Producing and Quality Attributes of Low Calories Dark Chocolate Using Different Intense sweeteners and Wheat Fiber Isolate

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Abstract This study aimed to produce sucrose free dark chocolate and low in calories in order to be suitable for diabetes and those wishing to lose weight or using it for other industrial purpose. Therefore, a suggested recipes were used to prepare low calories dark chocolate using four different intense sugar namely: steviosid, acesulfam-k, sucralose and inulin instead of sucrose with the addition of wheat fiber isolate. The prepared chocolate samples were compared to a sucrose sample (control) in which sucrose was used in its manufacture. Physical properties, chemical composition and sensory properties were determined as well as the caloric value. The results indicates that the caloric value was reduced by 28.46, 28.69, 28.65 and 36.14 % in the prepared samples using stevioside, acesulfam-k, sucralose and inulin, respectively, compared with the sucrose sample. A slight difference between the prepared samples and the sucrose sample has occurred in the physical properties and the chemical composition while the percentage of fiber content significantly increased in prepared low calorie chocolate samples. Although the inulin sample recorded the lowest caloric value among the rest of the prepared samples and the control one, it was more solid than the other samples as well as it showed some unpalatable taste by arbitrators, so the sample which prepared using stevioside was chosen to be the best one for the rest samples, where it has a reduction in the calories by 28.46%, compared to the sucrose one, with high quality properties. Effect of storage at (10 °C) on the stability of stevioside sample was studied by estimating its value of each free fatty acids and peroxide values every 15 days during the storage period (3 months). The results confirmed the high stability of the stevioside sample during the storage period.

Keywords: dark chocolate, low calories, sucrose free, intense sweeteners

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1. Introduction

According to Codex Alimentarius Commission [1] dark chocolate is a confectionery product containing not less than 35 % of total dry cocoa solids, 18 % of cocoa butter and 14 % of dry non-fat cocoa solids with addition of any sugar, the most common sweetener used in chocolate is sucrose, which causes chocolate to contain a large amounts of calories. In addition it is well known that sucrose is harmful to health and causes many diseases, among them cancer. Chocolate is classified as a high calorie food due to the high fat and sugar contents. Sugar substitutes have gained importance due to the increasing demand for diet and light products resulting from the growing effort to reduce the ingestion of calories of specific diets because of a growing concern about health in the last years [2]. Sucrose-free chocolates have become popular among consumers and manufacturers because of its reduced calorific values, and the fact that these are both non carcinogenic and suitable for diabetics [3].

Sucrose constitutes more than 40-50% of solids dispersed in chocolate fat, its functional properties such as sweetness, stability and mouth feel are important factors affecting the chocolate products [4]. The chocolate products are desired and eaten, due to their attractive flavors and appearances. The primary chocolate categories are dark, milk and white. The widely enjoyed chocolate-flavor, make it a favorite ingredient in bakery, ice cream, beverage, syrup manufacture and as a confection in itself. Moreover, chocolate is consumed all over the world, in all segments of society and by people of all ages [5,6]. The popularity of this food appears to mainly associate with its potential to arouse sensory pleasure and positive emotions [7,8].

Sugar alcohol was previously used to produce sucrose free chocolate as a result of many attempts to reduce sucrose intake, through time there was other attempts to replacement of sucrose by intense sweeteners [9]. Nowadays, there is increasing awareness of the link
between diet and health, [10]. For instance, alternative healthy sweeteners for common mono and di saccharide should be used to reduce the calories intake and therefore, contribute to prevention of obesity and help people with diabetes and/or other diseases [11,12].

Recent studies reported that daily consuming dark chocolate significantly lower urinary excretion of the stress hormone and catecholamine and release of neurotransmitters serotonin [13]. Also food safety agency suggested 200 mg of cocoa flavonoids should be consumed daily. This amount could be obtained by consuming 2.5g of high quality dark chocolate in order to maintain normal endothelium dependent vasodilation (normal blood flow). Although high intensity sweeteners are caloric free, some of these sweeteners impart undesirable flavor and aftertaste, especially bitterness that can limit their applications in foods and beverages [14].

The production of chocolate without sucrose is considered very important for the health point of view [10]. So this study aimed to produce free sugar dark chocolate and low calories using some intense sweeteners as inulin, stevioside, aceslfam-k and sucralose alternative to the sucrose. The physical properties, chemical composition, caloric value and sensory attributes of the prepared chocolate were estimated. The effect of storage for three months on the stability of the prepared chocolate was also studied.

2. Materials and Methods

2.1. Source of Materials

Dark natural chocolate was manufactured from natural raw materials according to Egyptian Standard No. 465-3 [15] . Ghanaian cocoa mass and cocoa butter was obtained from Cargill Cocoa Processing Company, Accra, Ghana. Cocoa powder was obtained from ADM international sarl, Des Moines, Iowa, USA. Sucrose was obtained from Dakahlia sugar Co, Dakahlia, Egypt. Whey powder was obtained from Polmlek group, Warsaw, Poland. Lethicin was obtained from Alexandria Company for seed processing and derivatives, Alexandria, Egypt. Vanillin was obtained from Redwood biotech CO., LTD, Shanghai, China. Wheat fibers isolate was obtained from JD food, Garancieres en Beauce, France. Grinsted P.S.101. Stevioside, sucralose and acesulfam-K were obtained from Merck KGaA, Darmstadt, Germany. All chemicals used in analysis were obtained from El-Nasr pharmaceutical Chemicals CO - Egypt and Al Safwa for Trading Est. Agent & Importer of Sigma Aldrich Chemicals - Egypt.

2.2. Preparation of Chocolates

Chocolate prepared according to Food and Drugs Administration (FDA) regulation. Chocolate was manufactured at Al-Ahmady factory, Alexandria, Egypt.

2.3. Physical Properties

The pH of chocolate was measured with pH meter AD/11 ADWA made in Europe - Romania, according to AOAC [16]. Chocolate's slip melting point was determined according to [16]. The slip melting point of the sample = water temperature when the sample column begins to rise in the capillary tube.

Hardness of the chocolate samples was evaluated with a Texture Analyzer (TA.XT plus). (Stable Micro Systems Ltd, Surrey, UK) equipped with a stainless steel needle (P/2) probe and the trigger force of 5g. Hardness (N) was defined as the maximum penetration force required for the needle to penetrate through the chocolate sample (12mm width × 12mm length × 15mm mm height) at room temperature (24°C) over an interval of 3mm at stable rate of 1mm/s. Measurement was determined in 3 replications and the mean value was used [17].

Color of chocolate samples was determined according to [18] using colorimeter (Minolta Model CM-2500D Spectrophotometer, Tokyo, Japan) calibrated with white reference standard. The SCE-mode (Specular light excluded) was used with the color expressed in terms of the CIELAB system. The measured parameters were L* for lightness, b* for yellowness and a* for redness [19].

The caloric value of chocolate samples was calculated from the results of the chemical composition of chocolate samples by the following equation: -

\[
\text{Caloric value (Kcal)} = \left(\frac{\%\text{Carbohydrates} \times 4}{100}\right) + \left(\frac{\%\text{Total fat} \times 9}{100}\right) + \left(\frac{\%\text{Total protein} \times 4}{100}\right) + \left(\frac{\%\text{Sugar} \times 4}{100}\right)
\]

2.4. Chemical Composition

Moisture content, crude ether extract (crude fat), crude fibers and ash content were determined according to [16]. Crude protein was determined using Kjeldahl method as described in [16] factor of 6.25 was used to convert nitrogen to crude protein content [20].

Nitrogen free extract was estimated by difference [100- % of (moisture + protein + crude fat + ash + crude fibers)].

2.5. Free Fatty Acid

Free fatty acids of stored chocolate samples were determined according to [16].

2.6. Peroxide Value

Peroxide value of stored chocolate samples was determined according to [16].

2.7. Sensory Evaluation

Sensory evaluation of the chocolate samples was carried out according to [21] using a scale for different parameters such as texture, color, smell, sweetness, bitterness, aftertaste and overall acceptability.

2.8. Statistical Analysis

The results of each experiment were analyzed statistically by one-way analysis of variance (ANOVA) using an SPSS Vol.6, No.4, pp.369-392 program.
Statistical significance was measured by ANOVA using the LSD test [22], diagrams were constructed in microsoft excel 2016 using the parameters collected on the computer.

3. Results and Discussion

3.1. Production of Low Calories Dark Chocolate

Several experiments have been conducted to produce low-calorie dark chocolate (free from sucrose) with high quality properties by using some intense sweeteners namely: stevioside, acesulfam-k, sucralose and inulin as sucrose substitutes. The recipe in Table 1 was chosen as the best one for the rest of the tested recipes. As shown in Table 1 sample (A) has been prepared with 45.16% sucrose to give usual sweet taste for chocolate (control sample). Meanwhile, 0.13,0.19,0.06 and 10.50% from each of stevioside, acesulfam-k, sucralose and inulin (as intense sweeteners) were used, alternative to sucrose, for the required sweetness to the prepared low calorie dark chocolate and free sucrose (samples B,C,D and E) respectively. Also, wheat fiber isolate has been added in a ratio between 26.83 - 29.95%, this percentage of proportion were selected after conducting pre initial experiments for selecting the best percentage which resulted in acceptable textural and molding ability for every treatments. Sorbitol was added at 15.27 - 17.00% to the proposed treatments B, C, D and E samples as shown in Table 1 to keep the balance of ingredients.

Table 1. The suggested recipes used to prepare low calories dark chocolate

| Samples Ingredients (%) | A (Sucrose) | B (Steviosid) | C (Acesulfam-k) | D (Sucralose) | E (Inulin) |
|--------------------------|-------------|---------------|----------------|--------------|-----------|
| Sugar alcohol (sorbitol) | 45.16       | 0.00          | 0.00           | 0.00         | 0.00      |
| Cocoa mass               | 20.06       | 20.03         | 20.03          | 20.05        | 17.95     |
| Cocoa butter             | 20.85       | 18.96         | 18.96          | 18.97        | 16.99     |
| Cocoa powder             | 9.04        | 9.03          | 9.03           | 9.03         | 8.09      |
| Whey powder              | 3.99        | 3.99          | 3.99           | 3.99         | 3.57      |
| Wheat fibers isolate     | 0.00        | 29.93         | 29.93          | 29.95        | 26.83     |
| Lecithin                 | 0.50        | 0.50          | 0.50           | 0.50         | 0.50      |
| P.G.P.R                  | 0.20        | 0.20          | 0.20           | 0.20         | 0.20      |
| Ethyl vanillin           | 0.07        | 0.07          | 0.07           | 0.07         | 0.07      |
| Stevioside               | 0.00        | 0.13          | 0.00           | 0.00         | 0.00      |
| Acesulfam-K              | 0.00        | 0.00          | 0.19           | 0.00         | 0.00      |
| Sucralose                | 0.00        | 0.00          | 0.00           | 0.06         | 0.00      |
| Inulin                   | 0.00        | 0.00          | 0.00           | 0.00         | 10.50     |

A: Control sample which sweetened using sucrose. B: Steviosid dark chocolate sample. C: Acesulfam-k dark chocolate sample. D: Sucralose dark chocolate sample. E: Inulin dark chocolate sample.

3.2. Physical Properties

Physical properties, chemical composition, caloric value and the acceptability of the prepared samples were determined.

Data in Table 2 represent the physical properties of the suggested low calories dark chocolate samples (sucrose free) which prepared using the previous recipe. It could be notice that there were a significant differences between control sample (A) and most of the prepared samples in most of studied physical properties. Data revealed that the pH value of the control sample (A) was 6.33 whereas the pH of the prepared samples (B, C, D and E) ranged between 6.41-6.43. pH values of Stevioside and acesulfam - k chocolate samples were close to that of the control one. These results are in agreement with [23]. For color values it is obvious that lightness values (L*) of B, C and D samples ranged between 24.70- 24.83 which are not far from the value of control sample (A) being (25.47). Inulin sample had the lowest value of lightness (22.87) among all the samples. These results are in agreement with the data published by [24]. The red color (a*) of the prepared samples are slightly lower in the prepared samples than the control one. Inulin sample had the lowest redness value among all the samples (5.03). Data published by [24] stated that the redness value of the inulin chocolate ranged between 4.95 - 7.48. Meanwhile, there was no difference between the low calories chocolate samples and the control one in the yellowness value. Data in Table 2 also present the hardness values (N) for the prepared samples comparing to the control one. There were slight differences between samples B,C and D value (14.30 - 14.47) and that of the control (14.21), whereas inulin sample had highest hardness value among the all the samples being (16.63). This result are in agreement with the data published by [25] and [26] but in contrast with [24] who reported that the hardness of the sucrose sample was 26.80 while the hardness if the inulin samples was between (21.50 - 27.82). In addition, data in Table 2 also show that slip melting point value was 33.77°C for the control sample (A) and was in the range of 34.63-34.83 °C for the prepared samples (B,C,D and E) . It could be noticed that stevioside (B) acesulfam - k (C) and sucralose (D) samples had slightly higher slip melting point value than the control one. The characterization of the melting properties of dark chocolate is very important property for the consumers [4,27]. Our results are in agreement with [23,24,25,26,28].

3.3. Proximate Chemical Composition

Data in Table 3 represents the proximate chemical composition of low calories dark chocolate samples comparing to the control one. It could be seen that the moisture content of all prepared samples are slightly higher than that of the control one, whereas there were no significant differences between samples B, C, and D being (0.9 - 0.91%) while sample E had the lowest moisture content among them being (0.80%). Data published by [29] mentioned that the moisture content for the milk chocolate control sample is 1.02% and for stevia samples ranged from 1.11% to 1.24%.

Data in Table 3 also show that there were a significant differences between chocolate samples in crude ether extract (crude fat) whereas control sample had the highest content (34.03%) followed by sucralose sample (31.86%), stevioside sample (31.80%), acesulfame-k sample (31.79%) and inulin sample (28.51%) respectively, fat
content in the control sample was higher than the content founded by [30] who reported that the fat content for the chocolate sample which made using sucrose was 23.20%.

Protein content ranged from 4.59 to 5.15 % in the prepared chocolate samples. No significant differences were noticed between the prepared chocolate samples and the control regarding to the protein content as shown in Table 3 except inulin sample which had the lowest content in protein (4.59%) compared with the others.

Control sample results is in agreement with the date published by [26]. On the other hand it is clear that addition of wheat fiber isolate resulted in increment in the fiber content in the prepared low calories dark chocolate samples (Table 3). The fiber content increased from 6.25% in the control sample to the range of 34.85% - 41.75% in the prepared samples. Moreover, the ash content significantly increased in the low calories dark chocolate samples (2.63 - 3.00%) comparing to the control sample (2.22%). This result may be due to the addition of the wheat fiber isolate.

Also, Table 3 shows that the nitrogen free extract (N.F.E) of low calories dark chocolate samples which prepared using intense sweeteners were significant decreased comparing with the control sample.

| Samples  | pH       | Color   | Hardness-s  | Slip melting point (°C) |
|----------|----------|---------|-------------|-------------------------|
| A        | 6.33±0.06| 25.47±0.06| 4.77±0.06 | 14.21±0.01 | 33.77±0.06 |
| B        | 6.41±0.06| 24.80±0.06| 4.73±0.06 | 14.30±0.10 | 34.63±0.06 |
| C        | 6.40±0.10| 24.83±0.12| 4.70±0.00 | 14.36±0.06 | 34.63±0.15 |
| D        | 6.43±0.06| 24.70±0.12| 4.67±0.12 | 14.47±0.12 | 34.63±0.06 |
| E        | 6.43±0.06| 22.87±0.15| 4.67±0.06 | 14.63±0.06 | 34.83±0.12 |

Means in columns sharing same letters are not significantly different, using the reversed LSD test at 0.05 levels
L*= Lightness. a* (redness). b* (yellowness). N= maximum penetration force to penetrate sample
A: Control sample which sweetened using sucrose.
B: Steviosid dark chocolate sample.
C: Acesulfam-k dark chocolate sample.
D: Sucralose dark chocolate sample.
E: Inulin dark chocolate sample.

Table 3. Proximate chemical composition of prepared low calories dark chocolate

| Samples  | Moisture (%) | Crude ether extract (%) | Crude protein (%) | Fibers (%) | Ash (%) | N.F.E (%) |
|----------|--------------|-------------------------|-------------------|------------|---------|-----------|
| A        | 0.75±0.01    | 34.03±0.43              | 5.10±0.01         | 6.25±0.01  | 2.22±0.01| 51.64±0.50 |
| B        | 0.90±0.03    | 31.80±0.13              | 5.11±0.02         | 34.87±0.02 | 3.00±0.03| 24.41±0.18 |
| C        | 0.90±0.03    | 31.79±0.31              | 5.13±0.01         | 34.85±0.02 | 3.00±0.06| 24.33±0.03 |
| D        | 0.91±0.01    | 31.86±0.61              | 5.15±0.01         | 34.89±0.01 | 3.00±0.04| 24.22±0.02 |
| E        | 0.80±0.01    | 28.51±0.22              | 4.59±0.01         | 41.75±0.01 | 2.63±0.12| 21.71±0.11 |

Means in columns sharing same letters are not significantly different, using the reversed LSD test at 0.05 levels.
N.F.E = Free nitrogen extract
A: Control sample which sweetened using sucrose.
B: Steviosid dark chocolate sample.
C: Acesulfam-k dark chocolate sample.
D: Sucralose dark chocolate sample.
E: Inulin dark chocolate sample.

3.4. Caloric Value

There is a noticeable significant reduction in the caloric value of the chocolate samples (B,C,D,E) comparing to the control samples (A) as shown in Table 4.

| Samples  | Caloric value (Kcal/100g) | Energy losses (%) |
|----------|---------------------------|-------------------|
| A        | 533.22±0.13               | 0.00              |
| B        | 381.44±0.01               | 28.46             |
| C        | 380.20±0.00               | 28.69             |
| D        | 380.40±0.06               | 28.65             |
| E        | 340.47±0.02               | 36.14             |

Means in columns sharing same letters are not significantly different, using the reversed LSD test at 0.05 levels.
A: Control sample which sweetened using sucrose.
B: Steviosid dark chocolate sample.
C: Acesulfam-k dark chocolate sample.
D: Sucralose dark chocolate sample.
E: Inulin dark chocolate sample.
The energy losses ranged between 28.46 % to 36.14% in all samples. These results are in agreement with the date published by [14] and [31]. Inulin sample had the lowest caloric value (340.47 Kcal/100g) compared with the control sample (533.22 Kcal/100g) whereas energy losses reached to 36.14%. Results of [24] stated that the inulin sample lost 25.20% of caloric value compared to the sucrose one. This reduction is due, mainly, to the use of the intense sugars as substitutes for sucrose and also to the added wheat fiber isolate to the recipe.

3.5. Sensory Evaluation

Data in Table 5 show the sensory attributes evaluation of the prepared low calories dark chocolate samples comparing to the control one (which made with sucrose) The presented data confirmed the high acceptability of all the prepared chocolate samples expect the inulin one. Although inulin sample had lowest caloric value among all the prepared samples but it was harder in texture compared with the other samples and had a noticeable after taste as shown in Table 5. Meanwhile stevioside sample had the highest degree in the overall acceptability among the prepared samples.

These results are in contrast with [24] who reported that the control sample and inulin sample have a same hardness, Flavor and taste in the sensory attributes evaluation.

3.6. Storage of Low Calories Dark Chocolate

From the previous results stevioside sample had the highest values for most of the quality properties especially that of the sensory evaluation test with reduction in its caloric by 28.46% comparing to the control sample (chocolate with sucrose). So, it was necessary to test its stability during storage, therefore stevioside sample was stored at 10 °C (the temperature that is used in factories during storage of chocolate) for 3 months. Free fatty acids and peroxide values were estimated every 15 days.

Figure 1, Figure 2 show the free fatty acids (FFA) and peroxide values of low calories dark chocolate (stevioside sample) stored at 10°C for 3 months compared to the control one. As shown in Figure 1, Figure 2 both of FFA% and peroxide values increased significantly during the storage period up to 90 days either for the control sample or for the low caloric dark chocolate but these values remained, at the end of the storage period, less than the permissible values, which indicate the stability of the samples during storage. Moreover, there were no significant difference between the control sample and the stevioside sample (low caloric dark chocolate) in the free fatty acids and the peroxide values during the storage period.

Mishra et al. [32] studied the shelf life of guava milk chocolate at different temperature, and reported that the chocolate which stored at low temperature (10°C) was found to be The best when compared with chocolate which stored at higher temperature (25°C).

| Samples | Hard texture (25 degree) | Dark color(15 degree) | Smell(10 degree) | Sweetness (20 degree) | Bitterness (20 degree) | Aftertaste (10 degree) | Overall acceptability (100 degree) |
|---------|--------------------------|-----------------------|-----------------|-----------------------|------------------------|------------------------|-----------------------------------|
| A       | 24.18±0.90              | 12.27±0.65            | 9.00±1.61       | 18.72±0.79            | 13.64±1.50             | 0.45±0.69              | 94.81±4.20                       |
| B       | 21.45±2.02              | 13.54±1.51            | 8.81±0.87       | 16.63±1.36            | 13.36±1.21             | 0.90±0.94              | 88.27±5.88                       |
| C       | 21.00±2.40              | 13.18±1.54            | 8.18±1.08       | 15.09±1.38            | 13.45±2.95             | 3.73±1.27              | 75.45±9.28                       |
| D       | 19.45±3.30              | 12.54±1.44            | 8.44±1.13       | 16.82±1.72            | 13.00±1.55             | 1.64±1.21              | 80.66±10.10                      |
| E       | 2.65±0.52               | 12.54±1.51            | 7.72±1.01       | 11.36±1.03            | 15.73±0.91             | 2.73±1.42              | 69.54±5.60                       |

Means in columns sharing same letters are not significantly different, using the reversed LSD test at 0.05 levels.
A: Control sample which sweetened using sucrose.
B: Steviosid dark chocolate sample.
C: Acesulfam-k dark chocolate sample.
D: Sucralose dark chocolate sample.
E: Inulin dark chocolate sample.

Figure 1. Free fatty acids content of low calorie dark chocolate samples during storage at 10°C for 90 days compared to the control sample.
This study aimed to produce sucrose free dark chocolate and low in calories, using different intense sweeteners instead of sucrose. From the results of the study, it could be concluded that using stevioside as substitute for sucrose with the addition of wheat fiber isolate in preparing low calorie dark chocolate resulted in calories reduction by %28.46 compared to the sucrose one. The stevioside chocolate had the highest quality attributes and impressed by the taste test panelists. A storage experiments of the stevioside sample was performed at cool temperature (10°C) for three months. The results indicated high degree of stability since the values of free fatty acids and peroxide were less than the permissible value during the 90 days of storage.

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Figure 2. Peroxide value of low calorie dark chocolate samples during storage at 10°C for 90 days compared to the control sample
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