Effect of Modified Cassava Starch in Reduced-fat Mayonnaise by Correlating Emulsion Stability with Anti-oxidation Reaction Using Gas Chromatography–Mass Spectrometry (GC-MS)

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Modified starches are essential firming additives in many food processes as they act as emulsion stabilizers. Presently, they are produced mostly from corn and potatoes, but cassava is a good alternative source to augment the rising global demand for starch. In this work, cassava starch from a native variety called *Manihot esculenta* Crantz, modified by facile heat-moisture treatment (HMT) method was used in reduced-fat mayonnaise to retain product consistency and texture but, more importantly, to contribute to the stabilizing effect of emulsions. Varying amounts of modified cassava starch in mayonnaise formulation were evaluated to correlate emulsion stability with a reduction in the amount of oil and the presence of anti-oxidation compounds that slow down chemical processes. The anti-oxidation process resulting from starch composition in the emulsion was measured by an analytical method using the combined gas chromatography and mass spectrometry (GC-MS). ²² complete factorial experiments were conducted in determining the significant effects and interactions between the amount of modified starch and oil. Correlations of these two factors with resulting stability as response variables were analyzed. Two-way ANOVA showed significant relationships in emulsion stability as directly proportional to the amount of oil in the emulsion as well as the modified starch. The results confirmed that individual factors both influenced the slowing down of emulsion breakdown of mayonnaise. However, it also revealed that modified starch had a greater influence on the concentration of acetic acid as an antioxidant than the effect of oil on the emulsion stability. Thus, this study confirmed that increasing the amount of modified cassava starch correlates to a significant occurrence of anti-oxidation reaction in reduced-fat mayonnaise. The combination of 0.60 wt% modified starch with 50 wt % oil achieved a higher stabilizing effect on emulsions as compared to other combinations. The results proved that predictive analysis of the capacity to prevent the auto-oxidation process is one way of predicting the long-term stability of the mayonnaise after 24 h.

Keywords: anti-oxidation, cassava, emulsion stability, gas chromatography, mayonnaise, modified starch

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INTRODUCTION

Starches are important ingredients in food processing to preserve its flavor and enhance its appearance, as well as improve its taste. Starch is most commonly used as a thickener or as a bulking agent that increases the bulk of the food without any nutritional value-added (Egharevba 2020). Several studies have investigated the use of starch for food applications such as potato, sweet potato, corn, and cassava (Fuglie et al. 2006). But its properties vary depending on the source and in unmodified form, its application to food is still very limited. Starch for food production requires better behavioral characteristics and functionalities than unmodified form, which led to its enhancement by modification procedure (Abbas et al. 2010).

There are two types of modification methods commonly used for starch in the food industry – namely, physical and chemical. Physical modification involves simultaneous actions of several parameters such as temperature, pressure, shear, and moisture on the starch (Ibrahim and Achudan 2011). On the other hand, chemical modification involves the insertion of functional groups into the starch molecule, which causes markedly altered physicochemical properties (Singh et al. 2007). Depending on its intended application, modification has beneficial effects on primary properties of starch such as an increase in water holding capacity, heat-resistant behavior, binding capacity, and improved thickening effect (Adzahan 2002; Miyazaki et al. 2006). In addition, this enhances the positive attribute, minimizes the defect, and achieves much functionality after modification (Abbas et al. 2010; Kozich and Wastyn 2012). These added attributes and functionalities of starch are flowability, elastic/viscous properties, texturizing, stabilizing, viscosity control, long shelf-life, increase shear stability, process tolerance, improve pH stability, acidic stability, retrogradation, gelling, amylase crystallization, freeze-thaw-stability, adhesion/cohesion, flocculation, and sensory properties (Abbas et al. 2010; Kozich and Wastyn, 2012).

Enhancement in starch brought about by modification methods became an important aspect in developing new food products (Abbas et al. 2010). For the food industry, special types of starch have beneficial effects on their products, so they preferred using the modified starch to native starch. Modified starches as food ingredients have functional uses namely as thickener, stabilizer, binder, and emulsifier (Abbas et al. 2010). In modified form, starch maintains the texture of the food, as well as acts as gelling agents in emulsions to provide stability and prevent the components from separating from each other, which have beneficial effects on the overall physical appearance and flavor of the food products (Egharevba 2020).

One particular food application of starch is the reduced-fat or low-fat mayonnaise, which is gaining more popularity than the traditional full-fat mayonnaise (Nor Hayati et al. 2009; Dolz et al. 2007). The health benefits obtained are reduced fat content and lower calorific value (Worrasinchai et al. 2006). Fat as one of the main ingredients of mayonnaise has a significant contribution to the stability of the product, which makes it difficult to replace (Mun et al. 2009). Mayonnaise is an oil-in-water emulsion consisting of closely packed foam of oil droplets (Depree and Savage 2001). The very strong interactions of the droplets with one another are due to the close packing, which are weakened by reduction of oil proportion that results in instability of emulsions (Depree and Savage 2001). The breakdown of emulsion leads to the spoilage of the mayonnaise. This reaction occurs when a very large area of oil is exposed to an aqueous phase, which may contain substantial dissolved oxygen that initiates the oxidation process (Depree and Savage 2001). This in addition to the air bubbles containing oxygen during the blending process that became trapped in the emulsion (Depree and Savage 2001). The availability or presence of oxygen is one of the causes of auto-oxidation (oxidative rancidity) over a period of time (Feiner 2016). In emulsions of mayonnaise, the presence of oxygen causes the auto-oxidation of fat components (Depree and Savage 2001). The acetic acid in vinegar is an aqueous solution (5–10% acetic acid), which is one of the ingredients of mayonnaise that produces antioxidant effects (Bakir et al. 2016; Fushimi et al. 2001; Qui et al. 2010) as well as an antimicrobial barrier (Alves Gomes et al. 2017). Antioxidant activity helps in the reduction or removal of oxygen in the product to prevent the auto-oxidation process. In addition, many bacteria thrive for survival on this oxygen in any given environment so reduction of oxygen prevents deterioration and rancidity (de Maionese 2012). This could explain in parts why antioxidant properties could correlate positively with antimicrobial activity. Fat substitutes were seen to be the solution at first but there were some technical issues when used for the production of low-fat mayonnaise in terms of its product quality such as texture, flavor, and physical stability (Mirzanajafi-Zanjani et al. 2019). Some of the fat substitutes commonly used to stabilize the emulsions are inulin, pectin, microcrystalline, cellulose, carrageenan, and some thickeners (Depree and Savage 2001). To make the fat substitute an ideal replacement in reduced-fat mayonnaise, the product should be comparable to the quality attributes of the full-fat mayonnaise. One of the solutions to a very low-fat content of mayonnaise is to use a water-soluble gelling agent such as modified starch to stabilize emulsions (Depree and Savage 2001).

In this study, modifying cassava starch by simple HMT was considered a more viable option and a suitable fat substitute to produce low-fat mayonnaise. One of the most commonly used modified starches as a stabilizer of
emulsion in reduced-fat mayonnaise is corn starch. But another good alternative source to current sources such as rice, wheat, potato, and sweet potato is cassava starch, which was explored for this particular application. The viability of producing modified starch using cassava, which is abundant in many parts of the world could address the increasing demand of end-users in the food and non-food industries and at the same time lower the cost. With the modification of cassava starch, it will also add value to the product that will increase demand for its use as a food additive, which can compete with other starch products and could also benefit farmers that produce cassava. In terms of suitability of modified starch in low-fat mayonnaise, there have been several studies that were carried out to measure the stability of mayonnaise (Depree and Savage 2001; Karas et al. 2002; Ibrahim and Achudan 2011; Jacobson et al. 2000), but there was no study conducted – to the best of our knowledge – that used modified starch from cassava as gelling-agent. It would be a significant step in the production of modified cassava starch to know its effect on the emulsion when the amount of oil is reduced to the suggested limits and retain its stability. Hence, this study determined the anti-oxidation reaction affected by different modified cassava starch compositions with reducing oil content in mayonnaise formulation after curing. The presence of acetic acid as an antioxidant was measured by an analytical method using the combined features of GC-MS. Factorial experiments were conducted in determining the significant effect and interaction between the amount of modified starch and oil in preventing the breakdown of emulsions. Correlations of these two factors and resulting emulsion stability as response variables were analyzed.

MATERIALS AND METHODS

Materials

The ingredients used for the preparation of mayonnaise were egg yolk, vinegar, salt, sugar, spices, and oil. The oil used was Jolly soya bean oil, which was purchased from a local supermarket. Soya bean oil was used because it was found out from a previous study that it was less susceptible to oxidation compared to corn oil and fish oil (Hsieh and Regenstein 1992). Fresh eggs instead of dried or frozen yolk obtained from a nearby market were used in the preparation of mayonnaise, which has better emulsion-forming properties based on previous studies (Depree and Savage 2001). The other ingredients like Marca Piña™ vinegar, McCormick® mustard, Victoria™ refined sugar, and non-iodized (no brand) salt were also purchased from a nearby market. The modified cassava starch used in the study was processed using a native variety called Manihot esculenta (Crandt, which is abundant in Cagayan de Oro City in Northern Mindanao, Philippines. It was processed to food-grade starch by the Chemicals and Energy Division (CED) of the Industrial Technology Development Institute (ITDI) under the Department of Science and Technology (DOST). The modification of native cassava starch was the HMT method, which is a hydrothermal treatment that changes the physicochemical properties of starches by facilitating starch chain interactions within the amorphous and crystalline domains and/or by disrupting starch crystallites (Hoober 2010). In the works of Carandang et al. (2017), they produced heat-moisture treated starches from cassava because of the simple process and the available facility for this type of process. HMT is categorized under physical modification of starches that improves the functional and physicochemical properties suited for food application (Mathobo et al. 2021). HMT holds an advantage over other methods of modification as it results in modified properties of starch without rupturing the granule and involves the addition of restricted levels of moisture (Adebowale et al. 2005). The modified cassava starch was analyzed for its selected physicochemical properties such as dry matter, pH, moisture and ash content, water solubility and absorption index, swelling power, and viscosity as baseline properties prior to this study to determine if it is comparable with the standard of commercial starch for food applications. These properties were obtained using the appropriate methods used for starch such as the International Starch Institute 01-1e method for determination of the dry matter, International Organization for Standardization (ISO) 1666: 1996 for determination of moisture content, and ISO 3593:198 for determination of ash content. Water absorption index (WAI) and water solubility index (WSI) were obtained based on the procedure described in the works of Stojceska et al. (2008). The starch sample was suspended in water at room temperature for 30 min, stirred, and then centrifuged for 15 min. Then, the supernatant was decanted into evaporating dish. WAI was obtained as the weight of the gel after removal of supernatant per unit weight of the dry sample. WSI is the weight of the dry sample in the supernatant expressed as a percentage of the original weight of the sample. The swelling power was also be obtained using this method by obtaining the weight ratio of the wet sediment to the initial dry sample. For the pH level of the sample starch, it was directly measured from the slurry of starch in water or the hydrated starch at room temperature measured for at least 5 min to get a constant pH value. For the pasting characteristics – which includes final viscosity (viscosity at the end of cooling) – a rotational viscometer was used by subjecting the starch suspension to control heating, holding, and cooling processes at constant shearing, as described in the works.
of Sharma et al. (2009). The gelatinization parameters are represented by transition temperatures and gelatinization enthalpies in the paste, which were determined using differential scanning calorimetry (Perkin Elmer, DSC 4000), as described in the works of Coral et al. (2009). The sample starch was placed in a hermetically sealed pan and stabilized at room temperature, then the sample was heated in a ramp at 10 °C/min using an empty pan as a reference under a nitrogen environment. The heat-moisture treated cassava starch used in the production of reduced-fat mayonnaise has a WSI of 0.004–0.01, WAI of 1.70–1.72, swelling power of 2.73–4.37, dry matter of 84.58–89.94%, pH of 4.25–4.99, moisture of 7.67–15.20%, ash of 0.08–0.15%, peak viscosity of 1690–3203 cP, trough viscosity of 925–1378 cP, final viscosity of 1317–2029 cP, breakdown of 763–1856 cP, setback of 392–651 cP, pasting temperature of 66.5–73.65 °C, peak time of 3.8–4.6 min, gelatinization enthalpy of 364.13–411.08 J/g, and gelatinization temperatures of 33.31–33.61 °C (onset), 82.23–89.29 °C (peak), 173.26–176.50 °C (end) (Carandang et al. 2017). The properties of native cassava starch, commercial corn starch, and modified cassava starch are presented in Table 1 for comparison.

### Preparation of Reduced-fat Mayonnaise

The reduced-fat mayonnaise was prepared in lab-scale proportion using the ingredients: soya bean oil, water, vinegar, egg yolk, spice, salt, sugar, and the modified cassava starch. The amount of oil and modified starch in the low-fat mayonnaise were varied in different proportions. The ingredients were weighed using an analytical balance (Shimadzu AUX 220, Japan) according to the proportions determined based on the amount of oil and modified starch prior to mixing. The proportions are summarized in Table 2.

The first part involved mixing egg yolk, salt, sugar, and vinegar together in the osterizer at low speed for 2 min to homogenize the mixture. Then, mustard was added to the mixture and mixed again for 1 min. For the next step, the water and modified starch was added to the mixture and stirred for another 1 min. After which, the oil was added drop-wise to the mixture while continuously mixing at a low speed to emulsify the mixture. After all the oil was added, it was further homogenized for 4 min at a higher speed of mixing.

The reduced-fat mayonnaise samples prepared were transferred in clean containers with a tight cover and proper labels. The samples were stored and cured for 24 h in room conditions prior to obtaining test samples for analysis by GC-MS. The mayonnaise samples were prepared in two replicates based on the experimental design.

### Analytical Method Using GC-MS

The concentration of acetic acid (in ppm) from the mayonnaise samples after 24 h of curing was determined using Thermo Focus GC-Polaris Q MS GC-MS (Thermo Fischer Scientific, Texas, USA) with an HTA autosampler equipped with 30-m (DB-5) Rxi®-5ms capillary column (0.30 mm i.d., 1.00 μm film thickness, SN No. 1131330) and ultra-high purity helium as carrier gas. Prior to tests, the gas chromatography with mass spectrometer was standardized and calibrated. Acetic acid concentration of 0.5, 25, 50, 75, and 100 ppm based on analytical standard (≥ 99.8% GC, Fluka, Germany) was analyzed.
to prepare the calibration curve, which was later on used for determining the concentration of the acetic acid in the samples of mayonnaise.

High-performance liquid chromatography grade methanol (Duksan, Korea) was used in the preparation of standard solutions for the calibration curve. Ultrapure water from Millipore (Billerica, MA) system was used in the preparation of mayonnaise samples that were injected in the GC-MS. A 65 μm polydimethylsiloxane/divinylbenzene (Supelco, St. Louis, MO, Belleponte, PA) solid-phase microextraction fiber was used for the extraction of acetic acid volatiles in mayonnaise.

**Experimental Design**

The emulsion stability depends on two main ingredients, oil and modified cassava starch, which both have significant influence in achieving the stability of emulsion in reduced-fat mayonnaise. According to Depree and Savage (2001), full-fat mayonnaise contains 70–80 wt % oil, which results in an oil-in-water emulsion having a closely packed foam of oil droplets that strongly interacts with each other. But reducing the amount of oil in low-fat mayonnaise weakens the interactions and the emulsion becomes unstable (Depree and Savage 2001). With very low-fat content, a water-soluble gelling agent such as modified starch in the aqueous phase is used to form a strong enough gel to stabilize the product (Depree and Savage 2001). These components, which are responsible for the physical stability of reduced-fat mayonnaise, were identified as the two important factors used in the experimental design. Since there are two factors identified in the study, the appropriate experimental design was $2^2$ factorial for screening experiments that have not been studied before. Factorial design is more practical and efficient since it allowed the smallest number of runs for the two factors investigated. For the screening of factor levels, it was conducted by reviewing the results of previous studies on the formulation of low-fat mayonnaise. In one of the previous studies, it was found that the minimum proportion of oil that is acceptable is 40%. For emulsion containing less than 40 wt % oil, it was highly unstable and when stored at a lower temperature, it showed physical separation after 1 day (Depree and Savage 2001). This became the basis for the low level of factor A (amount of oil). For the high level, in consideration of the formulation of traditional mayonnaise, it uses 70–80 wt % of oil. So lowering the oil content below 70% is already considered low-fat. In a previous study, it was found that between 50–55 wt %, the oil requires the addition of gelling-agent to prevent the breakdown of emulsion (Depree and Savage 2001). From this result, the high level was identified as 50 wt % oil.

Since there was no previous study conducted that used modified starch from native cassava in low-fat mayonnaise, the results of the previous studies on modified starch from corn, yam, and potato became the bases for the high and low levels of modified starch. The low level used was 0.20 wt % and the high level was 0.6 wt %. In the pretesting conducted prior to the experiment, since the starch is in powder form and very light; even at a smaller amount, the starch is very bulky. The amount of 0.6 wt % was approximately equivalent to 0.30–0.40 g, which was already a reasonable amount when added to the mixture. For the selection of response variable, previous studies showed that emulsion stability is directly related to oxidation of the mayonnaise. It was found that the increase in emulsion stability resulted in slower chemical processes, especially auto-oxidation, which is an important indicator of rancidity and breakdown of emulsions (Depree and Savage 2001). Based on this, the emulsion stability was expressed in terms of the concentration of the most prominent compound of interest that was present in the GC-MS analysis, which serves as an anti-oxidation compound.

Pretesting of mayonnaise sample prior to experimental runs showed that the most prominent compound was acetic acid based on the appropriate analytical standards of the GC-MS. The acetic acid present in the mayonnaise stabilizes the emulsions of oil-in-water depending on its concentration, which showed significant reduction after oxidation occurs in the mayonnaise. This became the basis for the response variable, which is the concentration of acetic acid (in ppm) present in the mayonnaise after curing for 24 h. The curing period is important in examining the extent by which the anti-oxidizing compound level was reduced after a certain period of time, which is – in this case – 24 h after preparation of the reduced-fat mayonnaise as an early indicator of emulsion breakdown. The final design of the experiment is summarized in Table 3.

<table>
<thead>
<tr>
<th>Table 3. Experimental design.</th>
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<td>Factor</td>
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<td>A: Amount of oil (g)</td>
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<tr>
<td>B: Amount of modified cassava starch (g)</td>
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To handle potential sources of error, the experiments were implemented in two independent replicates and in a completely randomized design. The experimental runs were conducted in accordance with the randomized sequence of the treatments generated using the Design Expert software (version 7.0.0). The responses were taken per run and recorded throughout the randomized experimental runs.
Statistical Analysis
Statistical analysis was carried out using the Design Expert software (version 7.0.0). The results of the experiments were analyzed using a two-way analysis of variance (ANOVA) with replicates to determine the significant effects of the two factors, as well as the interaction effect between the two factors in the response variable. Other diagnostic procedures to validate the results of the ANOVA were checked and considered in the study to satisfy all the assumptions of the statistical test. The model graphs of the effects were also used as the basis for the findings on the two factors.

RESULTS
The concentration of acetic acid (in ppm) from the GC-MS results was calculated by the calibration curve for each combination of the low- and high-level factors. The results of the experiments are summarized in Table 4, which shows the two factors (amount of oil and modified starch) and the corresponding responses (concentration of acetic acid) analyzed after 24 h curing period following the experimental randomized runs to eliminate bias.

The analyzies of the results revealed the significant effects of the individual factors and interaction between the two factors on the response variable. The data were evaluated using ANOVA with replicates, which is shown in Table 5. Based on the results of the ANOVA, the two factors (A and B) have a significant effect (p-value < 0.05) on the concentration of acetic acid. However, the interaction between the two factors is not significant (p-value > 0.05).

To validate the results of ANOVA, the required diagnostics were evaluated if all the assumptions were satisfied, as shown in Figure 1. The three assumptions of ANOVA were satisfied based on these graphs. The normal plot of residuals as shown in Figure 1a is a straight line where almost all of the data points lie near or on the line, which indicates that no abnormalities occurred and satisfies the first assumption. For the second graph in Figure 1b, the plot satisfied the second assumption by having a constant variance across predicted response values. The vertical spread of the residuals is approximately the same across all of the predicted values, which are acceptable. The third assumption was satisfied based on the last graph in Figure 1c, which showed no specific pattern from the data points plotted in the randomized run order.

| Table 4. Acetic acid concentration in different samples of reduced-fat mayonnaise. |
|-----------------------------------------------|----------------|----------------|----------------|
| Standard run | Experimental randomized run | Amount of modified starch (wt %) | Amount of oil (wt %) | Concentration of acetic acid (ppm) |
| 8 | 1 | 0.60 | 50 | 55.55 |
| 1 | 2 | 0.20 | 40 | 34.24 |
| 5 | 3 | 0.20 | 50 | 42.56 |
| 4 | 4 | 0.60 | 40 | 47.88 |
| 3 | 5 | 0.60 | 40 | 53.25 |
| 2 | 6 | 0.20 | 40 | 39.02 |
| 7 | 7 | 0.60 | 50 | 58.44 |
| 6 | 8 | 0.20 | 50 | 40.92 |

| Table 5. ANOVA results of two factors on the response variable. |
|----------------|----------------|----------------|----------------|----------------|
| Source | Sum of Squares | dF | Mean Square | F Value | p-value | Prob > F |
| Model | 492.61 | 2 | 246.31 | 38.21 | 0.0009 | Significant |
| A: Amount of starch | 426.03 | 1 | 426.03 | 66.08 | 0.0005 | Significant |
| B: Amount of oil | 66.59 | 1 | 66.59 | 10.33 | 0.0236 | Significant |
| AB | 0.87 | 1 | 0.87 | 0.11 | 0.7556 | Not significant |
| Residual | 32.23 | 5 | 6.45 | | | |
| Lack of fit | 0.87 | 1 | 0.87 | 0.11 | 0.7556 | Not significant |
| Pure error | 31.36 | 4 | 7.84 | | | |
| Cor total | 524.85 | 7 | | | | |
The result of ANOVA revealed that the proportion of oil in the low-fat mayonnaise has a significant effect ($p = 0.0236 < 0.05$) in the concentration of acetic acid as an indicator of a stabilized emulsion, which prevents the oxidation of the mayonnaise. The plot of the factor (amount of oil) against the concentration of acetic acid is shown in Figure 2.

For the second factor, which is the proportion of modified starch, the result of ANOVA revealed the same findings with the proportion of oil, which showed that it has a significant effect ($p = 0.0005 < 0.05$) on the concentration of acetic acid in the mayonnaise. The plot of the factor (amount of modified starch) against the concentration of acetic acid is shown in Figure 3.

**DISCUSSION**

Traditional mayonnaise or also called full-fat mayonnaise is a mixture of egg, vinegar, salt, spices, water, and oil. It contains about 70–80% of oil, which is mixed with the other ingredients to form the oil-in-water emulsion. The emulsion consists of spherical oil droplets packed together within the continuous water phase, which allows it to interact very strongly with one another that forms a network (Depree and Savage 2001). This formation of a network of small droplets in a continuous phase will prevent coalescence of the oil droplets (Powrie and Nakai 1985), wherein the interaction is mostly responsible for the emulsion stability (Depree and Savage 2001).

In the preparation of low-fat mayonnaise, the reduction in the amount of oil has a significant effect on the stability of the mayonnaise. This results in weaker interaction
between the droplets and affects the stability of the emulsion (Depree and Savage 2001). With a weaker interaction, low-fat emulsions tend to break down easily, which affects its long-term stability. This was investigated with the addition of water-soluble gelling agents such as modified cassava starch to increase stability and prevent the breakdown of emulsion by correlating the remaining concentration of acetic acid concentration as an antioxidant that reduced auto-oxidation to prevent deterioration and rancidity.

**Effect on Textural and Physical Features**
The reduced-fat mayonnaise, which was prepared in lab-scale proportion with a reduced amount of oil and modified cassava starch as an additional ingredient that was varied in different proportions is shown in Figure 4. By visual inspection, it showed that the same consistency, texture, and bulk property were retained as indicated by its ability to stick in the spatula without falling as compared to the commercially-produced mayonnaise. The physical properties like color and single-phase homogeneous state without separations of emulsions and absence of water were also evident compared to traditional mayonnaise.

**Correlation of Acetic Acid with Anti-oxidation Reaction**
The results of determining the concentration of acetic acid in the reduced-fat mayonnaise with 40–50 wt % oil and 0.20–0.40 wt % modified cassava starch added as emulsion stabilizer ranged from 36.63–56.99 ppm, as shown in Table 6.

The average concentration of acetic acid in the mayonnaise samples with 0.20% modified cassava starch is 39.19 ppm, while in the samples with 0.6% modified starch is 53.78 ppm. For the samples with 40 wt % oil and 50 wt % oil, the average concentrations of acetic acid are 43.60 ppm and 49.37 ppm, respectively. This showed that there was a correlation that exists with both factors: the amount of oil and modified cassava starch. The relationship revealed that an increasing amount of modified starch showed an increasing amount of acetic acid concentration after curing for 24 h. The same relationships exist with an increasing amount of oil with acetic acid. The correlation of varying compositions of oil and modified starch revealed that having a lower amount of both the modified starch and oil at 0.20 and 40 wt %, the concentration of acetic acid is at its lowest value of 36.63. This means that in terms of the antioxidant effect it has the lowest stabilizing level. Increasing the amount of modified cassava starch from 0.2–0.6 wt % but still retaining the amount of oil at 40 wt %, the increase was very significant from 36.36–50.56, which is 39%. Unlike the increase in oil from 40–50 wt% at constant 0.2 wt% of modified starch, which only has incremental values from 36.63–41.74 at 14% increase in the concentration of acetic acid. The stabilizing level of a difference of 0.4 wt % modified cassava starch was far greater with more acetic acid that prevents instability. For oil with an increase of 10 wt % at a higher amount of modified starch of 0.6 wt %, the increase of 12.72% acetic acid was measured which was even lower than the increase at 0.2 wt % modified starch at 14%. This revealed that the gelling agent effect of modified cassava starch

*Figure 4. Reduced-fat mayonnaise samples at different starch and oil compositions: a) 0.2/40, b) 0.2/50, c) 0.6/40, and d) 0.6/50.*

*Table 6. Correlation of concentration of acetic acid with the amount of modified cassava starch and oil.

<table>
<thead>
<tr>
<th>Amount of modified starch (wt %)</th>
<th>Amount of oil (wt %)</th>
<th>Concentration of acetic acid (ppm)</th>
<th>Correlation with amount of modified starch</th>
<th>Correlation with amount of oil</th>
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<tr>
<td>0.6</td>
<td>50</td>
<td>56.99</td>
<td>Increasing concentration of acetic acid</td>
<td>Increasing concentration of acetic acid</td>
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<tr>
<td>0.2</td>
<td>50</td>
<td>41.74</td>
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<td>0.6</td>
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in the aqueous phase became the primary stabilizer and prevented the breakdown of emulsions due to a higher amount of acetic acid that reduced the auto-oxidation process. Generally, these relationships revealed the increasing anti-oxidation reaction occurred when varying the oil and modified starch amounts were increased.

**Effect of Reduced Amount of Oil**

Figure 2 showed that increasing the proportion of oil in the emulsion of reduced-fat mayonnaise resulted in an increase in the concentration of acetic acid. The average concentration of acetic acid at 40% wt oil is 43.60 ppm, which increased to 49.40 ppm when the proportion of oil is increased to 50%. The increase in the average concentration of acetic acid is 13.30%, which can be observed with the spread of the two data points from 40–50% where the line that connects the points is not very steep. The reduced amount of oil greatly affects the ability of the emulsions to retain stability, with the lowest percentage of concentration of acetic acid providing anti-oxidant activity.

**Effect of Adding Modified Cassava Starch**

Figure 3 showed similar behavior with the effect of the proportion of oil in the emulsion of low-fat mayonnaise. The concentration of acetic acid increased when the proportion of modified starch was also increased. The average concentration of acetic acid at 0.20 wt % modified cassava starch is 39.94 ppm, which increased to 53.78 ppm when the proportion of modified cassava starch was increased to 0.60 wt %. The increase in the average concentration of acetic acid is 25.73%, which was almost twice the increase in the concentration of acetic acid in the proportion of oil factor. This can be observed from the graph with a steeper line that connects the two data points from 0.20–0.60%. This level of acetic concentration available with the increased amount of modified cassava starch made a big difference in the emulsion stability, which could withstand the auto-oxidation process twice the capacity of the oil for a longer time of product stability.

In comparing the effects of these two factors – oil and modified cassava starch – the effect of modified starch on the concentration of acetic acid is greater than the effect of the oil in the emulsion in terms of the concentration of acetic acid. These results proved that the addition of modified starch as a gelling agent is appropriate and beneficial in low-fat mayonnaise, which contributes to the stability of the emulsions in the mayonnaise samples. The correlation of concentration of acetic acid retained in the low-fat mayonnaise with the amount of oil and modified cassava starch could be measured to conduct predictive analysis of the capacity to prevent the auto-oxidation process, as well as predict the long term stability of the mayonnaise after 24 h.

**CONCLUSION**

This study showed the effects of the different combinations of oil and modified cassava starch in producing a commercially comparable low-fat mayonnaise. Both factors, the amounts of oil and modified cassava starch in the reduced-fat mayonnaise have significant effects on the concentration of acetic acid in the emulsions. This confirmed that both factors have influenced in slowing the process of oxidation, which prevents the breakdown of emulsion for the long-term stability of the food product.

Mayonnaise having oil as a major ingredient is susceptible to spoilage due to the auto-oxidation of the unsaturated and polyunsaturated fats present in the oil. The occurrence of this auto-oxidation of fats can be prevented with the considerable amount of oil used in the emulsion that stabilizes the mayonnaise. It was observed that emulsion stability is directly proportional to the amount of oil in the emulsion. This was validated when the amount of oil is reduced from 50% to 40% in the emulsion, which resulted in a decrease in the acetic acid concentration. When the acetic acid is reduced, then there is more tendency for the emulsion to easily break down due to auto-oxidation.

While the reduction of oil affects the stability, the addition of modified starch will enhance the emulsion stability. It can be seen from the results that the higher the amount of modified starch in the aqueous continuous phase, the amount of acetic acid was increased, which prevents emulsion breakdown. And since the modified starch has greater effects on the concentration of acetic acid as compared to the amount of oil-based on the statistical analysis, then it is the main factor that can influence the emulsion stability in reduced-fat mayonnaise rather than the oil when reduced its effect on emulsions.

In conclusion, the combination of 0.60 wt % modified starch and 50 wt % oil was the best combination to achieve higher stability of the emulsion. However, since there is no significant interaction between the two factors, the amount of modified starch can be further investigated at a fixed proportion of oil (50 wt %). The contribution of the modified cassava starch showed an increase in the concentration of acetic acid, which doubles the amount compared to the contribution of the oil. This will further optimize the amount of modified starch that can be used to achieve the greater stability of emulsions.

**RECOMMENDATIONS**

The limited time and resources of this study led to constraints in the scope of the experimental works. Thus, it is the recommendation of this study to undertake a follow-up study on the analysis of the mayonnaise samples after 7
and 15 days to verify the long-term effect of the modified starch in the stability of the reduced-fat mayonnaise. It should also involve other tests on the phase separation and turbidity.

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STATEMENT ON CONFLICT OF INTEREST

There are no conflicts of interest to declare.

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