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The Effect of Fermentation Time on The Characteristic of Chips Made from Grated Cassava (*Manihot uttilissima*).

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ABSTRACT

The influence of fermentation time of grated cassava on the characteristic of the resulting chips was investigated using a range of fermentation times from zero to thirty hours. Completely randomized design with two replicates was used in this study. Statistical analysis was conducted using ANOVA and significantly different values were submitted to Duncan's New Multiple Range Test (DNMRT) at 5% significance level. It was discovered that fermentation time had a significant influence on fat content of both raw and fried chips, and also carbohydrate content. The optimal fermentation time was found to be 18 hours (based on physical properties).
Keywords: fermentation, chips, cassava, postharvest processing.

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INTRODUCTION

Cassava chips, known locally as "*opak*", is a favorite Indonesian snack prized for its crisp texture. This food is made from grated cassava flavored with additions that may include salt, leek and garlic. After mixing, the dough is steamed and then shaped, dried and fried before eating.

Cassava is widely grown in the tropical and sub-tropical areas of Asia, Africa and Latin America as the third most important source of calories in the tropics after rice and maize [1, 2]. Cassava roots are essentially a carbohydrate source and are very rich in starch [3]. Cassava gives a carbohydrate production which is about 40% higher than rice and 25% more than maize, among the starchy staples [4, 5].

Cassava produced is for human consumption (fresh or processed) [5]. Cassava is a high carbohydrate food containing, the percentage compotition of 35% carbohydrate, 60% water, 1% protein, 0.5% fat, and 1% ash [6]. 40 mg phosphorous, 33 mg calcium, 30 mg vitamin C, 0.70 mg iron and 0.06 mg vitamin B1, with 146 kcal [7]. Based on [5] reported 100 g cassava contain of moisture, starch, fiber, protein, and other substances including minerals is (70%0, (24%), (2%), (1%) and (3%) respectively. Consequently, cassava is poor protein and other macro nutrients [5]

In Lareh Sago Halaban, Lima Puluh Kota Regency, West Sumatera-Indonesia grated cassava is usually fermented before the chips are shaped. This causes the mixture to increase in volume and so more chips can be made from the same quantity of dough and produces a distinctive aroma. The starch in grated cassava-degraded by microorganisms with relevant combination of starch hydrolases and related enzymes [8]. These enzymes are in general called amylases, are produced by amylolytic; Amylase producing microbes in cassava are generally molds that gluco-amylase, α amylase and β amylase to break down the starch to glucose, maltose, and dekstrin [9, 10] . This research was conducted to discover how, over time, the actions of the microbes in fermenting cassava changed the physical and chemical characteristics of the final product.

MATERIAL AND METHODS

Fermentation process

Fermentation was carried out in sealed semi porous woven plastic rice sacks at room temperature for pre-determined periods of time. Fermentation of the samples was begun at appropriately staggered times so that all of the samples could be analyzed simultaneously.

Analysis

Moisture content was estimated using the Gravimetry Method.

Approximately 2 gram of samples were oven dried in porcelain cups at 100-105^{o}C until a constant weight was reached. They were then stabilized in a desiccator. Water content was calculated from weight difference [11].

Ash content

2 gram samples were dried at 110^{o}C for one hour, cooled in a desiccator then charred on a hot plate until no more smoke was released, they were then heated at 500^{o}C-600^{o}C for 2 hours to reduce them to ash [12].

Protein content was estimated using the Kjeldahl Method.

A one gram sample is placed in a flask mixed with 1.9 grams of selenium. 25ml of concentrated sulfuric acid is added. After digestion is complete the resulting clear fluid is allowed to cool. The resulting material is rinsed with 10 ml of distilled water and placed in a distillation flask. 25ml of 30% sodium hydroxide is added and then the mixture is distilled. A 100 ml Erlenmeyer flask containing 25 ml of 3 % H3BO3 and 3 drops of a mixture of methyl red indicator and methyl blue under the tip of the condenser which is submerged under the H3BO3

solution. After 10ml of distillate is obtained it is titrated with 0.02 N chloride acid solution. Until a blue color is obtained. A control blank is processed alongside the one containing the sample for comparison. Protein is calculated from the amount of resulting dry matter [13].

Fat content was estimated using the Socxhlet Method.

A 5 gram sample is wrapped in filter paper and placed in a pre-weighed socxhlet flask then the equipment is assembled. After 5-6 hours of reflux with the extraction solvent the flask will contain the extracted fats and the remaining solution which is heated in an oven at 105^0C until the fluid has evaporated. The flask containing the fat is cooled in a desiccator and then weighed [14].

Carbohydrate content

Carbohydrate content was obtained by using carbohydrate by difference [13].

Absorption of frying oil.

The amount of oil that was absorbed when the chips were fried was calculated by measuring the volume of oil before and after frying [14].

Crude fiber content

Crude fiber content analysis was conducted by hear treatment of the acids and bases method. The principle of this analysis is washing the sample with acids, bases, boiled water and 95% alcohol to obtain a crude fiber residue [13].

Yield

The weight of the dried cassava chips was measured then expressed as a percentage of the initial weight of the dough.

Size of the chips produced

Diameters of chips were measured in five different places with *vernier calipers* and averaged [14].

Total plate count

Standard agar plate method was used to count the number of microbial colonies. A 5g sample of dough was serially diluted six times to obtain a $1:10^6$ dilution then spread across the plate and incubated for 24 hours [15].

Experimental design

This research design was Complete Random Design (CRD) with 3 replications. Fermentation times used were A= 0 H (control), B= 6 H, C= 12 H, D= 18 H, E= 24 H and F= 30 H. The resulting data was analyzed using analysis of variance with Duncan's New Multiple Range Test (DNMRT) at a 5% level being used where significant differences were detected. The statistical package used was SPSS 16.0 for Windows.

RESULT AND DISCUSSION

Chemical Properties

In the fermentation process microorganism produce enzymes that decompose organic material to release energy, cell building materials and other metabolites [16]. The resulting chemical changes in the fermented grated cassava include a breaking down of starch to sugar, alcohols, organic acids, carbon dioxide

and water by amylase [17]. Are enzyme who has the ability to break down the glucoside bond in starch polymer known as hydrolysis of starch [18]. Normal starch is composed of two glucans, are amylose and amylopectin. Amylose is a primarily linear molecule with thousand glucose with –α1,4 glucosida, and the highly branched, high molecular weight glucan or amylopectin. Amylopectin is a glucan with α-(1-4) glucosidic linkages containing α-(1-6) branch points [18, 19, 20].

In the appropriated conditions, this fermentation process occurs spontaneously from the microorganisms in the grated cassava [21]. Microorganismes are grown on substrate that is rich in strach, generally have the potential to produce amylase enzymes [18, 22]. Amylolytic enzyme active towards starch, pollulan, glycogen and other related oligo-and polysaccharides [8].

Microorganisms that grow on a carbohydrate substrate faster than those break down protein or fat. The length of fermentation has an influence on the carbohydrate content of the cassava. [16, 17, 23] reported that microorganisms tend to attack carbohydrate first and then protein then finally the fat. As the fermentation process breaks the starch down to sugars then to alcohols and organic acids one would expect the total carbohydrate to decrease. The percentage of carbohydrate content however increased with the length of fermentation. This is most likely to be the result of reduction in water content, protein, fat and ash content (by difference analysis).

The longer of fermentation process the lower of protein and fat content in the grated cassava as the microorganisms begin to decompose the protein and hydrolyze the fat into glycerol and fatty acids. During the food processing processes of fermentation, steaming and drying fat is broken down [24]. The fat content of the fermented grated cassava is significantly less than the control indicating that fat hydrolysis had occurred.

Found that crude fiber content of fermented cassava flour (modified cassava flour) is higher than in unfermented flour [25]. Fermentation can only result in a breakdown of crude fiber if the appropriate cellulolytic microorganisms are present to produce the enzymes needed to break down the lignin binding cellulose [26]. In this study crude fiber content appeared to increase during fermentation but the change was not statistically significant. This indicates that the action of any cellulolytic microorganisms in the dough is insignificant.

The water content of the resulting chips does not change significantly as a result of the fermentation process. The grated cassava could be expected to have an increased water content as more starch is broken down as water is a byproduct of this process [16] for another fermented cassava product*; tape.* However, unlike the totally anaerobic tape fermentation, the conditions of fermentation for the cassava dough allow for evaporation to occur which may balance out this gain. Any previously unevaporated water that has been released from the cells that have decomposed by the fermentation process will be more easily removed in the subsequent steaming and drying of the chips [27].

No significant change in ash content was observed with fermentation time. Ash contained chips indicated mineral content (calcium, phosphorus and iron) in cassava. According [28], in cassava contains 73.7 mg/100 g minerals. The minerals in a tuber of cassava is 3% [5]. Chemical properties of cassava chips are show on Table 1.

Tabel 1. Chemical Properties of Chips

Treatment	Carbohydrate (%)	Protein (%)	Fat (%)	Crude fiber (%)	Water content (%)	Ash content (%)
0 H	88.6 a	4.6	1.8 a	2.7	4.8	0.2
6 H	89.9 ab	4.0	1.5 b	2.8	4.4	0.2
12 H	90.1 abc	4.0	1.5 b	2.9	4.2	0.2
18 H	91.4 bc	3.7	1.0 b	3.0	3.7	0.2
24 H	89.3 abc	4.1	1.3 b	3.0	5.0	0.3
30 H	90.4 c	4.0	1.2 b	3.0	4.2	0.2

Description: Numbers followed by lower case letters are not the same, statistically significantly different (5%)

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Physical Properties

The yield of chips from the mixture ranged between 47.3 and 54.6% with no significant increase in yield with time of fermentation. During fermentation the microorganisms break down starch and improving gelatinization that occurs during mixing and steaming (resulting in a more compact mixture) due to holes that form in starch granules as a result of fermentation. This is discussed by [29], who stated that it was the holes in starch granules caused by fermentation resulted in a more compact structure in the dough allowing the chips to be pressed into thinner shapes without breaking.

Amount a dough rises is linked to the degree of gelatinization due to microbial action [30]. Gelatinization of starch granules that contain holes could be expected to result in a larger volume of dough than with starch granules that have not yet begun to decompose/dissolve. It was observed that the longer of fermentation time more the cassava dough appeared to rise but this volume difference was not statistically significant. It may be that the fat content of the dough is sufficient to hinder the gelatinization process by coating the surface of the granules and preventing water penetration. Physical properties of chips cassava show on Table 2.

Tabel 2. Physical Properties

Treatment	Yield (%)	Degree of chips development (%)
0 H	47.3	34.0
6 H	50.8	41.2
12 H	52.5	42.0
18 H	54.6	49.2
24 H	53.0	47.6
30 H	51.8	44.7

The oil is absorbed during the frying process was measured as was the fat content of the resulting chips. The fat absorbed in the frying process and the fat content of the fried chips was significantly lower in chips that had been fermented longer as show in Table 3.

Tabel 3. Fat Content of fried chips and absorption of cooking oil

Treatment	Fat of chips cassava (%)	Oil absorption (%)
0 H	26.8 a	24.9
6 H	23.9 a	22.5
12 H	23.6 ab	22.1
18 H	23.0 ab	21.6
24 H	20.3 ab	18.9
30 H	20.0 b	18.7

Microbiology Analysis on Grated Cassava

Table 4. Displays the number of microorganisms present based on the total plate count. The number for unfermented dough can be assumed to be the number naturally present in the cassava or introduced during initial processing before any increase due to fermentation occured. This increases in the first 18 hours of fermentation as the microorganisms replicate. The variety of microorganism most evident at this stage is amylolytic as shown by the milk white colonies surrounded by yellow areas (show on figure 1). However the count reduces after 24 hours showing that the microorganism have entered a stationary phase of growth. At the 30 hour fermentation stage there was a sudden increase in total plate count, possibly due to contaminants.

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Tabel 4. Total Plate Count

Treatment	Total Plate Count (cfu/g)
0 H	$7,3 * 10^3$
6 H	$1,1 * 10^4$
12 H	$1,5 * 10^4$
18 H	$1,6 * 10^4$
24 H	$1,4 * 10^4$
30 H	$2,3 * 10^4$

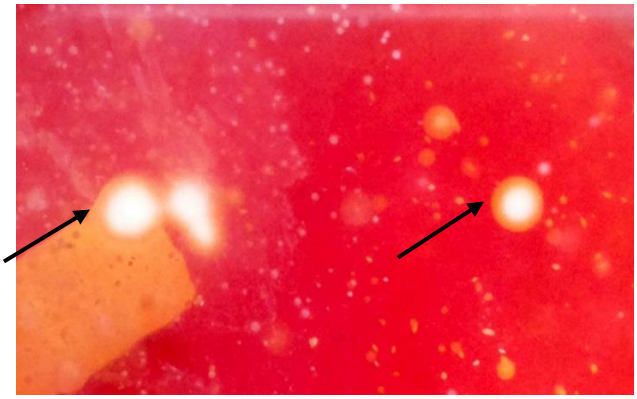

Figure 1. Amylolytic colonies on starch agar media

CONCLUSION

This study shows that fermentation time of grated cassava influences carbohydrate content and fat content both before and after frying. The optimal fermentation time with maximize physical properties was 18 H. This resulted in raw chips containing 91.4% carbohydrate, 3% crude fiber, 3.7% protein, 1% fat, 3.7% moisture content and 0.2% ash. The yield was 54.6%, and degree of chips development 49.2%. After frying the chips contained 23% fat, and oil absorbed 21.6 % and the total plate count of microorganisms was $1,6*10^4$ cfu/g.

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