



α -Glucosidase Inhibition and Glycemic Index of Chocolate Incorporated with Selected Spices

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Authors' contributions

This work was carried out in collaboration between both authors. Author DS carried out the experiment and prepared the manuscript. Author DB supervised the research and helped in drafting the article for final approval of the version to be published. Both authors read and approved the final manuscript.

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ABSTRACT

The present study was carried out to evaluate the α -glucosidase, α -amylase, sucrase inhibition and glycemic index of chocolate prepared by incorporating selected spices *i.e.*, fenugreek, black cumin, coriander and cinnamon in the form of powders at different equal levels of substitution at 1%, 1.5% and 2%. The spices incorporated chocolate showed α -glucosidase inhibition in the range of 27.36 to 48.28% at 50 to 150 μ l concentration. The glycemic index of developed chocolate was found to be lower than the control chocolate, which was attributed to the incorporation of spices. The results of the present study revealed that the chocolate prepared by incorporating different spices is low glycemic in nature.

Keywords: *Chocolate; cinnamon; glycemic index; α -glucosidase; α -amylase.*

1. INTRODUCTION

Chocolate is one of the most desired confectionery products, widely consumed by all generations throughout the world [1]. Chocolate can be described as an emulsion consisting of non-fat particles (cocoa solids, sugar, and milk powder) dispersed in cocoa butter as a continuous phase [2]. Chocolates are categorized into three main types based on their compositions, which are dark chocolate, white chocolate and milk chocolate [3]. Dark chocolate has been shown to exert anti-inflammatory, cardioprotective, and neuroprotective effects; it also enhances nitric oxide bioavailability, thus improving both platelet function and blood pressure [4]. It is well known that polyphenols are associated with beneficial effects; therefore cocoa and dark chocolate have assumed significant importance [5,6]. Foods developed by incorporating spices have the potential to contribute to a healthier society. There has been mounting evidence in recent years that food choices are an important factor in reducing the risk of developing heart disease, metabolic diseases, cancer, and obesity. Hence, the present study was conducted to develop chocolate by incorporating spices and to evaluate their glycemic index and antidiabetic properties by *in-vitro* studies.

2. MATERIALS AND METHODS

2.1 Materials

The cocoa mass and cocoa butter used in the chocolate preparation were purchased from Morde Foods Private Limited, Pune. Lecithin used as a stabilizer was purchased from Venus Essence Private Limited, Chennai. Fenugreek, black cumin, cinnamon, coriander, and sugar were purchased from Sri MRV supermarket, Redhills, Chennai. The study was carried out in the College of Food and Dairy Technology, Alamathi, Chennai – 600 052.

The chocolate prepared by incorporating selected spices *i.e.*, fenugreek, black cumin, coriander and cinnamon in the form of powders at different equal levels of substitution at 1% (SPIC1), 1.5% (SPIC 2) and 2% (SPIC 3) was given in Fig. 1.

2.2 Estimation of Glycemic Index

The glycemic index of samples was determined according to the method given by Goni et al. [7].

Samples of 50mg were prepared and 10ml of HCl-KCl buffer (pH = 1.5) were added. Then, 0.2ml of a solution containing 1 g of pepsin in 10 ml of HCl-KCl buffer were added to each sample and incubated at 40°C for 1 hr in a shaking water bath. Volume was made up to 25ml with Tris-Maleate buffer (pH = 6.9). A 5ml of a solution of α -amylase in Tris-Maleate buffer containing 2.6 UI were added to each sample. Then, the samples were incubated at 37°C in a shaking water bath. 1ml aliquot samples were taken from each tube every 90 min from 0 to 3 hours. These aliquots were placed in a tube at 100°C and were shaken vigorously for 5 minutes to inactivate the enzyme and refrigerated until the end of the incubation time. Then, 3ml of 0.4M sodium acetate buffer (pH = 4.75) were added to each aliquot and 60 μ l of amyloglucosidase were used to hydrolyse the digested starch into glucose after 45 minutes at 60°C in a shaking water bath. Volume was adjusted to 10-100ml with distilled water. The triplicated aliquots of 0.5ml were incubated with Peridochrom Glucose GOD-PAP. The hydrolysis index (HI) was calculated as follows:

$$HI = \frac{\text{Area under the curve of the sample}}{\text{Area under the curve of white bread}}$$

The *in-vitro* glycemic index (GI) was determined by using the following equation as described by Goni et al. [7].

$$GI = 39.71 + 0.549 HI$$

2.3 Estimation of Antidiabetic Activity

2.3.1 α -glucosidase inhibition

α -glucosidase inhibitory effects in samples were determined according to the method given by Watanabe et al. [8]. Yeast α -glucosidase (0.7 U) dissolved in 100mM phosphate buffer (pH 7.0) containing 2 g/l bovine serum albumin and 0.2g/l sodium azide (NaN₃) which was used as enzyme source. Paranitrophenyl- α -D-glucopyranoside (PNPG) was used as substrate. The enzyme solution (1000 μ l) and 100 μ l of the test sample at various concentrations (50, 100 and 150n μ l) were mixed and incubated for 5 min. After incubation, 50 μ l of the substrate was added and further incubated for 5 min at room temp and the absorbance was measured using a spectrophotometer at 405nm. The reaction is monitored by an increase in absorption at 405 nm. Control was taken without the extract.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control at 405nm} - \text{Absorbance of sample at 405nm}}{\text{Absorbance of control at 405nm}} \times 100$$

2.3.2 α -amylase inhibition

α -amylase inhibitory effects in samples were determined according to the method described by Jung et al. [9]. The assay mixture containing 200 μ l of 0.02 M sodium phosphate buffer, 20 μ l of enzyme and the sample extracts in concentration range 50-150 μ l/ml were incubated

for 10 minutes at room temperature. Then, add 200 μ l of starch in all test tubes. The reaction was stopped with the addition of 400 μ l dinitrosalicylic acid and placed in boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was measured at 540nm. Control was taken without the extract.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control at 540nm} - \text{Absorbance of sample at 540nm}}{\text{Absorbance of control at 540nm}} \times 100$$

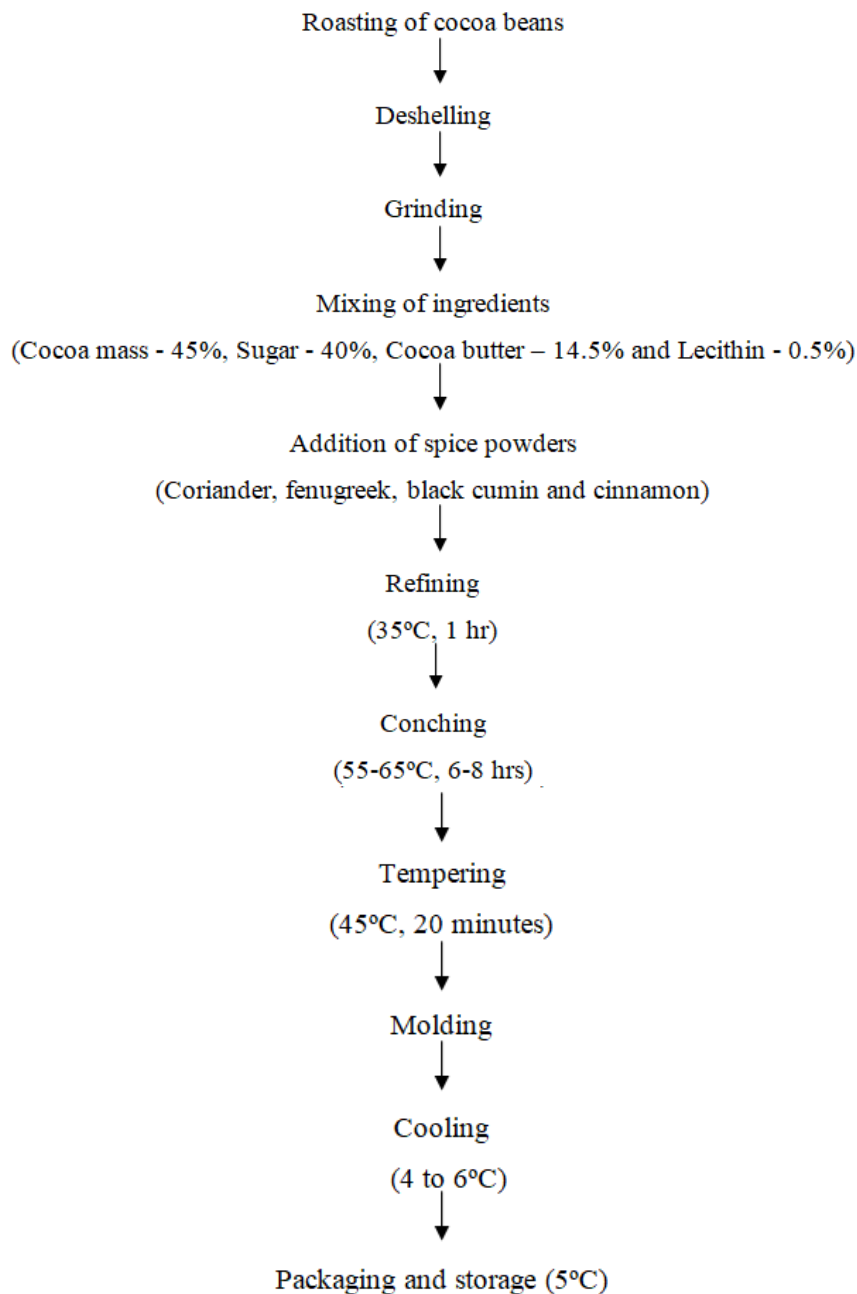


Fig. 1. Flow chart for the preparation of spice powders incorporated chocolate

2.3.3 Sucrase inhibition

The effect of the sample on sucrase activity was analyzed according to the method described by Honda and Hara [10]. The enzyme solution (10 μ l) and varying concentrations (50, 100 and 150 μ l) of the samples were incubated together for 10 minutes at 37°C, and the volume is made up of 200 μ l with maleate buffer (pH 6.0). The enzyme reaction is started by adding a 100 μ l sucrose solution (60mM). After 30 minutes, the reaction is stopped by adding 200 μ l of 3, 5-dinitrosalysilic acid reagent and treating the mixture in a boiling water bath for 5 minutes. Then, the absorbance was taken at 540nm. Control was taken without the extract.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control at 540nm} - \text{Absorbance of sample at 540nm}}{\text{Absorbance of control at 540nm}} \times 100$$

2.4 Statistical Analysis

All the experiments were carried out in six replicates and results are expressed as Mean \pm SE. The statistical analysis was performed by ANOVA using SPSS®20.0 software for Windows as described by Snedecor and Cochran [11].

3. RESULTS AND DISCUSSION

3.1 Antidiabetic Activities of Spices Incorporated Chocolate

The antidiabetic activities of spice powders incorporated chocolate (SPIC) by α -glucosidase, α -amylase and sucrase inhibition was illustrated in Figs. 2, 3 and 4 respectively. On analysis, there was a highly significant ($P \leq 0.01$) difference observed in the control and developed chocolate.

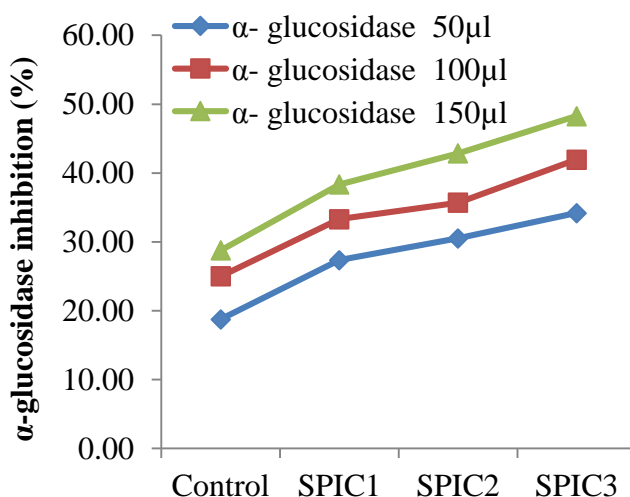


Fig. 2. α -glucosidase inhibition of spice powders incorporated chocolate

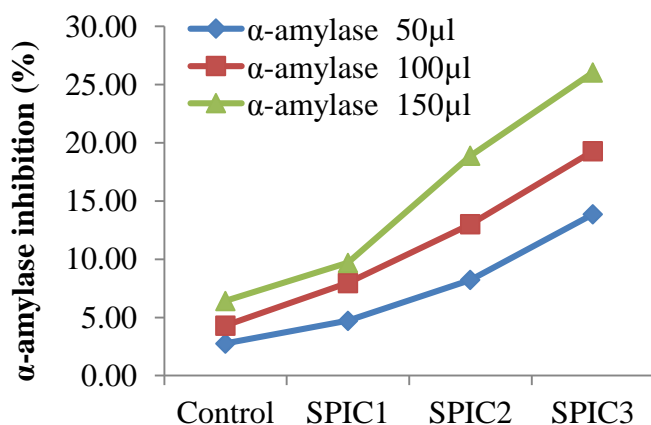


Fig. 3. α -amylase inhibition of spice powders incorporated chocolate

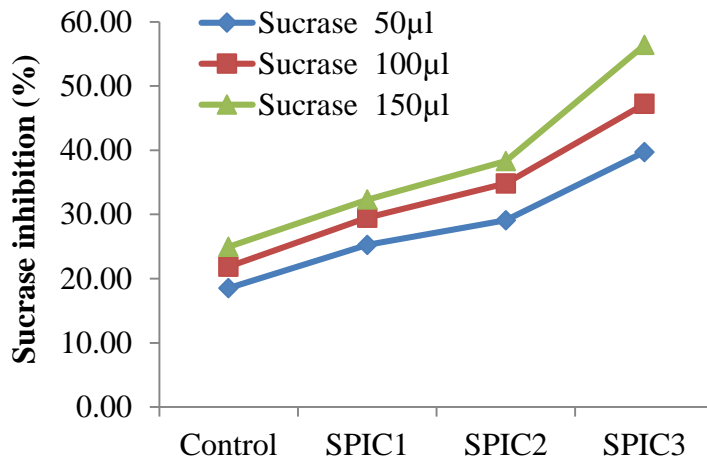


Fig. 4. Sucrase inhibition of spice powders incorporated chocolate

Table 1. Glycemic index of spice powders incorporated chocolate

| Chocolate variants | Glycemic index | | | F-value |
|--------------------|---------------------------|---------------------------|---------------------------|----------|
| | 0 min | 90 min | 180 min | |
| Control | 43.01±0.337 ^{bA} | 45.89±0.393 ^{cB} | 47.45±0.278 ^{cC} | 44.075** |
| SPIC1 | 45.14±0.299 ^{cC} | 43.38±0.322 ^{bB} | 41.46±0.264 ^{bA} | 38.839** |
| SPIC2 | 44.83±0.408 ^{cC} | 42.76±0.238 ^{bB} | 40.82±0.201 ^{bA} | 38.322** |
| SPIC3 | 41.95±0.271 ^{aB} | 40.97±0.334 ^{aB} | 39.72±0.349 ^{aA} | 11.429** |
| F-value | 18.239** | 38.039** | 155.486** | - |

Data expressed as Mean ± SE; n=6; ** - Highly significant difference ($P \leq 0.01$); Different superscripts within the same column (lowercase) and row (uppercase) differ significantly

From the Fig. 2, it was observed that the α -glucosidase inhibition was found to be in the range of 27.36 to 34.20%, 33.30 to 41.94% and 38.36 to 48.28% at 50µl, 100µl and 150µl respectively. The α -amylase inhibition was found to be in the range of 4.73 to 13.87%, 7.97 to 19.28% and 9.70 to 26.05% at 50µl, 100µl and 150µl respectively (Fig. 3). The sucrase inhibition was found to be in the range of 25.26 to 39.70%, 29.48 to 47.22% and 32.32 to 56.39% at 50µl, 100µl and 150µl respectively (Fig. 4). The variations found in the inhibition of α -glucosidase, α -amylase and sucrase in developed chocolate might be attributed to the addition of spices in different levels of substitution.

3.2 Glycemic Index of Spices Incorporated Chocolate

The glycemic index of spice powders incorporated chocolate (SPIC) was shown in Table 1. The glycemic index of developed chocolate was found to be ranged from 41.95 to 45.14, 40.97 to 43.38 and 39.72 to 41.46 at 0, 90 and 180 minutes respectively. The glycemic

index of control chocolate was found to be higher than the developed chocolate. The low GI of developed chocolate might be attributed to the inclusion of spices as they are low GI in nature.

4. CONCLUSION

The results reported in the present study indicated that the α -glucosidase and sucrase showed increased inhibition as the inclusion of spices increased. The glycemic index values reported in the present study suggested that the developed chocolate falls in the category of low GI foods.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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