl Acad Sci USA* vol **79** pp 1158-62.
- [15] Kumalasari I D, Sugahara T, Nishi K, Putra A B N 2014 Activation of macrophage stimulated by bengkoang fiber extract through toll-like receptor 4 *Food and Function Foods Journal RSC* vol **5**(7) pp 14-23.
- [16] Yokoi H, Hirose J, Hayashi S and Takasaki Y 2002 Flocculation properties of pectin in various suspensions *Bioresource Technology* vol **84** (3) pp 287-90.
- [17] Mei X, Mu T H and Han J J 2010 Composition and physicochemical properties of dietary fiber extracted from residues of 10 varieties of sweet potato by a sieving method *Journal of Agricultural and Food Chemistry* vol **58**(12) pp 7305-10.
- [18] Yamaguchi F, Shimizu N and Hatanaka C 1994 Preparation and physiological effect of low-molecular weight pectin *Bioscience, Biotechnology and Biochemistry* vol **58** pp 679-82.
- [19] Tian Cand Wang G 2008 Study on the anti-tumor effect of polysaccharides from sweet potato *Journal of Biotechnology* 136S S351.
- [20] Ingjerdigen M, Ingjerdigen K T, Patel T R, Alen S, Chen XY and Rolstad B 2008 Pectic polysaccharides from *Biophytum petersianum* Klotzsch, and their activation of macrophages dendritic cells *Glycobiology* vol **18** pp 1074-84.



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