The Extraction of Non-purified Insoluble Fiber (NIF) From Yam Bean and Its Application in Salt Stick.

By

Ms. Pornpan Into

A Special Problem Submitted to the Faculty of Biotechnology, Assumption University, in Part Fulfillment of the Requirements for the Degree of Bachelor of Science in Biotechnology

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Department	: Agro-Industry
Faculty	: Biotechnology
Academic year	: 1999

Advisory committee

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(A. Wunwisa Krasaekoopt)

Abstract

Yam bean was selected in order to produce non-purified in soluble fiber because of its low prices (5 baht/kg). Yam bean powder was produced by using dry milling method with 15.8% yield and then extracted by using 2 steps. The first step was isolation of highly soluble carbohydrate complex (hot water extract). The second step was isolation of moderately soluble carbohydrate complex (cold water extract). The percent yield of fiber extraction was 34.5%. Yam bean NIF powder consisted of 6.0% moisture, 3.4% protein, 1.3% ash, 36.8% carbohydrate, and 51.2% fiber. It was applied as fiber supplementary in salt stick by substitution in portion of wheat flour (all-purpose flour). The fiber content of this product increased by 9.9 times (from 0.3 to 2.8%). The panelists moderately liked this product with the preference score as 7.5, color; 6.9, flavor; 6.8, hardness, and 6.9, overall acceptance.

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Introduction

The role of dietary fiber (DF) in human nutrition and the relation between DF and diseases have attracted considerable in recent years. Because the composition of natural source of DF, such as cereals, fruits, and vegetables, providing adequate amount of DF for human through out the day declined. There are many sources of DF can be used in Thailand. Yam bean, called Mun-kaeo, is an interesting edible root because of very low price. So this project was aimed to extract (crude) non-purified insoluble fiber from yam bean and its application in salt stick production.

Objectives

I. To study the production of yam bean powder by using dry milling method.

II. To study the extraction of yam bean non-purified insoluble fiber powder and its properties.

III. To study an application of yam bean non-purified insoluble fiber powder in salt stick.

Literature Review

Fiber is plant material resistant to enzymes of mammalian digestive tract (indigestible properties). Fiber also describes as a certain fraction of vegetable food fiber derived from many varieties of foods high in dietary fiber including whole grain product, fruit and vegetable. The clinicians, nutritionists, and food promoted for its contributions to a healthy diet used for prevention of heart disease and cancer, etc.

There are two types of fiber, water-insoluble and water-soluble. Insoluble components are found primarily in cellulose, hemicellulose, lignin, cutin and waxes. Soluble fibers are basically gums, Beta-glucans, and pectin. No dietary fiber is derived from animal sources except for chitin. Base on infra-plant functions, fibers are divided into 3 major fractions as:

1. Structural polysaccharides are associated with the cell wall and include the noncellulose polysaccharides (hemicellulose and some pectin) and cellulose.
2. Structural nonpolysaccharides include predominantly lignin.
3. Nonstructural polysaccharides include the gums and mucilage, secreted by the cell and polysaccharides, such as carrageenan and agar from algae and seaweed (Southgate, 1982, 1976)

CHEMICAL COMPOSITION

The chemical components found in various fibers. The simplest is the polysaccharide cellulose – a glucose polymer without branching. The noncellulose polysaccharides are based on a variety of carbohydrates, and can contain a high degree of branching. Lignin is a highly complex nonpolysaccharide polymer, witch contains phenylpropane units derived from sinapyl, coniferyl, cinnamyl, and p-coumaryl alcohols.

Cellulose, a linear polymer of glucose with beta 1-4 links, is the main structural component of plant cell walls, it is considered relatively as insoluble fiber.

Pectin is composed primarily of D-galacturonic acid, although it can have other carbohydrates linked to it. Partial methylation of the carboxyl groups on the galacturonic acid imparts important properties to pectic substances. Pectins are found as part of the cell wall and act as intercellular cementing substances.

Hemicellulose is a heterogenous group containing a number of sugars in its backbone and side chains. It dissolves in dilute alkaline. Hemicellulose exhibits a wide range of solubility, with a greater solubility being associated with a high degree of branching. The chemical structures of polysaccharide dietary fiber were shown in Figure 1.

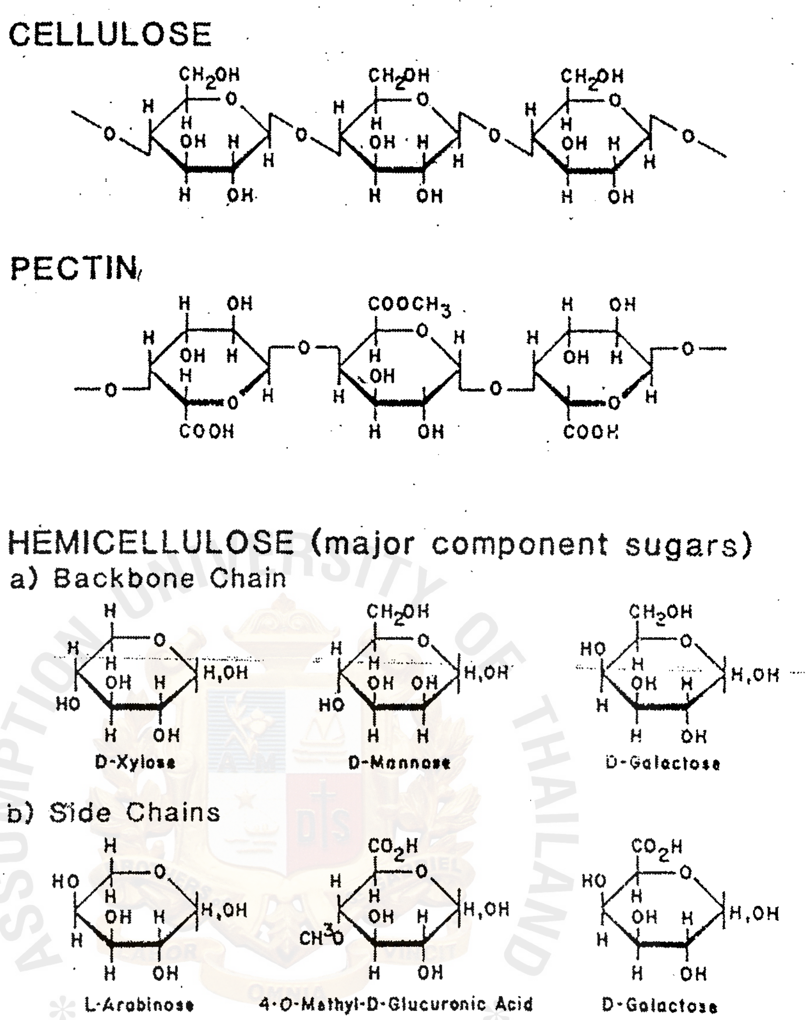

Figure 1: Chemical structure of some carbohydrate complex

EXTRACTION OF FIBER

Extraction methods may be used to isolate various fractions, which are then gravimetrically quantified. For merely the Association of Official Analytical Chemists (AOAC) method, crude fiber analysis is based on extraction which acid and alkali. This procedure does not accurately estimate the fiber content, nor can it be quantitatively related to the fiber content of food. Neutral and acid detergents are the bases of another extraction method, the Neutral Detergent Fiber procedure (NDF) developed by Van Soest (1963) for animal feed, this method was modified for analysis of human foods (1967) which are high in fat, protein, and starch. It is useful for estimating the content of insoluble structural polysaccharides and lignin. A modified NDF is an official method of the American Association of Cereal Chemists.

Procedural efficiency of starch removing from test samples is very important interfere the analysis. The crude fiber method achieves estimation of cellulose and lignin contents in foods, although crude fiber represented the sum of lignin, cellulose, and hemicellulose.

PHYSICAL PROPERTIES

These following properties are responsible for elicitation specific fiber functions. (Kay, 1982)

1. Bacterial Degradation

Bacterial degradation, which is reverting to only the polysaccharide fraction, is the first of these properties. DF cannot be enzymatically degraded to varying degrees of degradation within the large bowel, the degree of degradation varies considerably among the polysaccharides e.g. pectins, mucilages, and gums appear to be completely, whereas cellulose is only partially broken down. The extent of bacterial degradation has several important implications:

1. The short-chain fatty acid by-products may influence the physiological response to fiber. (Pomare et al., 1985)

2. The fermentation process can lower the pH of the large bowel and may affect microbial metabolism.

3. Bacterial cells can account for a significant portion of fecal weight and thus contribute to fecal bulk.

2. Water-Holding Capacity (WHC)

This property is significantly enhanced of sugar residue with free polar groups; the pectins, mucilages, and hemicelulose have the greatest WHC. Hydrations of fiber results in formation a gel metrics, higher viscosity of the small intestinal content and have critical effects on nutrient absorption into the gel metrics and by increasing in viscosity of the intestinal content. Although WHC has also been related to increased fecal bulk. The relationship is not straightforward. The relationship is not straightforward because of the bacterial degradation of fiber within the colon.

3. Absorption of Organic Molecules

In clouding bile, cholesterol, and toxic compounds e.g., lignin is potent bile acid absorbent. Pectin and other acidic polysaccharide also seem to sequester bile acid, in contrast, cellulose has little bile-acid binding ability. Bile- acid absorption is measured as the ability to increase fecal bile acid and steroid excretion. It has been correlated to the plasma cholesterol lowing effect of certain soluble noncellulose polysaccharides, such as pectin and guar gum. Although the ability of some fibers to bind toxic compounds has been extensively studied, it has been proposed as a protective mechanism of fiber against gastrointestinal cancers.

4. Cation Exchange

The residual mineral availability and electrolyte absorption, associations with some diets high in fiber are undoubtedly due to the binding of minerals and electrolytes on fiber source. The number of free carboxyl groups on the sugar residues and uronics acid content of polysaccharides appear to be related to the cation exchange properties of fibers.

THE IMPORTANCE OF DIETARY FIBERS

The importance of dietary fiber (DF) has more appreciated as its physiological effects which can be divided into two groups (Bijlani, 1985). DF directly affects bowel function from ingestion through defecation. High fiber diets tend to be a natural appetite suppressant, which could lead to weight loss. It also indirectly modifies postprandial glycemia and lipid metabolism.

DF trends to slow down the rate of ingestion. Soluble fiber has more effective in reducing hunger urges than insoluble fiber.

DF consumption influences the digestion and absorption of nutrients in small bowel. DF exerts influence on secretory and brush-border enzymes.

The consumption of DF has the potential to reduce the reabsorption of certain bile acids.

DF is a major component of the material delivered to the large bowel and accelerates bowel transit and increases fecal bulk. Fiber may act as partial barrier to diffusion of nutrients from the lumen to gut muscosa. This slow absorption results in a more prolonged blood glucose response, and aids in maintaining a more glucose level (Asp et al., 1981)

The high-carbohydrate, high-fiber diet, with its proportionately reduced fat levels, in term of caloric intake, results in improved glucose metabolism, because of the reduction in free fatty acids in the blood. (Hales and Randle, 1963). High levels of serum free fatty acids impair insulin efficiency (Anderson and Sieling, 1981)

Consumption of high-fiber diet over a considerable period of time leads to an increase in the number of insulin receptors on target tissues and an increase in glucose metabolism enzyme activity (Anderson and Sieling, 1981; Asp et al., 1981).

The other adverse effects of a high-fiber diet include abdominal fullness, flatulence, reduce gastric emptying and in some cases diarrhea, a gradual increase in DF may help to minimize such symptom.

DIETARY FIBER LINKS TO DISEASE

Dietary fiber and weight control

There are several mechanisms for DF's action have been proposed:

- A reduction in the caloric density of the diet or a decreased efficiency of caloric absorption.
- Increased chewing of food slows the rate of intake.
- Increase satiety associated with the extra volumes of the bowel content.
- Changes in blood glucose and hormones.

Dietary fiber and bowel disorders

One of the most consistent effects of DF is to increase fecal weight and water content, thereby making the stools softer and bulkier. Conversely, a lack of DF appears to be one of coarse of a wide variety of bowel disorders.

Dietary fiber and cancer of the large bowel

The government agencies in both the United States and Japan have made dietary recommendations for possible cancer prevention: The diet should include dietary fiber-rich foods, a variety of fruits and vegetables, legumes, and seeds.

Dietary fiber and Gallstones

Gallstone disease (cholelithiasis), the problem is that the bile contains more cholesterol than it can solubilize with the detergent system of bile. Many people with supersaturated bile still main free of gallstones. And it appears that patients main free of gallstones experience nucleating factors which trigger cholesterol crystallization (Burnstein et al., 1983) which are absent in nongallstone formers (Kesaniemi and Grundy, 1983). A dietary fiber-rich diet appears to protect against gallstone formation.

YAM BEAN (MUN-KAEO)

The yam bean, scientifically classified as Pachyrhizus erosus (L.) and P. tuberousus (Lam.), is also called Jicama, Mexican yam bean, Ahipa, Mexican water chestnut, Di gwa (Chinease), Sinkamas (Filipino), Kusuiemo (Japanese), Seng kuang (Malay), Cu^da^u (Vietnamese).

The yam bean is a member of the bean family, *Liguminosae*; subfamily: *Papilionodieae.* The bean stems come from exotic origins: P. erosus originated in Mexico, while P. tuberosus, a larger root species believed to be selected for larger root size, has its origins in the Amazon Basin.

The yam bean is a short-day plant, and is a high-protein, low fat dietary staple in many areas. The nutrient contents per pound of the yam bean: 46 calories, 88% moisture content, 1.6% protein content, 0.2% fat content, 10%carbohydrate, and 1.3% fiber content.

The yam bean is grown primarily for its root, which is quite crisp when it is peeled and sliced. It can be eaten raw, garnished with chili pepper and lemon juice, salted, with dips, or stir-fried as a replacement for water chestnuts. In addition, it is also used as a source of starch because the yam bean is a legume, it fixes nitrogen and produces pods and seeds. Although the immature pods are eaten by some, any existing hairs are removed by rubbing in sand, it is suggested that the pods can not be eaten. Mature pods and seeds contain ROTENONE, a potent poison.

The seeds should be started indoors, in an area with plenty of sun and warmth. They may be transplanted after the danger of frost has passed, in a 12" by 24" sunny, warm spot with loose, well-drained soil. Because the yam bean is a short-day plant, it may not flower should be removed since it will reduce the root size. The optimal root size is four to six inches; large root, shrinkage may be "woody". Due to high moisture content within the root, shrinkage may occur in open, dry storage. The roots may be safety stored in the refrigerator, or left in the ground with the tops of the beans cut off, until it is used. If employing the latter method, the roots must be removed before the first frost, because ground frosts would probably injure the roots and will kill the tops. One rai of land needs 8 kilograms of seed and will yield about 2-6 tons of roots with worthiness from 50-80 bath per 10 kilograms at market sales.

Material and Method

Material

1. Yam bean (Mun-Kaeo)
   Pachyrhizus erosus (L) *urban.*

Equipment

1. Balance
2. Knife
3. Chopping board
4. Slicer
5. Blender
6. Aluminium tray
7. Hot air oven, Memmert Model 600
8. Sieve (60, 100 mesh)
9. Plastic bag
10. Water bath
11. Shaker
12. Glass equipment
13. Wash bottle

Chemical reagent

1. water
2. 0.01g EDTA

Method

A. To study the production of yam bean powder by using dry milling method

1. Weigh yam bean corms.
2. Peel, trim, and wash yam bean corms.
3. Cut yam bean corm into small pieces and slice.
4. Dry the sliced yam bean in oven at 50-60°c.
5. Grind and sieve. (60 mesh)
6. Keeping in plastic bag.

B. To study the extraction of yam bean non-purified insoluble fiber procedure

1. To study the isolation of highly and moderately soluble complex carbohydrate.
   - 1.1 Wash the yam bean powder with water (pH 7.0-7.5) by using the ratio of powder to water as 1:10.
   - 1.2 Shake vigorously for 6 hours at room temperature.
   - 1.3 Filter and Wash with 20ml of 90°C water, which containing 0.01g of EDTA.
   - 1.4 Shake in 80°C water bath for 30 minute.
   - 1.5 Filter and repeat 3.1, 3.2, and 3.3 two times. Filter the residual and dry in the oven at 50-60°C.
   - 1.6 Grind and sieve. (100 mesh)

2. To study some properties of yam bean non-purified insoluble fiber powder (NIF)

    2.1 Chemical analysis

        2.1.1 Moisture content (A.O.A.C., 1994)

        2.1.2 Ash content (A.O.A.C., 1994)

        2.1.3 Protein content (A.O.A.C., 1994)

        2.1.4 Reducing sugar content (A.O.A.C., 1994)

        2.1.5 Crude fiber content (A.O.A.C., 1994)

C. To study the application of yam bean NIF powder in salt stick

1. Production of yam bean NIF salt stick

    1.1 Produce salt stick by using the formulation in Table 1.

    1.2 Replace all-purpose flour with yam bean NIF powder as 5 and 10 % (treatment 1 and 2, respectively).

    1.3 Add yam bean NIF powder directly in all-purpose flour 5 and 10% (treatment 3 and 4, respectively).

Table 1: The formations of salt stick produced by using yam bean NIF powder

Ingredients (%)	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Sugar	3.0	3.0	3.0	3.0	3.0
Salt	0.9	0.9	0.9	0.9	0.9
Milk powder	1.26	1.26	1.26	1.26	1.26
Fat	13.5	13.5	13.5	13.5	13.5
Active dry yeast	1.5	1.5	1.5	1.5	1.5
Water	58	58	58	58	58
All purpose flour	75	70	65	75	75
Cake flour	25	25	25	25	25
Yam bean NIF powder	0	5	10	5	10

Procedure

1. Dissolve sugar and salt in water.
2. Add the sugar and salt solution in the mixture of all purpose flour, cake flour, yam bean NIF powder, milk powder, and active dry yeast. Knead slightly until develop to a smooth dough.
3. Place the dough in a warm-moist place for 30-45 minute.

4. Punch and roll out the dough with a rolling pin about 0.2-0.3 cm thickness.
5. Allow the rolled out dough to relax for 3-5 minute
6. Cut into small pieces about 10 grams each and make up a rod shape.
7. Place the sticks on slightly greased pan.
8. Brush the top of sticks with salted fat solution.
9. Bake at 425°- 450°F until light brown.

1. Sensory evaluation

The 9 point hedonic scale is used to evaluate yam bean NIF salted stick by 15 panelists.

Result and Discussion

A. The extraction of yam bean fiber powder.

1. The production of yam bean N I F powder by using dry milling method.

The tuber part or corm of yam bean was used for the production of yam bean powder. The result was shown in Table 2. During preparation step, the peeling loss of yam bean was 13.5%. The percentage of yield was 15.8%. Some properties of fresh yam bean and yam bean powder were shown in Table 3. Yam bean powder contained 6.7% moisture, 18.4% protein, 36.9% fiber, 8.6% ash, and 31.9% carbohydrate content.

Table 2: Peeling loss and yield yam bean powder of production.

	%
Peeling loss	13.5
Yield	15.8

Table 3: Some chemical properties of fresh yam bean and yam bean Powder.

Composition	Fresh yam bean (%)	Yam bean powder (%)
Moisture content	82.4	6.7
Protein content	1.5	18.4
Fiber content	3.0	36.9
Ash content	1.6	8.6
Carbohydrate content	11.5	31.9

2. <u>The extraction of Non-purified Insoluble Fiber (N I F) from yam bean powder.</u>

The extraction of yam bean powder was composed of 2 steps. The first step was the isolation of highly soluble carbohydrate complexes and the second step was the isolation of moderately soluble carbohydrate complexes. The results were shown in Table 4. It was noticed that the yield of fiber extraction was 34.5%. The yam bean NIF powder contained 6.0% moisture content, 3.4% protein content, 51.2% fiber content, 1.3% ash content, and 36.5% carbohydrate content. The fiber content of yam bean NIF powder was dramatically higher than yam bean powder by 14.3%

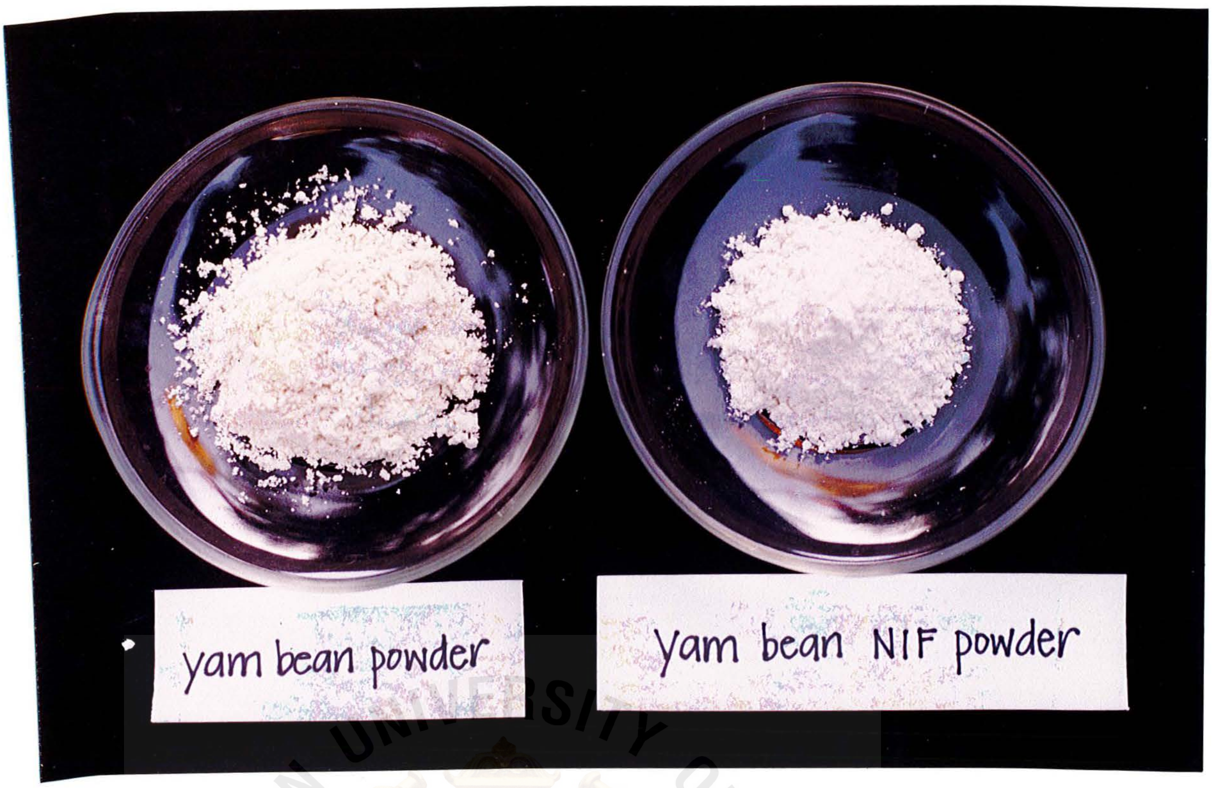

Figure 2: Yam bean non-purified insoluble fiber (NIF) powder and yam bean powder produced by dry milling method.

Table 4: Some chemical properties and yield of yam bean NIF powder.

	%
Yield of extraction	34.5
Moisture content	6.0
Protein content	3.4
Fiber content	51.2
Ash content	1.3
Carbohydrate content	36.8

Because of low price of raw material (5 baht/kg), the price of yam bean non-insoluble fiber was 91.7 baht/kg.

B. The application of yam bean N I F powder in salt stick production

The yam bean N I F powder was used as one ingredient of salt stick production in order to increase fiber content of product. There were 2 methods of using yam bean NIF powder, substitution in the portion of wheat flour and addition in the formulation, at 5% and 10%. After that the salt sticks of each formulation were compared with the original recipe (control) by 15 panelists. The sensory evaluation was shown in Table 5.

Table 5 : Sensory evaluation of salt stick which produced by using yam bean N I F powder as fiber supplementary

Treatment	Color	Flavor	Hardness	Overall acceptance
1. Control	7.8^{a*}	6.5	7.0^{a}	6.8^{ab}
2. Substituted by 5% NIF	7.5^{a}	6.9	5.7^{ab}	6.9^{ab}
3. Substituted by 10% NIF	7.2^{a}	6.3	6.2^{ab}	6.4^{b}
4. Add with 5% NIF	7.1^{ab}	6.4	6.5^{bc}	7.1^{a}
5. Add with 10% NIF	6.6^{b}	6.3	5.7^{c}	5.7^{c}

*The same letter means no significant difference at 95% level confidence.

From the Table 5, it was noticed that there was no significance difference only in flavor and there were significance differences in color, hardness, and overall acceptance of salt stick products. It might cause that the flavor of salt stick was influenced directly by margarine in the formulation.

The color of salt stick from five treatments had significant differences. The preference score of treatment 1, 2, and 3 were 7.8, 7.5, and 7.2, respectively. These were slightly higher than that of treatment 4 (7.1). The color score of treatment 5, added with 10% of N I F, was the lowest (6.6). It might cause that the difference in color of 5 treatments directly resulted from baking process rather than the effect of yam bean NIF powder. Moreover, during the dough making step the color of dough of 5 treatments was the same.

For hardness, control gave the highest (7.0). It was slightly higher than treatment 2 and 4, which were no significant differences. Treatment 5 was the lowest (5.7). It was slightly lower than that of treatment 2 (5.7). Between substitution and addition of yam bean NIF powder in salt stick products, the panelists preferred the substitution product because the addition of yam bean NIF powder directly to the formulation increased solid content, affecting on the hardness of product at the same amount of water.

For the overall acceptance, it was observed that there were no significant differences between treatment 1 and 2, 6.8 and 6.9, respectively. While treatment 4 provided the highest score (7.1) and treatment 5 provided the lowest score (5.7), that was slightly lower than that of control (6.4).

To sum up, the treatment 2, which wheat flour was substituted by 5% yam bean NIF powder, was the best formula because its preference score of all attributes did not significantly differ from that of control. These were 7.5 color, 6.9 flavor, 6.8 hardness, and 6.9 overall acceptance. It was showed that the panelists moderately liked this product.

Table 6: The comparison of fiber content in salt stick of five treatments.

Treatment	Fiber content (%)
1. Control	0.3
2. Substituted by 5% N I F	2.8
3. Substituted by 10% N I F	5.4
4. Add with 5% N I F	3.1
5. Add with 10% N I F	5.1

The fiber content of control was 0.3% which came from only wheat flour in the recipe. When some of wheat flour was replaced by yam bean NIF powder or yam bean NIF powder was directly added into the formula, fiber content of finished products increased. Addition of fiber powder into the original recipe, fiber content of the products were slightly higher than that of substitution of wheat flour with fiber content in the formula, at the same level of fiber powder used.

Treatment 2, the panelists liked most, contained 2.8% fiber content. It was 9.9 times higher than control.

Conclusion

I. The percent yield of yam bean powder production was 15.8%. This powder contained 6.7% moisture, 18.4% protein, 36.9% fiber, 8.6% ash, and 31.9% carbohydrate.

II. For fiber extraction, the percentage of yield was 34.5%. This yam ban NIF powder contained 6.0% moisture, 3.4% protein, 1.3% ash, 36.8% carbohydrate, and 51.2% fiber. The price of yam bean NIF fiber was 91.7 baht/kg.

III. For the application of yam bean non-purified insoluble fiber in salt stick product, the suitable level of yam bean NIF powder in the formulation was 5%, substitutes in the portion of wheat flour. The panelists moderately liked this product and the score of preference in each attribute were 7.5, color; 6.9, flavor; 6.8, hardness; and 6.9, overall acceptance.

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Appendix A

Chemical Analysis

1. Moisture Content Analysis (A. O. A. C., 1990)

1.1 Apparatus

- Moisture can
- Hot air oven (Memmert Model 600)
- Desiccator

1.2 Method

1. Place moisture cans in a hot air oven for 20-30 min and cool down in a desiccator before weighing and recording the weight.
2. Weigh samples approximately 5.00 g (4 digits) in aluminum cans that we absolutely know the weight of each can.
3. Dry in hot air oven at 100 ^{O}C for 3 hour or until get constant weight.
4. Cool down in desiccator.
5. Weight and record the result.
6. Calculate % Moisture Content.

1.3 Calculation

$$\% \text{ Moisture content} = \frac{\text{Weigh loss} * 100}{\text{Weigh of sample}}$$

2. Protein analysis (A. O. A. C., 1990)

2.1 Chemical Reagents

- Catalyst mixture of $CuSO_4.5H_2O$ and Na_2SO_4
- Conc. Sulfuric acid
- Sodium hydroxide
- 4% Boric acid
- Bromocrysol green and methyl red indicator
- Std. H_2SO_4 0.0046M solution

2.2 Method

1. Weight sample 0.7-2.2 g (4digits) and put into Kjeldahl digestion flask.
2. Add 8 g of catalyst mixture and concentrate hydrochoric acid 80 ml.
3. Digest the mixture by slowly bringing to boil until no bubbles appear (about 2 hr.)
4. Remove from the digestion apparatus and cool down to room temperature.
5. Slowly add 400 ml. Of H_2O and some glass beads.
6. Slowly add 75 ml. Of 50% NaOH solution and instantly connect the flask to the distillation apparatus, which has immense one end beneath 4% boric acid solution containing few drops of indicator mixture.
7. Slowly boil the content to release ammonia gas into the acid solution.
8. Titrate the distillate solution with std. H_2SO_4 0.0046 M. and determine % of total nitrogen in the sample.

2.3 Calculation

$$\% \text{ Total N} = \frac{\text{Weight of N} * 100}{\text{Dry weight of sample}}$$

% Protein = % Total N * conversion factor of starch

Conversion factor = 5.7

3. Crude fiber analysis (A. O. A. C., 1990)

3.1 Chemical Reagent

- Light petroleum ether
- H_2SO_4
- NaOH
- HCl
- Ethyl alcohol
- Diethyl ether

3.2 Method

1. Weigh 3.0 grams of sample to 4 digit-number.
2. Extract fat with petroleum ether 3 times by stirring the sample.
3. Air dry in the sample
4. Add 200 ml. Of 0.255 N. H_2SO_4 solution and a few glasses bead and boil for 1 min.
5. Cover the flask watch glass.
6. Gently for 30 min. (maintain volume by adding distilled water)

7. Filter through Bucher funnel containing #54 filter paper that has already been poured with boiling water with applied suction (Filtration should be completed with in 10 min.)
8. Wash with distilled water until the filtrate free of acid.
9. Transfer the residue into the same flask by mean of a washing bottle containing 200 ml. Of 0.313 N. NaOH.
10. Repeat refluxing with alkali solution the same as with acid. Filter by mean of suction.
11. Wash the residue with water until the filtrate is free from alkali.
12. Transfer the residue back to the same flask.
13. Repeat the reflux with 200 ml. Of 1% HCl and filter.
14. Wash the residue with water.
15. Wash it twice with 95% ethyl alcohol.
16. Wash it 3 times with diethyl ether.
17. Transfer the residue to the unknown weight dried ashless filter paper.
18. Dry at 100 ^{O}C to a constant weight (A)
19. Incinerate at 500-550 ^{O}C for 3 hr.
20. Weigh the ash (B)
21. Calculate % crude fiber.

3.3 Calculation

Weight of crude fiber = A-B

$$\% \text{ Crude fiber} = \frac{(A-B) * 100}{\text{Weight of sample}}$$

4. Ash Analysis (A. O. A. C., 1990)

4.1 Method

1. Place clean marked crucibles in a muffle furnace at 550-600 ^{O}C for 1-hr. Cooled crucibles to room temperature in desiccator.
2. Weigh them as quickly as possible to prevent moisture absorption.
3. Weigh 2 g. (4 digits) of dry sample into the prepared, preweight crucible.
4. Burn the sample with bunsen until free from smoke.
5. Place the crucibles in muffle furnace set at 550 ^{O}C. Burn off the samples until they become completely free from carbons to be a light gray or white ash.
6. Transfer the ash sample crucible to a desicccator and cool to room temperature. When cooled weigh the crucible as quickly as possible to prevent moisture absorption

4.2 Calculation

$$\% \text{ Ash} = \frac{\text{weigh of residue} * 100}{\text{Weigh of sample}}$$

Appendix B

Statistical Analysis

Appendix Table 1: Questionnaire for 9 points hedonic scale.

Name……………………………………….. Date………………….

Sample: Salt stick

Please test these samples. Rinse your month every time before taking different samples. Please rate the samples according to your preference by using the following aspects.

1 = Extremely dislike 6 = Slightly like

2 = Very dislike 7 = Moderately like

3 = Dislike 8 = Very like

4 = Slightly dislike 9 = Extremely like

5 = Neither dislike nor like

Sample code	color	flavor	Hardness	Overall acceptance
542				
934				
435				
281				
683				

Comments ………………………………………………………………..

Appendix Table 2: Statistical analysis of hardness of salt stick supplemented with yam bean N I F powder.

SOV	Df	SS	MS	Fcal	Ftab
SST	4	15.40	3.85	5.52	2.54
SSB	14	20.06	1.43		
SSE	56	39.04	0.70		
Total	74	74.50			

Appendix Table 3: Statistical analysis of color of salt stick supplemented with yam bean N I F powder.

SOV	Df	SS	MS	Fcal	Ftab
SST	4	12.68	3.17	3.59	2.54
SSB	14	10.41	0.74		
SSE	56	49.50	0.88		
Total	74	72.60			

Appendix Table 4: Statistical analysis of flavor of salt stick supplemented with yam bean N I F powder.

SOV	Df	SS	MS	Fcal	Ftab
SST	4	3.60	0.90	1.19	2.54
SSB	14	8.35	0.60		
SSE	56	42.29	0.76		
Total	74	54.24			

Appendix Table 5: Statistical analysis of overall acceptance of salt stick which supplemented with yam bean N I F powder.

SOV	Df	SS	MS	Fcal	Ftab
SST	4	18.86	4.71	6.14	2.54
SSB	14	12.69	0.91		
SSE	56	42.98	0.77		
Total	74	74.53			