

# Effect of Storage Conditions on Quality of Prebiotic Dark Chocolate

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## ABSTRACT

**Introduction:** A prebiotic such as inulin is a well-known functional plant food ingredient. It is capable of stimulating growth of beneficial bifidobacteria in the intestine thus protecting against intestinal infections, preventing constipation, increasing mineral absorption, reducing the incidence of colon cancer, and producing B vitamins. Inulin added to food therefore has to be stable during food processing especially against heat treatment, low pH and Maillard reaction.

**Methods:** Newly developed dark chocolate, DC-1, containing inulin (replacing sugar component) as an added value, was stored at 18°C, 60% relative humidity and 25°C, 80% relative humidity (RH) to determine shelf life stability compared to control dark chocolate, DC-0 (with high content of sugar). Sensory evaluation (quantitative descriptive analysis), water activity ( $a_w$ ), microbiological content and presence of inulin after storage of the prebiotic chocolate under both conditions were evaluated to determine shelf life. **Results:** The DC-1 chocolate had at least 12 months of shelf life at 18°C, 60% RH with better acceptance than DC-0; moreover, it did not experience microbiological and inulin content changes. At 25°C, 80% RH, the growth of *Aspergillus* sp. was observed on the surface of both DC-0 and DC-1 with  $a_w > 0.50$  after a 2-month storage. **Conclusion:** Shelf life stability of DC-1 is almost similar to DC-0.

**Keywords:** Inulin, dark chocolate, shelf life, water activity and sensory evaluation.

## INTRODUCTION

Problems related to storage stability are common to the food industry, and therefore storage studies are an essential part of product development and improvement or maintenance programmes (Marta & Josenete, 2006). Ordinary chocolate normally has a shelf life of 12 months and

such food products are recognised as microbiologically, chemically and organoleptically shelf stable (Man, 2002). The shelf life of chocolate is a period of time during which it will retain acceptable appearance, aroma, flavor and texture. The shelf life of chocolate depends on several parameters including: storage temperature and humidity, availability of oxygen in the

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immediate environment, packaging material used, as well as the addition of other ingredients such as fats, nuts etc (Nattress *et al.*, 2004). The type of packaging material used for chocolates varies; generally, aluminium foil, composite films and paper or plastic trays are used. The packaged chocolates are known to keep their quality up to 5 months when stored at 10–18°C and 60–70% relative humidity.

The actual storage period, however, may be extended for a longer duration in the distribution network/ retail market. 'Normal' storage temperature and relative humidity suggested is 18°C, 60% RH respectively, while storage at 25°C, 80% RH has been considered a harsh condition (Man & Jones, 2000).

Chocolate is known to provide nutritional benefits. The global confectionery market including chocolate products was estimated to exceed USD73.2 billion per annum and the annual global consumption of chocolate confectionery was estimated at 6.5 million tonnes (CAOBISCO, 2004). Evidence suggests that consumption of dark chocolate increases serum HDL cholesterol by 11.4% (Mursu *et al.*, 2004). Replacing the whole content of sugar and fat in chocolate is of high interest in order to add nutritional value. Bakery and dairy industries have been using inulin or prebiotics as a substitute for fat and sucrose which allow for an improvement in both taste and texture (Guven *et al.*, 2005; Mandala, Polaki & Yanniotis, 2009).

Inulin also offers nutritional advantages due to its prebiotic properties as it is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improving the health of the host (Gibson & Roberfroid, 1995). Inulin added in food therefore has to be stable during food processing especially against heat treatment, low pH and Maillard reaction. It is also important that such alternatives do not

cause significant changes in the sensory characteristics of the product (Bolini-Cardello, Da Silva & Damasio, 1999). Consumers tend to purchase a product on the basis of the sensory experience which it delivers, for instance, sweetness, softness, chocolateness, odour, flavour, aftertaste, etc. For this reason, sensory evaluation is undoubtedly the most appropriate type of test for evaluating changes during storage trials (Kilcast, 2000).

Traditionally, chocolate is regarded as being microbiologically stable and safe to eat. Owing to the inherent low water activity of chocolate, it is unlikely to support the growth and proliferation of bacterial pathogens (Baylis *et al.*, 2004). However, spoilage can occur as a result of the growth of osmophilic yeasts and xerophilic molds on chocolate which is categorised as a high sugar product (Brown, 1976). Species of *Aspergillus*, *Eurotium*, *Chrysosporium* and *Wallemia* are the most frequently occurring xerophilic fungi.

Such problems require us to carefully formulate and process the prebiotic dark chocolate in order to retain the inulin activity during long term storage as a major sugar replacer with incorporation of smaller amounts of isomalt (polyol). Therefore, our study aims were to determine and compare shelf life stability between prebiotic (with added inulin) and control (without inulin) dark chocolates at different temperatures by means of chemical and microbiological analysis, as well as by sensory evaluation. The incorporation of inulin and isomalt in sucrose-free prebiotic dark chocolate and their influence on a product's shelf life are little reported.

## METHODS

### Materials

As previously described by Norhayati *et al.* (2008), prebiotic dark chocolate was prepared using 3 in 1 concher machine (200 kg capacity) containing ingredients such as

cocoa solid, cocoa butter, milk components, emulsifier, a flavour component and sweeteners (inulin and a small amount of isomalt). Addition of small amounts of isomalt was due to the less sweet tasting inulin powder. A control dark chocolate (DC-0) which has sucrose as a sweetener (an original recipe) was also prepared for comparison purposes. Among other ingredients used include cocoa solid purchased from Selbourn Food Services at Pelabuhan Klang, Malaysia, milk powder from Promac Enterprises Sdn. Bhd., cocoa butter from Malaysia Cocoa Manufacturing Sdn. Bhd., isomalt from Nutrisweet & Food Specialties Sdn. Bhd., and prebiotic inulin extracted from chicory root (Sensus, The Netherlands). These chocolates (DC-0 and DC-1), in chocolate boxes lined with bubble plastic, were stored at 18°C, 60% RH (in a chiller cabinet, designated as DC-1a and DC-0a) and 25°C, 80% RH (in a controlled humidity chamber, designated as DC-1b and DC-0b) for 12 months.

### Microbiological analysis

Development of fungal growth was also observed with naked eyes especially on chocolate surface with white or mycelia spot in relation to storage at high humidity. In addition, other microbiological tests such as *Salmonella*, *Escherichia coli* and Total Plate Count (TPC) were also been carried out before evaluating shelf-life of the chocolate to ensure that no contamination had occurred (IOCCC, 1990). The experiment was stopped if samples in any storage condition showed the existence of fungal growth on their surfaces. Fungi grown on the chocolate samples were observed under a light microscope at 200x magnification after proliferation on Potato Dextrose Agar (PDA).

### Water activity ( $a_w$ )

Determination of water activity was carried out using  $a_w$  meter (AwC203-RS-C,

Novasina, Switzerland) on chocolate sample contaminated with growth of fungus and compared with a fresh sample. The samples need to be cut into small pieces of similar sizes without touching the surface with fingers. Calibration was done using control salt at specified relative humidity (11.3% - 98%) and temperature, 25°C. Results from three replications of each sample were taken after stabilisation for 20 min at 25°C (Man, 2002).

### Inulin determination

DC-1, before and after storage, was analysed for inulin content using HPLC to observe whether inulin content decreased or degraded subsequent to prolonged storage. About 1.0 g of a homogenised sample was weighed into a 200 ml beaker, treated with ca. 100 ml of boiling water at pH 6 to 8 and kept at 85°C with continuous magnetic stirring on a hot plate for 15 min. After cooling to room temperature, the volume was made up to 100 ml and the solution was filtered through a 0.20 mm membrane filter before injection (Zuleta & Sambucetti, 2001) into the HPLC.

The HPLC instrumentation consisted of a Waters 1525 Binary HPLC Pump, Waters 717 plus Autosampler (injector with a 50 ml sample volume), an Aminex HPX 42A (Bio Rad) anion exchange column and Waters 2414 Refractive Index detector. Deionised water at 85°C was used as the HPLC mobile phase at a flux rate of 0.6 ml/ min. Calibration curves were plotted with 0.005 to 1 g/ 100 ml of inulin as standard. All determinations were carried out in triplicate of three independent experiments.

### Sensory analysis

Evaluation of sensory attributes for each sample stored at different storage conditions included appearance or colour, odour, hardness and taste using quantitative descriptive analysis. These attributes were selected by trained panelists after several

training sessions. Each attribute was evaluated according to a numerical scale, 1-5, with 5 being better than standard (acceptable); 4 being same as standard (acceptable); 3 showing slight difference, nothing undesirable (tolerable); 2, inferior to standard (rejected); 1, much inferior (rejected). The score was based on its comparison with a fresh sample of newly prepared chocolate at the time of tasting (Man & Jones, 2000). One attribute having a reject score means that the stored chocolate must be rejected; thus in this study average 3 and above scores were used as acceptance levels. Evaluation of the sensory properties was carried out by 12 trained panelists. Sensory analysis was carried out in air-conditioned booths with white light. Crackers and taste-free water provided for palate cleansing.

### Statistical analyses

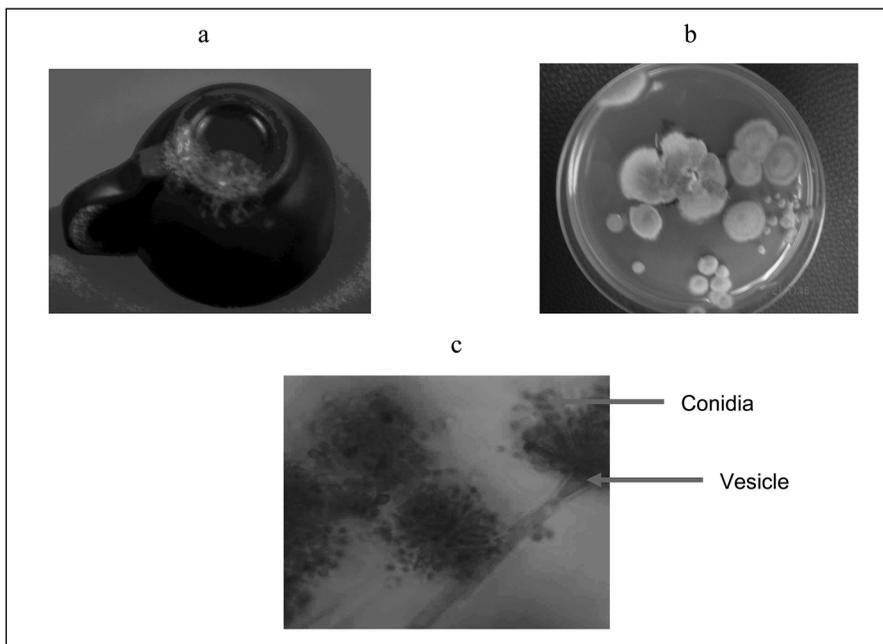
All data obtained from three replications of analysis were analysed using SPSS Inc. software (version 14.0). A two-factor analysis

of variance using the General Linear Model procedure was used to determine significant difference between samples and each month of storage with a significance level of  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Microbiological analysis

Microorganism contamination did not occur before initiation of shelf life testing. Both chocolates (DC-1a and DC-0a) were able to withstand the storage conditions of 18°C, 60% RH without obvious (naked eye) development of sugar blooms (whitish spots) during the 12-month storage. However, for chocolates DC-1b and DC-0b stored at 25°C, 80% RH (Figures 1a, b and c), we had to stop the analysis before the product reached the third month of storage due to fungal growth observed on the surface of both chocolates. The colours at the centre of the colony on PDA agar turned from yellow to green (Figure 1b). The erect hyphal branch developing from the foot cell are the conidiophores which enlarge at the



**Figure 1.** Growth of fungus, *Aspergillus* sp. on the surface of dark chocolate samples, DC-0b stored for 2 months at 25 °C, 80% RH.

apex to form a vesicle (Figure 1c). The fertile area of the vesicle gives rise to a layer of cells that produce mitotic spores called conidia or conidiospores.

The appearance of a bloom reflects a defect to chocolate quality but it is still safe to consume. However, in the presence of fungal growth at storage condition of 25°C, 80% RH identified as *Aspergillus* sp. (Ascomycota group), the chocolate is definitely unsafe to consume. This may be due to fast initial condensation occurring at higher environmental humidity (80% RH). Our finding also shows that a high humidity storage condition at an elevated temperature does enhance fungal growth on the chocolates when the samples reached a storage period of 3 months.

Temperature and relative humidity are important extrinsic factors in determining whether a food will get spoilt. Generally, at higher relative humidity, microbial growth will be initiated more rapidly (Prescott, Harley & Klein, 1993) and food will be spoilt at a faster rate. Consequently, both samples of DC-0b and DC-0b used in this study were rejected for being microbiologically unsafe (end of shelf life). Cordier (1994) also stated that occurrence of condensation was one reason which had the potential to cause the growth of microbes especially fungus. Therefore, suitable room designs and pipe insulation or equipment in the environment during product storage will be able to reduce the risk. Meanwhile, Lees & Jackson (1995)

reported that confection products wrapped inside a closed container during storage with an equivalent relative humidity of more than 70% will stimulate the proliferation of fungus. Therefore, prebiotic dark chocolate in this study had to be disposed due to unsuitable storage condition which caused a defect in chocolate quality, making it unsafe for consumption.

### Water activity

Table 1 shows the comparison of water activity data for freshly made prebiotic (DC-1 fresh) dark chocolate, prebiotic chocolate stored at 25°C, 80% RH (DC-1b), fresh control dark chocolate (DC-0 fresh) and control chocolate stored at 25°C, 80% RH (DC-0b). Only DC-1b and DC-0b were tested for water activity content due to the presence of fungal growth. Both types of dark chocolate stored at 25°C, 80% RH (DC-1b and DC-0b) had higher water activity value ( $a_w > 0.5$ ) than freshly made chocolate ( $a_w < 0.4$ ).

Determination of water activity on chocolate samples with fungal growth showed that  $a_w > 0.5$  was a critical factor during storage at 25°C, 80% RH. Such a storage condition stimulates growth of *Aspergillus* sp. although chocolate is considered a low water activity product. In the same way, grains, nuts and spices, all of which have relatively low water activity, are regularly attacked by moderately xerophilic species of *Aspergillus* (Lacey, 1994).

**Table 1.** Water activity values ( $a_w \pm$  sd) for dark chocolates kept at 25°C, 80% RH compared to freshly made dark chocolate

Sample	Water activity value ( $a_w$ )
DC-0b	0.52 ± 0.03 <sup>a</sup>
DC-1b	0.51 ± 0.05 <sup>a</sup>
DC-0 fresh	0.37 ± 0.01 <sup>b</sup>
DC-1 fresh	0.35 ± 0.01 <sup>b</sup>

<sup>a,b</sup> Means followed by different letters in the same column shows significant different mean values at  $p < 0.01$

DC-0b = Control dark chocolate

DC-1b = Prebiotic dark chocolate stored at 25°C, 80% RH

DC-0 fresh = Freshly made control dark chocolate

DC-1 fresh = Freshly made prebiotic dark chocolate

Other researchers report that abiotic factors such as temperature, presence of moisture or other source of nutrients are determinants of filamentous fungi ecology (Marin *et al.*, 1998; Klein and Paschke, 2004). Leong, Hocking & Scott (2006) also state that there is a complex relation between  $a_w$  and temperature, state of fungus (whether in the form of dormant spores or actively growing mycelium), and presence of nutrients or microflora from the environment.

### Inulin content

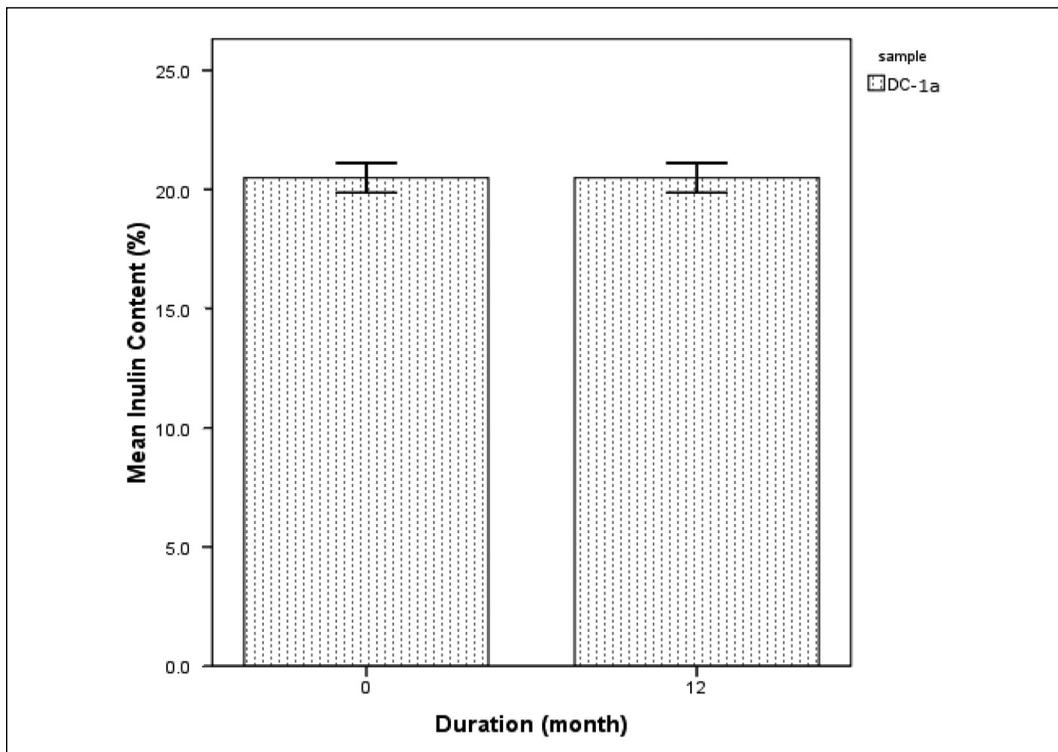
Figure 2 shows a comparison of inulin content of the DC-1a (chocolate with inulin) before and after 12 months storage at 18°C, 60% RH. This was performed to determine whether prolonged storage will result in significant changes in the added inulin. The results showed that there was no significant difference ( $p>0.05$ ) in inulin content before

and after prolonged storage (12 months) at 18°C, 60% RH.

After 12 months of storage at 18°C (60% RH), all the initial inulin was still significantly ( $p<0.05$ ) available in the DC-1a when determined using HPLC technique as described by Zuleta & Sambucetti (2001). Bohm *et al.* (2005) report that inulin from chicory or Jerusalem artichoke started to significantly degrade when exposed to temperatures between 135°C and 195°C for 60min; thus the selected storage condition in this study should not affect inulin performance in chocolates. Commonly, in chocolate making, the temperature will not exceed 100°C, thus inulin added to chocolate should not degrade.

### Sensory analysis

Table 2 indicates that the hardness and appearance/ colour attribute of DC-0a and



**Figure 2.** Comparison of mean inulin content  $\pm$  sd for prebiotic dark chocolate before and after 12-month storage at 18° C, 60 % RH.

**Table 2.** Mean value of hardness, taste, odour and appearance/ colour scores for control (DC-0a) and treated (DC-1a) dark chocolate stored at 18°C, 60% RH for 12 months.

Month	DC-0a				DC-1a			
	Hardness	Taste	Odour	Appearance/ Colour	Hardness	Taste	Odour	Appearance/ Colour
0	3.57 <sup>a,b</sup>	3.71 <sup>a</sup>	4.00 <sup>a</sup>	3.57 <sup>a,b</sup>	3.14 <sup>a</sup>	3.29 <sup>a</sup>	3.57 <sup>a</sup>	3.71 <sup>a</sup>
2	3.43 <sup>a,b</sup>	2.57 <sup>a</sup>	3.57 <sup>a</sup>	4.29 <sup>b</sup>	3.86 <sup>a</sup>	3.14 <sup>a</sup>	3.57 <sup>a</sup>	4.14 <sup>a</sup>
4	3.86 <sup>b</sup>	2.71 <sup>a</sup>	3.57 <sup>a</sup>	3.86 <sup>a,b</sup>	3.43 <sup>a</sup>	3.43 <sup>a</sup>	3.43 <sup>a</sup>	4.00 <sup>a</sup>
6	3.14 <sup>a,b</sup>	3.00 <sup>a</sup>	2.86 <sup>a</sup>	4.14 <sup>a,b</sup>	3.43 <sup>a</sup>	3.43 <sup>a</sup>	3.57 <sup>a</sup>	3.43 <sup>a</sup>
8	3.14 <sup>a,b</sup>	2.71 <sup>a</sup>	2.86 <sup>a</sup>	3.14 <sup>a</sup>	3.71 <sup>a</sup>	3.71 <sup>a</sup>	3.00 <sup>a</sup>	3.71 <sup>a</sup>
10	2.43 <sup>a,b</sup>	2.57 <sup>a</sup>	3.14 <sup>a</sup>	3.86 <sup>a,b</sup>	2.86 <sup>a</sup>	3.86 <sup>a</sup>	3.14 <sup>a</sup>	3.57 <sup>a</sup>
12	2.29 <sup>a</sup>	2.86 <sup>a</sup>	3.57 <sup>a</sup>	3.29 <sup>a,b</sup>	2.71 <sup>a</sup>	3.10 <sup>a</sup>	3.29 <sup>a</sup>	3.29 <sup>a</sup>

<sup>a,b</sup> Means followed by different letters in the same column show significant difference at  $p < 0.05$

DC-0a = Control dark chocolate stored at 18°C, 60% RH

DC-1a = Prebiotic dark chocolate stored at 18°C, 60% RH

**Table 3.** Mean value of hardness, taste, odour and appearance/colour scores for control (DC-0b) and treated (DC-1b) dark chocolate stored at 25°C, 80% RH for 2 months.

Month	DC-0b				DC-1b			
	Hardness	Taste	Odour	Appearance/ Colour	Hardness	Taste	Odour	Appearance/ Colour
0	3.43 <sup>a</sup>	3.29 <sup>a</sup>	3.43 <sup>a</sup>	3.14 <sup>a</sup>	3.00 <sup>a</sup>	3.43 <sup>a</sup>	3.43 <sup>a</sup>	3.14 <sup>a</sup>
1	3.43 <sup>a</sup>	3.00 <sup>a</sup>	3.29 <sup>a</sup>	3.00 <sup>a</sup>	2.86 <sup>a</sup>	3.29 <sup>a</sup>	3.14 <sup>a</sup>	3.00 <sup>a</sup>
2	3.14 <sup>a</sup>	3.29 <sup>a</sup>	3.71 <sup>a</sup>	3.29 <sup>a</sup>	Nil	Nil	Nil	Nil

<sup>a</sup> Means followed by same letters in the same column show no significant difference at  $p > 0.05$ .

DC-0b= Control dark chocolate stored at 25°C, 80% RH

DC-1b= Prebiotic dark chocolate stored at 25°C, 80% RH

Nil: No evaluation

DC-1a stored at 18°C, 60% RH for 12 months was significantly different ( $p < 0.05$ ). DC-1a had better scores for almost all attributes compared to DC-0a, especially for taste and odour. *T*- test also showed significant difference ( $p < 0.05$ ) for DC-0a which had a taste attribute mean score of 2.88 compared to DC-1a which had a higher mean score of 3.41.

Table 3 shows that there was no significant difference ( $p > 0.05$ ) for all attributes of DC-0b during early storage until the second month (25°C, 80% RH) but testing for DC-1b had to be stopped after a month due to fungal growth observed on the chocolate surface. Storage temperature of 18°C with relative humidity of 60% RH was

a suitable storage condition for prebiotic dark chocolate (DC-1a) and control chocolate (DC-0a) to prolong shelf life. Until the 16<sup>th</sup> month, the attributes for DC-1a were better accepted by the panelists (data not shown) compared to DC-0a. Given the assurance of product safety, sensory evaluation is undoubtedly the most appropriate technique for evaluating changes during storage trials (Kilcast, 2000).

In addition, sensory evaluation is a very important methodology for shelf life prediction of microbiologically stable products (Hough *et al.*, 2003). However, DC-0b stored at 25°C, 80% RH had better shelf life and was acceptable (scored more than 3.0) after a 2-month storage period compared

to DC-1b (totally rejected). A very high RH (80%) caused the sample to become damp which made the panelists score DC-1b lower than 3 for hardness after 2 months of storage and the dampness may stimulate fungal growth. Unlike DC-1a (60% RH); the hardness attribute was scored below 3 at months 11 and 12 but was insufficient to support fungal growth while the taste attribute was still acceptable.

## CONCLUSION

Our study suggests that dark chocolate containing inulin has a shelf life of 12 months at 18°C, 60% RH, without major degradation of inulin content in the chocolate (chemically stable), no fungal growth (microbiologically safe), and well accepted by sensory panelists who rated it better than the control chocolate in terms of taste attribute. Storage at 25°C, 80% RH with  $a_w > 0.50$  caused microbial spoilage to the chocolates. The above analysis may be useful as a control measure to determine prebiotic chocolate quality especially with regard to  $a_w$  value which should be maintained well below 0.50. Thus, the prebiotic dark chocolate may be an alternative sugar-free confectionary product at a very competitive unit price in the near future.

## ACKNOWLEDGMENTS

The author wishes to thank the Ministry of Science, Technology and Innovation (MOSTI), Malaysia for providing a research grant (IRPA 308821001). Our thanks are also extended to Malaysia Cocoa Board, Dr. Rosmin Kasran and Mrs. Wan Aidah Wan Ibrahim.

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