International Food Research Journal 21(5): 1825-1836 (2014)
Journal homepage: http://www.ifrj.upm.edu.my

Effect of different hydrocolloids on the physicochemical properties, microbiological quality and sensory acceptance of fermented cassava (*tapai ubi*) ice cream

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Article history

Received: 20 January 2014
Received in revised form: 14 March 2014
Accepted: 21 March 2014

Keywords

Ice cream
Fermented cassava
Guar gum
Xanthan gum
Carboxymethyl cellulose

Abstract

The aim of this work is to study the effect of hydrocolloids; guar gum (GG), xanthan gum (XG) and carboxymethyl cellulose (CMC) on the physicochemical properties, microbiological quality and sensory properties in order to investigate the potential of applying fermented cassava (*tapai ubi*) in ice cream. Fermented cassava ice cream (FCI) incorporated with the three types of hydrocolloid was prepared and the protein content, pH value, overrun, colour, hardness, microstructure, FTIR spectrum and sensory acceptance of all samples were determined. Fermented cassava ice cream incorporated with XG showed the highest protein content (14.88%), pH value (pH 6.07), and overrun value (4.27%) as compared to the fermented cassava ice cream incorporated with GG and CMC. Meanwhile, ice cream incorporated with GG possessed the highest L^* (94.43) and hardness (3693.15 g) value as compared to XG and CMC. The microstructure study showed that the difference in uniformity at the interface obtained with different types of the hydrocolloids added demonstrated the effect of fat absorption at the air interfaces. The FTIR spectrum investigated indicated that the addition of the fermented cassava to FCI had increased the OH group in the ice cream as compared to the control. All samples were microbial safe as the total plate counts in all samples were below the standard as prescribed in Food Act 1983 with no presence of *E. coli*. In conclusion, fermented cassava ice cream with XG showed the good quality in terms of its pH value, overrun, total plate count and overall acceptability.

Introduction

Nowadays, people frequently use convenience products as part of their lifestyle. Ice cream is one of the products that they are looking for to save their time to prepare meals, especially during festivals, open houses and small occasions. Ice cream is served as a dessert and it is appreciated by people of all ages since it was first invented in the 1500s, as stated by the US Library of Congress (2000).

There are two types of tapai that are commonly found in Malaysia, fermented glutinous rice (*tapai pulut*) and fermented cassava (*tapai ubi*). There are various names for tapai pulut, such as tape ketan, as it is commonly called by Indonesians, and *lao chao* by the Chinese, whereas fermented cassava (*tapai ubi*) is commonly called *tape ketella* in Indonesia, *tape telo* in Java and *peujeum* in Sudan. However, fermented cassava (*tapai ubi*) does not experience a high demand. According to Lan (1984), fermented cassava is only commercialized in small scale industries.

Recently, fermented glutinous rice is more popular compared to fermented cassava. People tend to choose fermented glutinous rice rather than fermented cassava because it has a better taste than fermented cassava. The incorporation of fermented cassava in ice cream is not meant to override the taste of the ice cream but presents a great opportunity to promote fermented cassava and encourage people to eat it when ice cream is used as the medium. Besides, it will create a new product variation of fermented cassava when it is incorporated into the ice cream.

The fermentation process is necessary in the production of fermented cassava (*tapai ubi*) while it provides better protein content compared to the cassava itself (Steinkraus, 1994). This shows that fermentation can add nutritional value to the raw food. Besides protein, other mineral contents will be provided by the fermentation process. In addition, the fermentation of cassava will improve the nutritional value of the cassava by reducing the antinutritional factors in it, such as tannin, trypsin inhibitor and oxalate (Holzapfel, 2002).

One of the crucial ingredients in making fermented products (*tapai*) is the starter culture, which is commonly called *ragi*. According to Gandjar (2003), a proper preparation of substrate and *ragi* will successfully produce good *tapai*. In addition,

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good storage conditions that are appropriate for the microorganisms will support the fermentation process. The fermentation of cassava will produce a sweet, alcoholic flavour with a pleasant aroma. Hence, it is expected that the incorporation of fermented cassava in ice cream will contribute to a new unique taste of ice cream.

Hydrocolloids such as guar gum, xanthan gum, carboxymethyl cellulose and carrageenan are commonly used as stabilizers in ice cream production in order to produce smoothness in body and texture, reduce ice and lactose crystal growth especially during periods of temperature fluctuation or known as heat shock and to provide uniformity to the product and resistance to melting (Goff, 1997). There are many studies conducted on the incorporation of hydrocolloids in ice cream (Mousavi *et al.*, 2003; Murtaza *et al.*, 2004; Soukoulis *et al.*, 2008; Moeenfard and Tehrani, 2008). However, there is limited study on the incorporation of hydrocolloids in ice cream made from fermented product. Fermented cassava ice cream (FCI) has its own uniqueness as the low pH of fermented cassava will affect the textural properties of the ice cream formed. Hence, the incorporation of hydrocolloids will help to improve the texture of this new product (Soukoulis *et al.*, 2010). This study used three different types of hydrocolloids which were guar gum (GG), xanthan gum (XG) and carboxymethyl cellulose (CMC) to provide a good texture of ice cream that is comparable with the ice creams that are available in the market. Therefore, the objectives of this study are to identify the effects of these hydrocolloids on the physical and chemical properties, sensory evaluation and microbiological quality, such as total plate count and the presence of *Escherichia coli* sp. in fermented cassava (tapai ubi) ice cream (FCI) incorporated with these three different hydrocolloids.

Material and methods

Materials

The main ingredient for fermented cassava ice cream (FCI) production is the cassava and the fermentation starter (*ragi*). The cassava (*Manihot utilissima*) used in this study was the sweet type of cassava from the family of *Euphorbiaceae.* Its maturity period was in the range of 10 – 12 months after planting. Cassava was obtained from a supplier in Kuala Terengganu, Terengganu, while the ragi was purchased from MARDI Serdang, Selangor, Malaysia. The ingredients for preparing the ice cream were bought from the local market, Kuala Terengganu, Terengganu, Malaysia. The three types of hydrocolloids; guar gum (GG), xanthan gum (XG) and carboxymethyl cellulose (CMC) were obtained from Sigma.

Table 1. Fermented cassava of ice cream formulations

Formulation	Ice cream (%)	Fermented cassava (*tapai ubi*) (%)
FI	75	25
F2	50	50
F3	25	75

Preparation of fermented cassava (tapai ubi)

Fermented cassava was prepared by using boiling method. In the preparation of fermented cassava, about 1200 g of peeled and cleaned cassava was weighed and boiled for about 15 mins to ensure that the cassava would not be too soft. Then, the boiled cassava was cooled on a clean, dry stainless steel tray before 0.1% (w/w) of fermentation starter (in powder form) was spread over the cassava and mixed well. Fermented cassava was obtained after the fermentation process of 48 to 72 h at room temperature. After that, it was kept in the chiller for 24 h prior to the preparation of the FCI.

Preparation of fermented cassava ice cream (FCI)

Ice cream production was prepared according to the method by Gisslen (2010) with some modification. Ice cream was prepared by mixing 10% egg as emulsifier and 14% sugar until thick and light. Approximately 50% mixture of fat and milk protein was scalded gradually and tempered with the mixture. The mixture was heated for about 20-30 sec until it reached 80^{o}C for the purpose of sterilization, before each hydrocolloid was added. Approximately 25% milk solid non-fat (MSNF) was added to the mixture and stirred quickly to stop the cooking process. The mixture was kept in the chiller for 1 h at 4^{o}C for the aging process and 1% vanilla essence was added. Fermented cassava was added prior to the freezing process, which was conducted for about 25 mins using a soft serve ice cream machine (GELATO PRO 3000, UK). The ice cream samples were then stored at -24^{o}C for hardening process.

Different formulations of FCI – Formulation 1 (F1) (25% *tapai ubi* added), Formulation 2 (F2) (50% *tapai ubi* added) and Formulation 3 (F3) (75% *tapai ubi* added) – were first developed (as presented in Table 1) and sensory evaluation on the overall acceptance was conducted in order to determine the best formulation of fermented cassava incorporated in the ice cream. The most accepted formulation by the panel was used as a sample to incorporate with the hydrocolloids. Four samples of FCI were evaluated in further analyses. These included the control (ice cream with 25% fermented cassava), FCI with GG added, FCI with XG added and FCI with CMC added. Each ice cream sample incorporated the same volume of hydrocolloid (0.3%) and the analysis was conducted in triplicate.

Chemical composition

Chemical composition for protein and fat content analysis were conducted according to the AOAC method (AOAC, 2000).

Determination of pH

The determination of pH was conducted according to the method by Hwang *et al.* (2009). About 10 g of the ice cream sample was dissolved in 90 ml distilled water. After homogenization, the pH was measured using a pH meter (CyberScan Series 600, pH-103 Merthom/Brinkmann, Brinkmann Instrument Inc., Westbury, NY), which was calibrated using the standard pH buffer solution, (pH 7 and pH 4). The values were presented as a means of triplicate analyses.

Determination of Overrun

Generally, the overrun is related to the air bubbles, which contribute to the soft texture and light body of ice (Thaiudom *et al.*, 2008). To determine the overrun, the ice cream mixture was put in a standard cup 200 ml and weighed. Once the mixture was made into frozen ice cream, the same volume of frozen ice cream was cut and put in the container. The overrun was calculated according to the standard equation, as below (Laaman, 2011).

$$\text{The overrun} = \frac{(W_A - W_B) \times 100}{W_B}$$

Where, W_A = weight of mix for fixed volume
W_B = weight of finished product for fixed volume

The same volume of sample was weighed before and after the ice cream mix was made into frozen ice cream. This was performed in triplicate and the results obtained were expressed as mean ± SD.

Determination of melting rate

Melting rate tests were conducted as described by Sofjan and Hartel (2004), with some modification on the weight of the sample. The melting rate was measured at room temperature. About 50 g of the ice cream sample was weighed and placed on a 2 cm cooling rack above a small aluminium bowl, which was used to collect the melted ice cream. The timing of the melt down rate began when the first drop of material that melted touched the bottom of the bowl. The amount of melted sample was recorded every 5 mins until the ice cream had completely melted. First dripping and complete melting times of the samples were determined in seconds. The melting rate was determined based on graphs of the melted portion as a function of time. This analysis was performed in triplicate and the results obtained were expressed as mean ± SD.

Determination of Colour

The colour changes were measured according to the method described by Hwang *et al.* (2009). The colours of the samples were determined using a Colorimeter (Model CR-400, Konica Minolta Sensing, Inc., Osaka, Japan). The colorimeter was calibrated with a white tile (L^* = 93.80, a^* = 0.3111, b^* = 0.3196). The sample was placed in a 25 ml plastic cup with upper diameter of 5.5 cm. The screen on the glass light projection tube was pressed on the sample for light penetration. The results were shown on the screen of the colorimeter and were recorded according to the value of lightness (L^*), greenness/redness (a^*) and blueness/yellowness (b^*). The results were expressed as mean ± SD.

Determination of hardness

The hardness of ice cream samples were measured according to the method as described by Hwang *et al*. (2009). The firmness of ice cream was determined using a texture analyser (Stable Micro System, Godalming, UK) with a load cell of 5 kg, cylinder probe (diameter 50 mm). The samples were cut into 2.5 cm cubes and compressed at a speed of 10 mm/s and return strain of 50 mm. The reading was the average of three determinations. The firmness of the samples was determined as the peak of compression force (in g), which was detected when the probe proceeded to penetrate into the ice cream. The values were presented as the means of triplicate analyses. All data was analysed using the Texture Expert software program.

Determination of Fourier Transform Infrared (FTIR)

Fourier Transform Infrared (FTIR) spectroscopy (Bruker Instruments, Billerica, MA) was used in order to identify the functional groups obtained in the FCI produced. In FTIR analysis, samples of the ice cream were placed on the sample compartment of the spectroscope. The FTIR spectra of samples were obtainedusingGolden-gateDiamondsinglereflectance ATR in a FTS 7000 FTIR spectrophotometer with a DTGS detector (Nicolet 380). The spectra of FCI were recorded using the absorbance mode from 1000 to 4000 cm^{-1} with a resolution of 4 cm^{-1} with 128 coadded scans (Pavia *et al*., 2009).

Microstructure study

The microstructure of the different formulations of FCI was investigated in order to compare the size of the air cells formed in each of the samples. Aluminium tube (inner diameter 10 mm, length 10 mm), commonly called a stub was used to place the sample. A small drop of ice cream sample was taken by using a clipper and spread thinly on the stub. The specimen was then immediately put into an electron microscope freeze drier (EMITECH K750X) with the end temperature -70°C for 5 h. Then, it was dried overnight in the desiccators containing silica gel. The dried specimen was sputter coated with a 30 nm layer of gold using an auto fine coater (JOEL JFC-1600). The specimen was viewed at 10 kV accelerating voltage with an objective lens aperture of 10 μm (Goff, 2000).

Total plate count

Sample homogenization was first conducted to determine the total plate count of the FCI sample. About 10 ml of the sample was homogenized using 90 ml sterile saline. Then, a serial dilution was prepared until the fourth dilution. After that, 0.1 ml of the diluted sample was pipetted into a plate count agar (PCA) plate. This procedure was repeated for the other dilutions. The inoculums on PCA plates were spread using a sterile glass spreader (hockey stick). The plates were inverted and incubated aerobically at 37°C for 24 h. The colony count was recorded using the criteria of counting rules (KGaA, 1994).

Presence of Escherichia coli

The presence of *E. coli* was detected using EC broth, which was previously grown on LST and BGLB broth tubes, incubated at 37 ± 0.2°C for 48 ± 2 hours. The entrapped gas in Durham tubes was checked after 48 hours. The presence of gas showed the presence of E. coli and vice versa (KGaA, 1994).

Sensory evaluation

The sensory evaluation was conducted by 30 untrained panellists. A 7-point hedonic scale, anchored by 'dislike extremely' and 'like extremely' was employed and the score test used flavour, body and texture (firmness), colour, sweetness, smoothness, odour resistant to melting and overall acceptability (Klesment *et al*., 2011).

Two stages of sensory evaluation were carried out in this study. The first stage of sensory evaluation was to determine the best formulation of different quantities of FCI produced. The samples included Formulation 1 (25% of *tapai ubi* added), Formulation 2 (50% of *tapai ubi* added) and Formulation 3 (75% of *tapai ubi* added). The second stage of sensory evaluation involved 4 samples which included the control, which is Formulation 1 (25% of *tapai ubi* added), sample GG (Formulation 1 with 0.3% guar gum), sample XG (Formulation 1 with 0.3% xanthan gum) and sample CMC (Formulation 1 with 0.3% CMC) in order to determine the most accepted FCI among the three different hydrocolloids used.

Data analysis

Numerical data obtained from all analysis were analysed using Minitab 14 for Windows version. The comparison of means for every sample was determined using one-way ANOVA and further analysed using the post-hoc test. The level of significance for this analysis was 95% with a 5% margin of error. The values obtained were presented in mean ± SD. For graphic data, the figures were imported into Microsoft Excel 2007.

Results and Discussion

Production of fermented cassava (tapai ubi) ice cream (FCI)

Three main steps during the production of FCI were involved in this study; preparation of fermented cassava (*tapai ubi*), FCI and production of FCI incorporating three types of hydrocolloids – guar gum (GG), xanthan gum (XG) and carboxymethyl cellulose (CMC). The preparation of FCI incorporating hydrocolloids was conducted when the best formulation of FCI was selected. The three types of hydrocolloid were mixed during the pasteurization process of the ice cream production.

During the preparation of fermented cassava (*tapai ubi*), fermentation starter (*ragi*) was spread when the boiled cassava was already cooled. This is because the microorganisms in the ragi might die if they had been spread on hot boiled cassava. The cassava was put in a dark container at room temperature to allow the fermentation process. According to Law *et al.* (2011), moulds in the *ragi* degrade the carbohydrate compounds in cassava while yeast converts the simple sugars into alcohol. Acids are produced during this process which softens the cassava. Meanwhile, the reaction between the acids and alcohol produces a pleasant aroma of the fermented cassava. Gandjar (2003) stated that the role of bacteria is minimal because their growth is already suppressed by the ingredients mixed in the rice flour during the making of *ragi.*

The results in Table 2 showed that the overall

Table 2. Acceptance level of different fermented cassava ice cream formulations

	Mean ± SD (n = 3)				
Formulation	Odour	Alcoholic flavour	Sweetness	Smoothness	Overall acceptance
F1(25% *tapai ubi*)	5.13±1.33[a]	4.67±1.61[a]	5.00±1.66[a]	4.87±1.31[a]	5.43±1.36[a]
F2 (50% *tapai ubi*)	4.50±1.25[a,b]	3.87±1.46[a]	4.47±1.41[a]	4.40±1.38[a]	4.53±1.41[a]
F3 (75% *tapai ubi*)	3.87±1.61[b]	2.60±1.16[b]	2.97±1.47[b]	3.30±1.51[b]	2.80±1.45[b]

*value with the same superscript within the rows are not significantly different ($p > 0.05$).

Table 3. The protein, fat content and pH of different ice cream formulations

	Mean±SD (n = 4)		
Formulation	Protein (%)	Fat (%)	pH
C	8.32±5.56[a]	12.68±1.12[a]	5.91±0.30[a]
GG	10.51±8.66[a]	12.17±2.43[a]	5.84±0.21[a]
XG	14.88±17.32[a]	10.96±0.11[a]	6.07±0.52[a]
CMC	10.06±6.80[a]	8.02±4.71[a]	6.42±0.74[a]

*value with the same superscript within rows are not significantly different ($p > 0.05$)

*C: Control, GG: Tapai ubi ice cream incorporating guar gum, XG: *Tapai ubi* ice cream incorporating xanthan gum, CMC: *Tapai ubi* ice cream incorporating carboxymethyl cellulose

acceptability was obtained from formulation F1 (25% of fermented cassava) which possessed the highest values for odour (5.13 ± 0.33), alcoholic flavour (4.67 ± 1.61), sweetness (5.00 ± 1.66) and smoothness (4.87 ± 1.31). The high score on F1 shows that the incorporation of fermented cassava (*tapai ubi*) in ice cream can be accepted by the consumers. Sample F3, which comprised 75% of fermented cassava, was highly affected the sweetness of the ice cream with the lowest score for the alcoholic taste of the fermented cassava. This was due to the the alcoholic taste of the fermented cassava had dominantly affect the taste of ice cream, thus, lowered its acceptability. Therefore, FCI with 25% of fermented cassava added (F1) was selected as the best formulation to carry out further studies. It has been described that fermentation process increased the total amount of soluble sugars, reducing and non-reducing sugar content, with a simultaneous decrease in its starch content (Khetarpaul and Chauhan, 1990). The three types of hydrocolloid were mixed during the pasteurization process of the ice cream production. According to Sharma and Hissaria (2009), hydrocolloids in the ice cream mixture stabilizes the emulsion, while, at the same time, help in the suspension of liquid flavour. This condition helps in the prevention of the creaming fat in the ice cream mixture. The addition of hydrocolloids controls the water contained in the ice cream mixture, thereby affecting the texture, slowing ice crystal growth, improving air incorporation, melting properties and preventing separation of ice cream mixture (Mousavi *et al*., 2003).

Chemical properties

Table 3 shows the pH, protein and fat content (%) of different FCI formulations. Based on the results obtained, FCI incorporating XG showed the highest protein content (14.88%) followed by ice cream incorporating GG (10.51%), ice cream incorporating CMC (10.06%) and ice cream without the incorporation of hydrocolloid (control) (8.32%). However, there was no significant difference ($p > 0.05$) in the protein content among all the ice cream formulations. FCI contained fermented cassava, thus, lowering its pH value. When hydrocolloid was added, the pH value of the FCI will changed. Therefore, it will affect the taste and acceptability of the FCI.

Choo *et al.* (2010), stated that the typical protein content of ice cream is approximately 4%. The Kjeldahl method was used, which involves the determination of total nitrogen content followed by the conversion of total nitrogen into crude protein content using a suitable conversion factor, 6.38. According to Choo *et al.* (2010), the nitrogen might come from the non-protein components. Hence, it might be the probable reason for the increase of protein content observed in the ice cream. In addition, Steinkraus (1994) reported that fermented cassava (*tapai ubi*) increased the protein content by at least 3% compared to the raw cassava. This factor might also be a reason for the increase in protein content in all the ice cream formulations prepared compared to the typical one, which has a protein content of 4%. Furthermore, the use of eggs as emulsifiers could also contribute to the higher protein content of the ice cream compared to the typical ice cream that commonly uses artificial emulsifiers. The interaction between hydrocolloid (polysaccharide) and protein in ice cream is important in stabilizing the emulsion properties of the ice cream.

Fat content in the ice cream was determined using Soxtec method (AOAC, 2000). The total fat content in the ice cream which obtained from milk and egg contributed to the smooth texture, creamy and rich body of ice cream. Although the hydrocolloids are hydrophilic, however the presence of the fat and hydrocolloids contributes to the emulsion properties of the ice cream in which it may affect the texture and the quality of the ice cream. Generally, there was no significant difference ($p > 0.05$) in the fat content among all the formulations. However, the control showed the highest fat content (12.68%) followed by GG (12.17%), XG (10.96%) and CMC (8.02%). Theoretically, the hydrocolloid binds with the water, emulsifier and fat contained in the mix to stabilize the mix when added to the ice cream mixture (Mousavi *et al*., 2003). Ice cream without the incorporation of hydrocolloids uses emulsifier, such as eggs to bind the water and fat in the mix. When hydrocolloids were added, the proportion of water, emulsifier and fat that bound was changed. This might be a reason for the decrease in fat content in the FCI formulations that incorporated with hydrocolloids.

Similar to the protein and fat content, the pH value

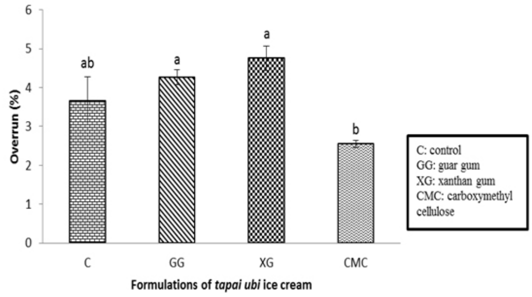


Figure 1. Overrun of different fermented cassava (*tapai ubi*) ice cream formulations (C: control, GG: guar gum, XG: xanthan gum and CMC: CMC incorporated)

*value with the same superscript are not significantly different ($p > 0.05$)

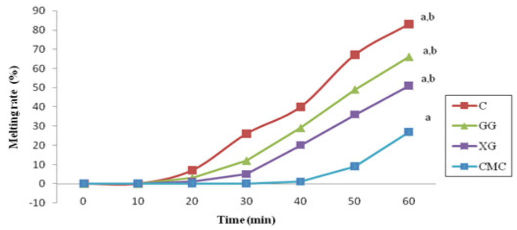


Figure 2. Melting rate of different fermented cassava (*tapai ubi*) ice cream formulations.

*value with the same superscript are not significantly different ($p > 0.05$)

*C: Control, GG: *Tapai ubi* ice cream with guar gum, XG: *Tapai ubi* ice cream with xanthan gum, CMC: *Tapai ubi* ice cream with carboxymethyl cellulose

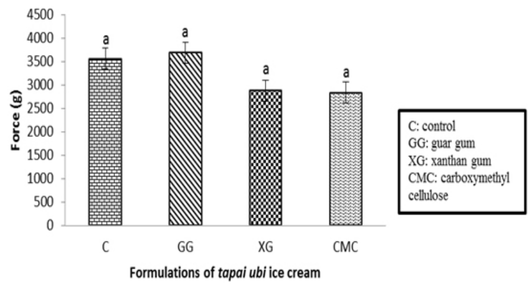
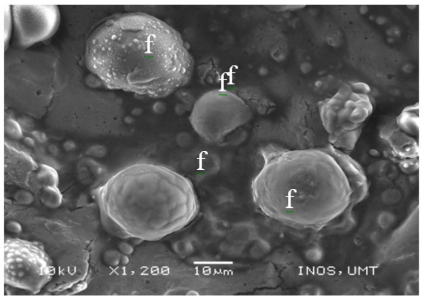


Figure 3. The hardness of different fermented cassava (*tapai ubi*) ice cream formulations. (C: control, GG: guar gum, XG: xanthan gum and CMC: CMC incorporated).

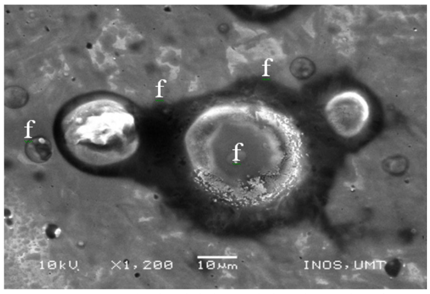
*value with the same superscript are not significantly different ($p > 0.05$)

of different ice cream formulations also showed that there was no significant difference ($p > 0.05$) between different formulations. Ice cream incorporating CMC showed the highest pH value (pH 6.42) followed by XG (pH 6.07), control (pH 5.91) and GG (pH 5.84), respectively. The normal pH of ice cream is pH 6.3 (Choo *et al.*, 2010). The lower value of pH compared to the normal pH might be due to the acid content in fermented cassava. According to Sharma and Hissaria (2009), guar gum is stable in solutions with a pH value between pH 4 to pH 11. Therefore, there was no significant difference between the pH value in the control, and the ice cream incorporating GG. The high pH value obtained in FCI with CMC was due to the treatment on the cellulose while preparing CMC in which cellulose alkaline react with monochloroacetic acid. The reaction of cellulose alkaline with monochloroacetic acid resulted in the high pH value (Izydorczyk *et al.*, 2005).



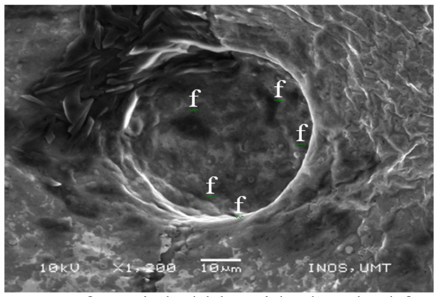
(a) Close up of an air bubble with absorbed fat (f) of ice cream (control)

Bar shown = 10 µm



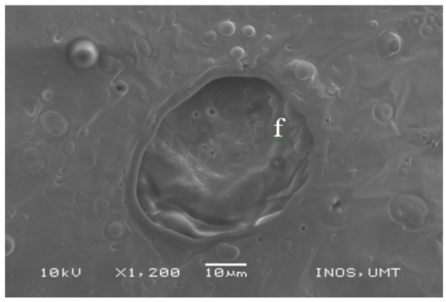
(b) Close up of an air bubble with absorbed fat (f) of ice cream with guar gum

Bar shown = 10 µm



(c) Close up of an air bubble with absorbed fat (f) of ice cream with xanthan gum

Bar shown = 10 µm



(d) Close up of an air bubble with absorbed fat (f) of ice cream with CMC

Figure 4. Microstructure of different fermented cassava ice cream formulations

Overrun

The FCI incorporating xanthan gum (XG) showed the highest overrun (4.76%) followed by GG (4.27%), control (3.67%) and CMC (2.56%). The overrun values of different FCI formulations were presented in Figure 1. There were no significant difference between control, GG and XG ($p > 0.05$), while CMC was significantly different ($p < 0.05$) to the GG and XG but not to the control. The overrun with no significant difference in the control, GG and XG may due to the whipping force applied to the ice cream mixture during freezing and the aeration process in the ice cream freezer, which was strong enough to break and distribute air bubbles into the same shape and size (Thaiudom *et al.*, 2008) in all ice cream mixes except the mix comprising the ice cream with CMC. The air bubbles in these mixes were immediately trapped in the unfrozen serum phase by crystallized fat partial coalescence and fixed by ice crystals during the freezing process. However, the air bubbles of ice cream containing CMC might experience less trapping and fixing than by those previously mentioned.

High overrun values indicate a good quality of ice cream. However, the values were varies according to the types of ice cream produced in several researches conducted previously. The overrun values obtained in this study was low as compared to other study. A research conducted by Moeenfard and Tehrani (2008) showed the overrun range of 39% - 47% in ice cream type frozen yogurt incorporated with different type and concentration of stabilizers while Murtaza *et al*. (2004) showed the overrun range of 53% - 58% in ice cream prepared with different stabilizers. The other research conducted by Soukoulis *et al.* (2008) showed the overrun range of 50% - 100% in ice cream with different hydrocolloids and κ-carrageenan. The result of overrun obtained in this study was very low as compared to the previous researches may due to the high starch content in cassava that affecting the properties of ice cream which causing little incorporation of air resulted in heavy ice cream (Moeenfard and Tehrani, 2008). When fermented cassava was added into the ice cream mix, it was difficult for the mixture to incorporate a lot of air, hence, lowering the overrun of the ice cream produced. Furthermore, the study conducted by Moeenfard and Tehrani (2008); Murtaza *et al*. (2004) and Soukoulis *et al.* (2008) did not used additional carbohydrate sources such as cassava, thus, their products had significantly higher overrun as compared to the overrun of fermented cassava ice cream.

Melting rate

The melting rates (%) of different FCI formulations were shown in Figure 2. Generally, the melting rate of different ice cream formulations was not significantly different ($p < 0.05$). The FCI with CMC incorporated presented the lowest melting rate (27.0%) followed by XG (51.0%), GG (65.5%) and the control (83.0%). One function of stabilizers in ice cream is to increase the melting resistance. Hydrocolloids significantly affect the melting quality due to their ability to hold water and enhance micro-viscosity. The influence of stabilizers on the thermal properties of ice cream, such as thermal conductivity, melting onset, and heat of fusion, could affect the melting rate (Bahramparvar and Tehrani, 2011). The melting rate obtained in this study was high for control, XG and GG added. The high the melting rate indicate the easier the ice cream to melt. The lowest percentage of melting rate obtained by FCI with CMC incorporation (27%) indicate a good melting resistance as it slow the melt down of the ice cream. Thus, this result showing that CMC incorporation significantly improves the melting rate of FCI from 83% to 27%.

Besides the nature of hydrocolloids, overrun also plays an important role in lowering the melting rate of ice cream. A higher overrun result in higher air content leading, to a higher apparent viscosity, greater fat destabilization and low melt down (Sofjan and Hartel, 2004). This is because air is a good insulator and undoubtedly slows the rate of heat transfer into the ice cream with higher overrun. The FCI with xanthan gum had greater overrun and the melting rate in this formulation was the second lowest among the others. This shows that the air cells in this formulation had a great ability to inhibit or slow down the heat transfer.

Xanthan gum hydrates quickly once dispersed and provides water binding. As it is heat resistant it possesses a good ability to disperse and bind water in the ice cream mix (Sofjan and Hartel, 2004). Therefore, the melting rate of ice cream incorporating xanthan gum is low. Guar gum, compared to xanthan gum, hydrates well in cold water (Sofjan and Hartel, 2004). When guar gum is incorporated into the hot ice cream mix, it does not disperse very well, and, thus, more free water in the mix forms ice crystals during freezing and storage. The ice crystals in ice cream with guar gum melt rapidly during heat transfer condition resulting in the higher melting rate of the ice cream. There has been limited research in the melting rate of ice cream incorporating hydrocolloids compared to the study on the viscosity of ice cream. However, in the study conducted by Soukoulis *et al.* (2008), the melting rate (gg^{-1} min) of ice cream incorporating guar gum was higher (1.32) than the ice cream with xanthan gum (1.31). The same pattern can be observed in this study.

Colour Measurement

The colour intensity of ice cream was demonstrated as L^*, a^*, b^* values. The L^* value indicates lightness while the a^* and b^* values indicate redness and yellowness, respectively. Table 4 shows

Table 4. L*, a*, b* values of different ice cream formulations

	Mean±SD (n = 4)		
Formulation	L*	a*	b*
C	93.10±0.62[b]	-2.72±0.17[a]	21.18±2.08[a]
GG	94.43±0.49[a]	-3.39±0.63[b]	19.20±0.99[a]
XG	93.10±0.93[b]	-2.25±0.23[a]	21.47±0.22[a]
CMC	93.37±0.30[b]	-2.41±0.09[a]	18.96±0.86[a]

*value with the same superscript within rows are not significantly different (p > 0.05)
*C: Control, GG: Tapai ubi ice cream incorporating guar gum, XG: *Tapai ubi* ice cream incorporating xanthan gum, CMC: *Tapai ubi* ice cream incorporating carboxymethyl cellulose.

Table 5. FTIR spectrum of fermented cassava (*tapai ubi*)

*Assignment	Wavelength (cm^{-1})
O – H stretching (3500-3100)	3469
O – H stretching (3500-3100)	3436
O – H stretching (3400-3200)	3368
C – H stretching (3000-2850)	2930
C ≡ N stretching (2180-2110)	2111
C – H stretching (1450-1375)	1424
C – H stretching (1375-1300)	1369
C – O stretching (1300-1000)	1150
C – O stretching (1300-1000)	1044
C – O stretching (1300-1000)	1033
C – O stretching (1300-1000)	1020
C – H out-of-plane bend (1000-600)	921
C – H out-of-plane bend (1000-600)	770
C – H out-of-plane bend (1000-600)	640

*References for band assignments presented in the text

the L*, a*, b* values for the different FCI formulations. Based on the result, it shows that different types of hydrocolloid significantly affect the lightness and redness of ice cream but not its yellowness. The control and XG had the same lightness (93.10) and were the lightest compared to the GG and CMC. The lightness of GG (94.43) was increases but its redness (-3.39) and yellowness (19.20) was decreased as compared to control. Moreover, XG showed an increase in the redness (-2.25) and yellowness (21.47), while CMC showed an increase in the lightness (93.37) and redness (-2.41) but not in yellowness (18.96).

The addition of GG in the ice cream will increase the concentration of the ice cream mixture (Sharma and Hissaria, 2009). Thus, the increased of the concentration of ice cream resulting to the increased of the ice cream lightness. Therefore the addition of GG in FCI presented in this study was the highest compared to control, XG and CMC added. In contrast, ice cream with xanthan gum showed no significant difference in lightness compared to the control ($p > 0.05$). The natural characteristic of xanthan gum is that it is quickly dispersed into the mixture when it is added (Naresh and Shailaja, 2006). This condition differed in the ice cream with GG, which became concentrated when guar gum was added, while xanthan gum dispersed water bound between molecules rather than concentrating the mixture. Although CMC also contribute in concentrating the ice cream mixture, however, it is pH dependent and unstable in acidic solutions (Izydorczyk *et al.*, 2005). This might be the reason for the increased lightness in the ice cream with CMC compared to the control, although it was not as light as the ice cream with GG.

Hardness measurement

Figure 3 shows the results obtained for the hardness of different FCI formulations. The incorporation of hydrocolloids showed no significant difference ($p > 0.05$) between the ice cream formulations. The GG was the hardest followed by the control, XG and CMC. In fact, the hardness of ice cream depends on the ingredients used and the process conditions (Soukoulis *et al.*, 2008). However, during this study, all ingredients and processes were controlled. Thus, the hardness presented in Figure 3 reflects the hardness of the ice cream affected by the incorporation of different hydrocolloids – GG, XG and CMC.

Moreover, the ice crystal size and ice phase volume may contribute to the hardness of ice cream (Klesment *et al.*, 2011). The FCI incorporation with guar gum conducted in this study seemed to be a poor cryoprotectant. However, this result is different from the research conducted by Soukoulis *et al.* (2008) which CMC was proved to be a poor cryoprotectant instead of guar gum due to its high hardness over the storage period. This condition might be due to the mixture of the hydrocolloids with κ-carrageenan used in their study in which k-carrageenan acts as a gelling agent and its ability to control recrystallization. Therefore, ice cream with the incorporation of guar gum and k-carrageenan had high hardness value. Meanwhile, our study was only used one type of hydrocolloid which leads to the different hardness of ice cream. In addition, the hardness of ice cream can be compared to its melting rate in which the harder the ice cream the higher the melting rate while softer ice cream has a lower melting rate. This condition is caused by the ice crystals formed during the freezing storage, which lead to a faster meltdown in the harder ice cream texture. In this case, the ice cream with guar gum, which was the hardest ice cream, was expected to have the largest number of ice crystals (Soukoulis *et al.*, 2008). However, based on the results of the melting rate in Figure 2, the control sample showed the highest melting rate instead of the ice cream with guar gum. This might be because the guar gum still functioned well to stabilize the ice cream despite the formation within it of many ice crystals.

Fourier Transform Infrared (FTIR)

FTIR spectrum of fermented cassava (tapai ubi)

Table 5 presents the FTIR spectrum of fermented cassava. Basically, fermented products contain alcohol compounds from the carboxyl and hydroxyl group. The presence of protein, as shown in the results, was due to the protein contained in the cassava. Siong *et*

Table 6. FTIR spectrum for different fermented cassava (*tapai ubi*) ice cream formulations

Assignment	Wavenumber (cm^{-1})			
	C	GG	XG	CMC
O – H stretching (3500-3100)	3440 - 3435	3483 - 3409	3433 – 3328	3468 – 3428
C – H asymmetric stretching (3000-2850)	2925 – 2920	2922 - 2922	2922 - 2920	2923 – 2922
C – H stretching (3000-2850)	2852 – 2851	2852 - 2852	2852 – 2851	2852 - 2852
C = O (1750-1730)	1742 – 1742	1742 - 1742	1742 - 1741	1742 - 1742
C = O stretching (1680-1630)	1638 -1634	1635 - 1635	1639 – 1638	1635 - 1635
C – H wagging band progression (1450-1375)	1417 – 1416	1416 - 1414	1459 - 1415	1416 - 1415
C – O (1300-1000)	1058 - 1055	1052 - 1052	1056 - 1053	1056 - 1052

*References for band assignments presented in the text
*C: Control, GG: *Tapai ubi* ice cream incorporating guar gum, XG: *Tapai ubi* ice cream incorporating xanthan gum, CMC: *Tapai ubi* ice cream incorporating carboxymethyl cellulose

al. (1997) stated that there is 0.8 g of protein in 100 g of raw cassava. O – H stretching bands at 3469 cm^{-1} and 3436 cm^{-1} showed the presence of water in fermented cassava (Pavia *et al*., 2009) while O – H stretching band at 3368 cm^{-1} was the hydroxyl group of this fermented product. The band is identified as the hydroxyl group in reference to Pavia *et al.* (2009) who stated that the O – H band absorbs infrared between 3400 cm^{-1} to 3200 cm^{-1}.

Furthermore, the C – H stretching bands at 2930 cm^{-1}, 1424 cm^{-1}, 1369 cm^{-1} are alkane groups absorbed by the infrared spectra (Skoog *et al*., 2007) while the C – O stretching bands appeared at 1150 cm^{-1}, 1044 cm^{-1}, 1033 cm^{-1} and 1020 cm^{-1}, which are the carboxyl group contained in fermented cassava (Pavia *et al*., 2009). The aromatic compound of the carbonyl group in the product was absorbed by infrared spectra at 921 cm^{-1}, 770 cm^{-1} and 640 cm^{-1}. According to Pavia *et al.* (2009), C – H out-of-plane bending vibrations appear between 900 cm^{-1} and 690 cm^{-1}.

FTIR spectrum of fermented cassava ice cream (FCI)

Table 6 depicts the FTIR spectrum for different ice cream formulations, control, GG, XG and CMC. As every ice cream produced incorporated fermented cassava (*tapai ubi*), the presence of the hydroxyl compound also needs to be discussed. It can be seen in Table 6 that the incorporation of guar gum, xanthan gum and carboxymethyl cellulose affected the wavenumber (cm^{-1}) of the same group of compounds. Table 6 shows that the addition of the fermented cassava to FCI had increased the OH group in the ice cream as compared to the control. The functional group of -OH that obtained from the GG added in FCI was the highest in intensity compared to FCI with XG and CMC added. Although the hydrocolloids addition affected the intensity of -OH group of the ice cream, we found that the structure of the ice cream was not affected. Besides that, the addition of the fermented cassava and hydrocolloids in ice cream did not affect the carboxyl, C-O group that present in the ice cream. There is no structure change with the addition of hydrocolloids in the ice cream although it gave different results on physical properties such as overrun, melting rate and hardness of the FCI. The changes that affected by the addition of hydrocolloids may influence the quality depending the values obtained for the physical properties of the ice cream.

According to Stuart (2004), the C – H stretching mode at 2920 cm^{-1} is the strongest band in the spectra while the wavenumber of the band is 'conformative-sensitive' and responds to the changes of the trans/gauche ratio in the acyl chains. The incorporation of different hydrocolloids in ice cream did not significantly affect the band of $–CH_2$ groups of the lipid. Therefore, the C – H stretching band of the lipid in GG, XG and CMC is within the range of the band in the control, that is, at 2925 – 2020 cm^{-1}. In addition, there is another C – H band of lipid at 1459 – 1414 cm^{-1}, as shown in all the ice cream formulations. This is supported theoretically by Stuart (2004) who found that the C – H wagging band progression of $–CH_2$ groups appeared at 1400 – 1200 cm^{-1}.

The shift of the wavenumber of amide I band in every ice cream formulation was due to the presence of phospholipids (Alzagtat, 2002). The interaction of protein and lipid can also be seen on C = O stretching band at the peak between 1742 – 1741 cm^{-1}. According to Alzagtat (2002), the presence of the peak at 1740 cm^{-1} showed the assignment of carbonyl (COOH) stretching group indication lipid molecules. The shift of the wavenumber of the carbonyl group in all ice cream formulations might be due to the presence of protein in the ice cream mixture. The shifting of the wavenumber can still be considered as the carbonyl group of the lipid because, according to Han *et al*. (1998), the absorption of the carbonyl group band occurs between 1770 and 1710 cm^{-1}. In addition, Alzagtat (2002) supported the shifting of the wavenumber in which the presence of lipid-induced changes in the secondary structure of the protein; in this analysis amide I, by shifting some of the bands from 1 – 3 cm^{-1} wavenumbers.

The presence of the hydroxyl group band in every ice cream formulation was due to the mixing of *tapai ubi* in the ice cream mixture. Pavia *et al*. (2009) stated that the hydrogen bonded hydroxyl group absorbed infrared between 3400 – 3200 cm^{-1}. The incorporation of different hydrocolloids had obviously shifted the O – H stretching band of the hydroxyl group in the ice cream formulations when compared to the control. Based on Table 6, the absorption of O – H stretching band of hydroxyl group in the ice cream decreased from 3435 cm^{-1} to 3409 cm^{-1} when guar gum was added. According to Mazumder *et al*. (2010), the O – H stretching band of guar gum appeared at 3407 cm^{-1}. When the guar gum was incorporated into the

Table 7. Microbiological quality of different ice cream formulations

Test	C	GG	XG	CMC
Total plate count (CFU/ml)	4.5 x 10^3	7.0 x 10^3	4.3 x 10^3	1.23 x 10^4
Presence of *E. coli sp.*	Absent	Absent	Absent	Absent

*C: Control, GG: *Tapai ubi* ice cream incorporating guar gum, XG: *Tapai ubi* ice cream incorporating xanthan gum, CMC: *Tapai ubi* ice cream incorporating carboxymethyl cellulose.

ice cream mixture, the band shifted to 3409 cm^{-1}.

The O – H stretching band of the ice cream with xanthan gum was between 3433 cm^{-1} and 3328 cm^{-1}. The assignment was due to the presence of xanthan gum and is supported by Gilani *et al.* (2011) who stated that the hydroxyl group of standard xanthan gum appeared at 3386 cm^{-1}. However, there is no evidence that the hydroxyl group is present in carboxymethyl cellulose, although the carboxyl group (C – O) is present in CMC at 1078 – 1021 cm^{-1} (Wang and Somasundaran, 2005). Based on Table 6, the C – O band of ice cream with CMC can be seen at 1056 – 1052 cm^{-1}, which falls between the ranges of infrared absorption of the carboxyl group.

Microstructure

Figure 4 shows the close up view of air bubbles with absorbed fat for the different formulations of ice cream. Fat at the air interface tends to be more in the form of discrete droplets (Goff, 2002). The ice cream with GG had a uniform absorption of fat and the size of air cells was smaller compared to the control. Sharma and Hissaria (2009) stated that the guar gum had a shear thinning characteristic that helped to maintain the smaller size of air bubbles during the freezing and stabilized the ice cream structure. The pseudoplastic property of guar gum made it able to bind to the fat contained in the ice cream mixture (Sharma and Hissaria, 2009). The small size of air cells in the ice cream is desirable and it's indicated a good quality of ice cream. As referred to Goff (2000), the fat content in ice cream will form a continuous mass surrounding the spaces which formerly held the air bubbles. Therefore the difference in uniformity at the interface obtained with different types of the hydrocolloids added demonstrated the effect of fat absorption at the air interfaces. The ice cream with XG also had a uniform absorption of fat and smaller air bubbles compared to the control. According to Naresh and Shailaja (2006), xanthan gum can quickly disperse into the mixture and bind the water between the molecules resulting in smaller air cells during the freezing of ice cream. Similar to guar gum, xanthan gum also has a pseudoplatic property that can bind fat molecules in the mixture (Zhao *et al*., 2009). Therefore, the size of air bubble was smaller in the ice cream with XG while the distribution of fat was more uniform compared to the control.

Table 8. Acceptance level of different ice cream formulations

	Mean±SD (n = 4)				
Formulation	Colour	Odour	Body and texture	Resistent to melting	Overall acceptance
C	4.83±1.09[a]	4.63±1.35[a]	4.17±1.64[a]	3.61±1.47[b]	4.46±1.26[a]
GG	4.73±1.39[a]	4.20±1.40[a]	3.55±1.50[b]	3.71±1.78[b]	4.29±1.36[a]
XG	4.97±1.10[a]	4.43±1.70[a]	4.20±1.65[a]	4.64±1.68[a]	4.75±1.35[a]
CMC	4.60±1.25[a]	4.77±1.22[a]	4.23±1.25[a]	4.04±1.60[a]	4.29±0.94[a]

*value with the same superscript within rows are not significantly different ($p > 0.05$)

*C: Control, GG: *Tapai ubi* ice cream incorporating guar gum, XG: *Tapai ubi* ice cream incorporating xanthan gum, CMC: *Tapai ubi* ice cream incorporating carboxymethyl cellulose.

Although it can be seen that the absorption of fat in the control and the ice cream CMC, the amount was not as great as in the GG and XG, while the size of the air bubble in ice cream with CMC was difficult to explain. It is known that carboxymethyl cellulose is unstable in acidic conditions (Izydorczyk *et al*., 2005). Therefore, it was observed that the fat bound by CMC was not as efficient as using guar gum or xanthan gum resulting in less uniform absorption of fat.

Microbiological quality

Table 7 shows the microbiological quality of different ice cream formulations. The FCI incorporating CMC showed the highest total plate count (1.23 x 10^4 CFU/ml) followed by GG (7.0 x 10^3 CFU/ml), the control (4.5 x 10^3 CFU/ml) and XG (4.3 x 10^3 CFU/ml). However, the total plate count in all ice cream formulations obtained was below the highest safety limit prescribed in the Malaysia Food Act 1983 and Regulation 1985, which is 5.0 x 10^4 CFU/ml. There are several reasons for the different readings of the total plate count for different hydrocolloids. One of the reasons is the difference in temperature fluctuation experienced by all ice cream formulations. Ice cream with a higher plate count, such as the FCI with CMC, might experience more frequent temperature fluctuation that allows the presence of microorganisms compared to the FCI with a lower plate count, such as FCI with XG. There was no presence of *E. coli* in any of the ice cream formulations. According to Kanbakan *et al.* (2004), the average microbial count can increase up to 7 times during the production, transportation and storage of ice cream. Therefore, good sanitation and hygienic practice should be implemented to get a better microbial quality of ice cream produced.

Sensory evaluation

Table 8 shows the acceptance level of FCI incorporating different hydrocolloids. There was no significant difference ($p > 0.05$) among any of the formulations in terms of colour and odour while there was a significant difference ($p < 0.05$) between GG and other formulations in terms of body and texture and resistance to melting. Ice cream with xanthan gum scored the highest acceptability in terms of its colour, resistance to melting and also overall acceptance. The decrease in the colour intensity of ice cream did not ensure its acceptability to consumers. Ice cream with xanthan gum, which was the lightest, was the most accepted. However, the colour of the ice cream with CMC was least accepted although it was not the darkest instrumentally.

Although the ice cream with CMC was the least accepted in terms of its colour, its odour was the most acceptable. This result shows that the natural odour of CMC blended well with the odour of the FCI. The acceptability of the body and texture of the ice cream with CMC in sensory test conducted was in the same agreement with the hardness value obtained as described in 3.6. The ice cream with CMC was the least hard, and hence, it scored the highest for the attribute of body and texture. Hydrocolloids that are good in cryoprotective action show the highest resistance to melting. In this study, xanthan gum had the most effective action. This agrees with the study conducted by Soukoulis *et al.* (2008) who found that xanthan gum was the most efficient cryoprotectant based on instrumental data.

Soukoulis *et al.* (2008) found that ice cream with xanthan gum scored the highest in terms of its vanilla flavour when compared to ice cream with guar gum, while Klesment *et al.* (2011) showed the same acceptance level. In this study, the odour of ice cream could describe its flavour. However, the study showed that FCI with CMC had higher acceptance in terms of its odour compared to the FCI with XG, however FCI with XG was greater than FCI with GG. This might be caused by the addition of *tapai ubi*, which affected the vanilla odour of the ice cream. In terms of its colour and hardness, the FCI with XG was more accepted than the FCI with GG. The results obtained in this study are in agreement with the study conducted by Soukoulis *et al.* (2008). According to Soukoulis *et al.* (2008), xanthan gum has a remarkable cryoprotective action that could maintain the hardness of ice cream even when it is stored for a long period of time. FCI contained fermented cassava, thus, lowering its pH value. When hydrocolloid was added, the pH value of the FCI will changed. Therefore, it will affect the taste and acceptability of the FCI.

Conclusion

Overall, each hydrocolloid had its own advantages in various physical and chemical properties as well as the sensory acceptability of ice cream. Although not all the properties were significantly affected by the incorporation of different hydrocolloids in ice cream, the ice cream with xanthan gum showed the best effect on overrun property and overall acceptability. Meanwhile, ice cream with guar gum possessed the highest value of lightness and hardness property. Result obtained showed that the incorporation of CMC only contribute to the good melting resistance. However, the ice creams produced were microbial safe because the total plate counts in all samples were below the standard as prescribed in Food Act 1983 with no presence of *E. coli.*

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