

## PHYSICOCHEMICAL PROPERTIES OF MODIFIED CASSAVA STARCH PREPARED BY APPLICATION OF MIXED MICROBIAL STARTER

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### ABSTRACT

*Modified cassava starch (MCS) is a product derived from cassava chips that uses a principle of modifying cassava chips in fermentation, which produces distinctive characteristics, so it can be used as a food ingredient with a very wide scale. Preliminary experimental results showed that MCS could be used as raw materials from a variety of foods, ranging from noodles, bakery, cookies and semi-moist food, since the application has a spectrum similar to wheat flour, rice and other starchy materials. Advantages of MCS has aroma and flavor better than regular cassava flour, white has more color than usual cassava flour, and has relatively low prices compared to wheat or rice flour. The purpose of this study was to examine the influence starter solution on physicochemical properties and rheology of MCS, those were swelling power, solubility and product texture. Cassava chips soaked in an enzymatic starter solution for 24-72 hours at a concentration of 2.0% (v/v). Cassava chips were dried, then ground and analyzed for physicochemical properties and their rheologies. The results showed that, soaking in 2.0% starter solution for 72 hours, resulting in swelling power and solubility at highest value, respectively 12.00 (w/w) and 10.5%. For comparison, the value of swelling power and solubility of wheat flour, respectively 10.0 (w/w) and 9.6%. However, a native cassava starch, only produced swelling power and its solubility level, respectively 7.5 (w/w) and 8.5%. MCS has been no longer developed and its applications in food industry might have a significant prospect in the future. Review on journal's papers of current decade has been done so as to observe the latest applications of MCS in the food industry. Hopefully this paper will assist anyone especially students who want to get information about the latest applications of MCS in the food industry. This paper will elaborate more about the definition of MCS by considering modification technique through fermentation and enzymatic treatment.*

**Keywords:** cassava, MCS, mixed culture, fermentation, physicochemical properties.

### 1. INTRODUCTION

Demand and domestic flour demand continues to increase every year. One effort to curb imports of wheat flour is developing a composite of local food maize, cassava and sweet potato flour is added locally so hopefully can potentially replace the role of imported wheat. The potential of cassava in some South East Asian countries is very large both in terms of the side as the main food sources of carbohydrates after rice and maize, as well as feed ingredients and raw materials industries. Judging from its contribution to GDP, cassava contributes the third largest food crop after rice and maize (Aptindo, 2010).

Cassava (*Manihot esculenta* Crantz or known also as *Manihot utilissima*) is a dependable food crop in Indonesia and its production and use ranks third after rice and wheat. Different traditional methods including extraction of

starch and fermentation have been used in addition to decorticating and milling to process cassava for the purpose of providing diverse materials. However, cassava has major drawbacks of poor starch and protein digestibilities that undermine its nutritional value. Thus cassava has been underutilized compared to wheat or rice. Therefore, a study was undertaken to determine if fermentation or enzymatic treatment can affect the digestion of starch in cassava flour (Rao and Hahn, 1984; Oyewole and Odunfa, 1988).

For those characteristics, which are unattainable with native starch, modified starch can be used for other industrial applications through a series of techniques, chemical, physical, and enzymatic modification. Thus, modified starch is native starch that has been changed in its physicochemical properties. Modifying starch is important to provide the following properties:

thickening, gelatinization, adhesiveness and/or film-formation, to improve water retention, enhance palatability and sheen and to remove or add opacity. The reasons why starch is modified are to modify cooking characteristics, reducing retrogradation, reducing paste's tendency to gelatinize, increasing paste's stability when cooled or frozen, increasing transparency of pastes and gels, improving texture of pastes and gels, improving adhesiveness between different surfaces (Oboh and Akindahunsi, 2003; 2005).

Preparation of flour is one of the traditional ways of preserving and adding value to cassava roots that is practised widely. However, cassava flour prepared using traditional methods is frequently of poor quality, thus making it unsuitable as a substitute for wheat flour. Cassava flour containing cyanide which is fortunately taken away in the process of soaking, fermenting and cooking. Though, cassava flour is gluten-free, however, it has no protein and is full of starch. Cassava root is normally processed before consumption as a means of detoxification, preservation and modification of fermented cassava products including modified cassava flour (Akingbala *et al*, 1991).

Modified cassava starch (MCS) is a product derived from cassava flour using the principles of modification of cassava cells by fermentation, in which the role of microbial enzymes dominating during the fermentation takes place. Technically, a very simple way MCS processing. It is similar to ordinary cassava flour processing, but accompanied by fermentation and then dried and milled into the MCS.

Microbial inoculants applied for fermentation produce cellulolytic enzymes that breaking down the cell walls of starches in such manner, resulting in the release of starch granules. The microbial strains also produce enzymes those hydrolyze starch into monosaccharides and then convert them into mainly lactic acid. The starch granule release process will lead to changes in the characteristics of the flour produced in the form of increased viscosity, gelation ability, power rehydration, and dissolves easily. The organic acids will be mixed with flour, so as when the flour is processed, it will produce aroma and distinctive taste that can cover the aroma and flavor of native cassava, and so the flavor of MCS be neutral that is likely preferred by consumers (Subagio, 2007; 2008).

One of the constraints in commercialization of fermented cassava products is that the quality of the products which vary from one processor to

the other and even from one processing batch to the other (Oyewole and Sanni, 1995). Factors which have been found to be responsible for this included differences in methods of processing from one processor to the other (Akingbala *et al*, 1991); variations in the temperature of fermentation as influenced by the season (Blansherd *et al*, 1994), the age and variety of cassava root used by different processors (Almazan, 1992; Idowu and Akindele, 1994).

Modified starch means hydroxyl groups of the starch has been transformed through a chemical reaction or by disrupting the native structure. Starch treated with certain properties for the purpose of producing better and to improve the properties beforehand, or to change some of the previous properties or other properties. This treatment may include the use of heat, acid, alkali, oxidizing agents or other chemicals included enzymes that will produce new chemical groups or changes in shape, size and structure of the starch molecules (An, 2005; Dzedzic and Kearsley, 1995; Greenwood *et al*, 1979; Koswara, 2006).

Modified starches exhibit different properties, such as viscosity reduction, decrease in iodine binding capacity, and reduction of granule swelling during gelatinization, intrinsic viscosity reduction, increased solubility in hot water under the gelatinization temperature, lowering gelatinization temperature, osmotic pressure (molecular weight), increased viscosity ratio of hot to cold viscosity. But just as the native starch, the modified starch is not soluble in cold water either (Koswara, 2006; Eliasson, 2004). In this case, which is included physicochemical properties of starch such as amylose and amylopectin content, viscosity, gelatinization, and swelling power (Murillo *et al*, 2008). Based on previous research, the physicochemical and rheological properties of modified starch products, such as, swelling power, solubility, carbonyl group and carboxyl particular, are changing (BeMiller and Lafayette, 1997).

Vatanasuchart *et al* (2005) has modified starch using lactic acid solution and UV irradiated and Sangseethong (2009) have made modifications based on hypochlorite oxidation with varying reaction conditions and a reaction time. Atichokudomchai *et al* (2000) hydrolyzed starch with hydrochloric acid and lactic acid and modified starch with a combination of lactic acid hydrolysis and UV photochemical reaction (Atichokudomchai, 2000; Pudjihastuti, 2010). Sobowale *et al* (2007) have used a strain of *Lactobacillus Plantarum* for cassava

fermentation. However, the modification that has been done has a weakness, because the protein content of the modified flour is still too low, and does not yet have a viscosity and rheological properties which meet to the nature of wheat flour, so that the modified starch may not substitute yet for wheat flour up to 100% (Akindahunsi, 2005; Akindahunsi *et al*, 1999; Sobowale *et al*, 2007).

In addition, the high price of wheat flour, makes flour based food industry, looking for an alternative source of carbohydrate raw materials cheaper substitute for wheat. The results of trials on substitution of wheat flour with MCS, showed that to produce good quality noodle, MCS can be used up to 15%, whereas for low-quality noodles, MCS can be used up to 25% (Misgiyarta, 2009). Trial results showed, that the mofaf can be used as raw material, both for substitution nor entirely, as a raw material for various kinds of food products such as bakery, cookies, cake, white bread, vermicelli and noodle (Subagio, 2006).

Microbial starter for fermentation of cassava chips, derived from microbial strains, which is prepared with the technology to produce stability and high effectiveness as the starter which were extracted from the three kinds of microbial food grade strains. They play an important role during the fermentation takes place, resulting in aroma and flavor and a good texture to the product. These microbial strains have the ability to break down starch containing in cassava chips into simple sugars, as well as degrade proteins into amino acids and peptides. Microbial strains for fermentation of MCS, are safe and do not produce toxins, so they are often referred to as food grade microorganism, those play a role in increasing a value of acceptance on this product as well to preserve foodstuffs, by the way of producing anti-microbial compounds such as organic acids, hydrogen peroxide, diacetyl, bacteriocins, ethanol, and low redox potential.

This research will be focused on modification of starch via fermentation of cassava chips using three kinds of enzymatic starter that play role in hydrolysis of cassava starch into simpler sugars and synthesize some of them furthermore into glycosides that influence the changing nature of its physicochemical.

## 2. MATERIALS AND METHODS

### 2.1. Fermentation Procedure

To obtain a uniform size of cassava chips, cassava tubers were chopped or thinly sliced or

shredded using a chopper or a grater. Cassava chips (1.0 kg) fermented by soaking in 2.0 L of tap water at ratio of (1:2, v/v) mixed with 2.0% (w/v) of an enzymatic starter solution containing extracted crude enzymes of *Lactobacillus plantarum*, *Xanthomonas campestris* and *Saccharomyces cerevisiae* for 1-3 days. Fermentation mixture was stirred by hand and covered with aluminum foil and then incubated at 25°C for 24-72 h. Once fermentation is complete, the residual starter solution was drained and the cassava chips were transferred onto aluminum trays and dried under the sun or dried by oven at 45°C for 48-72h to reduce moisture content of cassava chips up to 12-14%. The dried fermented cassava chips were then milled to obtain a good quality of MCS.

### 2.2. Determination of Reducing Sugars

Samples were prepared by weighing about 0.5g of flour into centrifuge tubes, then 10 ml of water was added, vortexes 3 times, boiled for 15min and then centrifuged for 10 min. The reducing sugars in the extracted material of the fermented and native flours from cassava samples were determined by DNS colorimetric methods with glucose as the standard and the absorbance values were read at 540 nm.

### 2.3. Determination of Soluble Proteins and Free Amino Acids

The quantitative measurements of free amino acids of the supernatant material from above of the regular and fermented flours from both the food grade cassava samples were performed using the ninhydrin reaction. The amounts of soluble proteins in the supernatant material were measured using the Lowry method.

### 2.4. Measurement of mesh of flour

Fifty grams of flour are screened through the appropriate sieve according to grade. While more accurate results can be obtained by using mechanical shaker of special design or other type of mechanical shaker, satisfactory results can be obtained by hand shaking.

### 2.5. Measurement of dry appearance

A sufficient sample of flour is taken to make a rectangle approximately 2.55 cm (1 to 2 inch) on a side and 1.6-3.5 mm (1/16 to 1/8 inch) high. The flour is placed on a white paper pad, laid out to these measurements with a spatula and one side evened off. Adjoining this side, a similar pile is made with a standard flour. A clean smooth piece of paper is laid over both piles and pressed gently with the spatula to make a smooth

upper surface. The two piles are compared by eye in a neutral light (i.e., by daylight) free from shadows and direct glare.

## 2.6. Measurements of pH and acidity

The suspension material was prepared by mixing 5g of regular and fermented flours from both food grade cassava samples with 50mls of water and pH readings were measured with a glass electrode. The titratable acidity was determined by titration with 0.1N NaOH to an end-point of pH=8.2. The titratable acidity was then expressed as the volume of sodium hydroxide solution required to neutralize 1 g of flour.

## 2.7. Determination of ash content

Approximately 5 g of flour are weighed into the ashing dish, which has previously been ignited, cooled and weighed. A sample is inserted in the furnace at about 500°C until a light gray ash results, or to constant weight. The sample is then cooled in a desiccator and weighed.

## 2.8. Determination of moisture content

Approximately 5 g of flour are weighed into a dish which has previously been dried in the oven, cooled, and weighed. The dish is uncovered, and dish, cover and contents are dried in the oven at 130°C for 4 hours. The dish is covered while still in the oven and then transferred to the desiccator and weighed when cool. The moisture is calculated and the loss of weight expressed as a percentage of the original sample.

## 2.9. Measurement of Solubility

Solubility was examined by dissolving 2 gram MCS into 40 ml of distilled water, then the solution was heated in a water bath at 60°C for 30 min. Supernatant and paste were formed, then separated using a centrifuge at 3000 rpm for 20 minutes. Furthermore as many as 10 ml supernatant was dried in an oven and a dry precipitated weight was recorded. The Solubility was calculated using the formula as follows;

$$\% \text{ Solubility} = \frac{\text{dry weight precipitated}}{\text{volume of supernatant}}$$

## 2.10. Measurement of Swelling Power

Swelling power is the power of flour to inflate. Swelling power was examined by dissolving 0.2 g of MCS into 20 ml of distilled water. The solution was heated using a water bath at 60°C for 30 min. Supernatant was separated using a centrifuge with a speed of 2500 rpm for 15 min. Swelling power was calculated by the formula;

$$\text{Swelling power} = \frac{\text{weight of slurry}}{\text{weight of dry sample}}$$

## 2.11. Measurement of viscosity

As a standard for comparison, the standard for grade A is always cooked with 10 g of flour per 150 g of distilled water. If the sample which is to be graded is thought to be approximately grade A, then it should be cooked with 11 g of starch per 150 g of water. If the unknown flour is thought to be grade B, then 17 g should be used. If the flour is supposed to be grade C, then 20 g should be used. In this way, if the unknown flour has a higher viscosity than the standard when cooked with 10 g of flour to 150 g of water, then the unknown flour would be grade A, B or C, according to the amount of flour used in the viscosity test.

## 3. RESULTS AND DISCUSSION

MCS is used in the food industry for one or more of the following purposes, those are (1), directly as cooked starch food, custard, and other forms; (2), As thickeners, using the paste properties of starch; (3), As fillers, contributing to the solid content of soups, pills, and tablets, and other pharmaceutical products, face cream, etc.; (4), As binders, to consolidate the mass and prevent it from drying out during cooking; (5), As stabilizers, owing to the high water holding capacity of starch.

Cassava consumption is considered a staple food in the tropics, however cassava has drawbacks, those are high levels of the toxic HCN and low levels of protein. One way of processed cassava flour is processed into tapioca flour which has a low protein content, so it is necessary to find a better method to increasing the quality of cassava flour. The method is currently being developed is a manufacture of MCS. The results were obtained ash content, fiber content, fat content, protein content and carbohydrate content fairly decent consumption.

The table above shows that there was no significant change (constant) in MCS that had been processed using liquid starter extracted from three microbial strains towards fiber content and ash. While the content of fat were high in mocaf, probably caused by the transformation of carbohydrates into fats (Lehninger, 1987), because some microbial strains can produce microbial fat during the fermentation process (Akindumila, Glatz1998). High protein content may be caused by ability of microbial strains were used, to secrete several extracellular proteins into the cassava starch

granules during the fermentation process had been taken place, so as to form a single cell proteins during the fermentation (Akindahunsi *et al*, 1999). However, a decrease in carbohydrate content may be caused by microorganisms those were used to ferment cassava chips, using a carbon source from carbohydrates to a metabolic process, including to produce a protein or fatty substances (Lehninger, 1987).

The amount of reducing sugars in flour samples from the food grade cassava varieties was determined. Results show that there was an increased amount of reducing sugar in the MCS from both cassava varieties. An increase in

reducing sugars during fermenting could be due to starch hydrolysis by hydrolytic enzymes such as  $\alpha$ -amylase. These results are in agreement with previous studies which indicated synthesis of hydrolytic enzymes, such as amylases; proteases, and phytases during fermenting.

A breakdown of protease resistant prolamines and an increase in the availability of minerals. Essential amino acids principally lysine, tryptofan and methionine were reported to increase during fermentation (Table 1 and 2).

**Table 1.** Approximate analysis on properties of MCS and native flour of cassava.

No	Type of Analysis	Fermented MCS			Unit	Method
		24h	48h	72h		
1	Moisture content	9.0	8.9	9.0	%	Gravimetri
2	Lipid	2.8	3.4	3.6	%	Soxhlet
3	Ash	1.4	1.6	1.7	%	Incineration
4	Protein	2.9	2.6	2.4	%	Kyeldhal
5	Fibre	15.9	16.0	15.4	%	Gravimetri
6	Starch	60.4	60.0	59.9	%	Spectro
7	Oligosaccharides	10.2	10.8	10.9	%	HPLC
8	Gelling time	24	25	26	menit	Brabender
9	Gelling temperature	66.2	67.5	68.0	°C	Brabender
10	Peak time	43	40	40	Menit	Brabender
11	Peak Temperature	93	92	94	°C	Brabender
12	Peak viscosity	660	668	669	BU	Brabender
13	Viscosity at 93°C	638	640	642	BU	Brabender
14	Viscosity at 93°C, 20 min	20	21	22	BU	Brabender
15	Viscosity at 50°C	635	640	642	BU	Brabender
16	Setback Viscosity	+300	+310	+320	BU	Brabender
17	Dextrin Equivalent	2.5	3.5	4.0	%	Spectro

**Table 2.** Approximate analysis of physical and chemical properties of MCS.

No	Parameter	Unit	MCS	Native	Method
1	Total energy	Kcal/100g	354.70	370.58	Calculation
2	Moisture content	%	11.51	7.07	Gravimetri
3	Ash	%	0.44	1.16	Gravimetri
4	Total lipid	%	2.85	0.70	Soxhlet
5	Protein	%	2.00	0.56	Kjeldhal
6	Carbohydrate	%	86.99	89.07	By different
7	Reducing sugar	%	0.51	0.50	Luff school
8	Viscosity	cp	70	61	Oswald
9	Consistency	MPa°S	52-55 (Hot pasta) 75-77 (Cold pasta)	20-40 (Hot pasta) 30-50 (Cold pasta)	Viscometer
10	Fibre	%	1.32	1.91	Gravimetri
11	Acidity	ml N NaOH/100g	2.67	0.80	Titrimetri

Cassava roots contain cyanogenic glycosides, ie linamarin and lotaustralin. They are decomposed by linamarase liberating hydrogen cyanide (HCN). Table 3 showed that there has been a

decline in levels of toxicity of HCN in fermented MCS. This is likely due to microbial culture used, which was capable in breaking cyanogenic glycoside and its derivatives into harmless

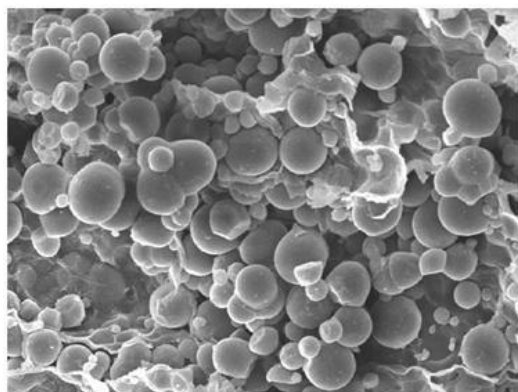
compounds. In addition to the processed cassava process involves washing with hot water, fermentation or drying process, can reduce levels of HCN in cassava proportionally (Cereda and Mattos, 1996; Akindahunsi *et al.*, 1999). The longer of fermentation, the levels of HCN content is also declining. Although some studies showed some negative aspects with this process, especially generation of cyanide, the good news

is this toxin, can be removed either by heating the flour, although heating may reduce the amylase content in the tubers. In some ethnic cultures of some Asian countries, traditional wine and fermented foods are prepared from fermented cassava tubers indicating that fermentation and other traditional processes can reduce the potential cyanide to lower levels considered nontoxic.

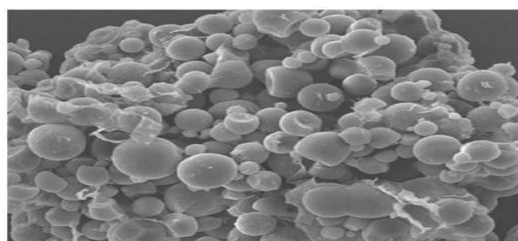
**Table 3.** Influence of fermentation of cassava chips to decreased levels of HCN in MCS.

Sample	Cassava chips	Liquid state fermentation using 2% starter solution				
		Day-1	Day-2	Day-3	Day-4	Day-5
HCN (mg/kg)	7.5	4.350	2.850	3.270	3.375	3.165

Microscopic observation using scanning electron microscope (SEM), as shown in Figures 1 and 2, indicated that unfermented cassava starch did not undergo breakage on its starch granules. While of MCS sample was observed indicating the breakdown of starch granules which seemed to opening. According to Murillo *et al* (2008), starch degradation can cause surface erosion of the starch granule and lead to enlarge its surface. During fermentation, some viable microbial cells were able to produce amylolytic and cellulolytic enzymes which could break down cell walls and hydrolyzed starch granules, and therefore they seemed to be opened and microscopically observed that they were swollen and hollowed.



**Figure 1.** The appearance of microscopic granules of native cassava starch.



**Figure 2.** Granules of MCS after 72h fermentation on scanning electron microscopy.

The amounts of soluble proteins and free amino

acids in the cassava varieties were analyzed. Results showed a slight increase in the amount of soluble protein in fermented flours. It showed a significant difference between MCS and native cassava starch. The interaction between the treatments showed an effect on the amount of soluble protein for both samples. An increase in soluble protein could be due to both solubilization of mocaf during fermentation and structural changes in storage protein (prolamines and glutelins) during fermentation, hence making them available to enzymatic attack.

A study supports the above argument whereby their results indicated an increase *in vitro* protein digestibility during fermentation and a combined treatment effect significantly would improve digestibility. Also studies all showed an increase in soluble protein during fermentation process. Although reasons for the increase in an *in vitro* protein digestibility on lactic acid fermentation are not known, rapid lowering of pH may have an effect on the structure of the proteins thus rendering them more accessible to pepsin.

A reference to lactic acid as having a softening effect on the cereal proteins suggesting that the structure of the protein was changed in some way by the effect of the lactic acid. This could explain the improvement in an *in vitro* protein digestibility coinciding with a rapid drop in pH and the corresponding increase in titratable acidity during fermentation.

Increasing in level of free amino acids during fermentation processes is due to a number of reasons. Bhise *et al* (1988) pointed out that during malting the storage proteins of the grain undergo partial hydrolysis by endogenous proteases to soluble proteins and free amino acids that are more susceptible to pepsin attack. Also the bacteria that are produced during fermentation increased proteolysis and degrade

protein into peptides and amino acids that are readily utilized by the bacteria. Mohammed *et al* (2000) pointed out that during their growth cycle, bacteria can also synthesize amino acids from metabolic intermediates. In their studies, they analyzed amino acids and conducted an *in vitro* pepsin digestibility during injera processing and found that fermentation increased both.

Study results showed that there was a decrease in pH levels in the cassava flour samples due to fermentation and submersion pretreatments. A decrease in pH levels and a corresponding increase in titratable acidity (TA) is due to the production of acids, most likely lactic, acetic or formic acids by the microorganisms, particularly bacteria. This is in agreement with study results by Friend *et al* (1995) and Cepeda *et al* (2000) who reported that lower pH values in tortilla were caused by the relative low amount of baking powder used and fumaric acid. The results further show more variability in values of titratable acidity (TA) across the cassava flour samples than for the pH. The result showed that while submersing had no effect on the TA values, while fermentation showed the effect. According to Friend *et al* (1995) this could possibly be due to the differences in chemical composition in flour and hence different buffering effects.

Wet noodle prepared from a mixture of wheat flour and MCS had increased a cooking yield and swelling index along with increased levels of MCS flour. The addition of MCS flour will lead to increase levels of carbohydrates which could increase the swelling index, but increased the value of cooking loss.

Absence of gluten content in mocaf, causing the noodle that was produced had low elasticity. Therefore, the higher addition of MCS in the dough therefore the gluten content in it was the less, so it produced a noodle product that was fragile and easily broken. Noodles with a low content of gluten will generate fragments that do not bind strongly dough during processing or cooking, thus affecting the value of cooking loss. The results showed that the use of MCS up to 30% could produce a noodle with the value of cooking yield of 9.76% and cooking loss of 0.00% and swelling index of 1,976.

**Table 4.** Nutritional value of food product made from wheat, MCS and mix of wheat and MCS.

Component (%)	MCS (100%)	Wheat (100%)	Wheat-MCS (70 : 30)
Water (%)	58.45	51.73	55.30
Carbohydrate	60.00	34.98	31.11

Protein (%)	2.20	8.32	6.44
Fat (%)	0.65	3.62	2.82
Ash (%)	1.50	1.04	1.18

#### 4. CONCLUSION

We concluded that *Lactobacillus plantarum*, *Saccharomyces cerevisiae* and *Xanthomonas campestris* those are non-pathogenic to humans, could increase levels of protein and fat and lower levels of HCN in MCS through fermentation. Substitution of wheat flour with MCS, in the manufacture of wet noodles exhibited significant effect on changes in moisture content, ash, carbohydrate, protein, fat and elasticity of noodles. Formulation treatment approach control (100% wheat flour), which was a mixture of wheat flour 70% and MCS 30%, showed following nutritional value; moisture content 55.45%, ash 1.18%, carbohydrate 31,11%, protein 6.44%, and fat 2.82%, where protein content and ash content of the resulting wet noodles has closed to quality standards of wet noodles (SNI 01-2897-1992).

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