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## $\alpha$ -Glucosidase, $\alpha$ -amylase inhibition and glycemic index of ice cream incorporated with spices

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### Abstract

The present study was carried out to determine the  $\alpha$ -glucosidase,  $\alpha$ -amylase, sucrase inhibition and glycemic index of ice cream prepared by incorporating selected spices *viz.*, fenugreek, black cumin, coriander and cinnamon in the form of powders at different equal levels of substitution (1%, 1.5% and 2%). The spices incorporated ice cream showed  $\alpha$ -glucosidase inhibition in the range of 6.34-15.36%, 8.36-18.04% and 12.47-22.69% at 50 $\mu$ l, 100 $\mu$ l and 150 $\mu$ l respectively. The glycemic index of developed ice cream was found to be lower than the control ice cream. The results of the present study revealed that the ice cream prepared by incorporating different spices is low glycemic in nature.

**Keywords:** Ice cream, cinnamon, black cumin, glycemic index,  $\alpha$ -glucosidase

### 1. Introduction

Ice cream is a frozen dessert made by mixing different ingredients including milk, cream, milk solids non-fat, sugar, stabilizers and emulsifiers, in addition to flavors and colorants. Composition of ice cream varies depending on markets and locations [fat 8-20%; milk solids non-fat 8-15%; sugar 13-20%; stabilizer and emulsifier 0-0.7% and total solids 36-43%] (Arbuckle, 2013) [5]. Ice cream is consumed by different age groups (Munhoz *et al.*, 2010) [14]. Due to the high prevalence of obesity and type-2 diabetes among children and adolescents, people are now more aware of their health and hence conscious of their diet. Several studies have been carried out in developing new functional ice creams with ingredients such as probiotics (Aboufazli *et al.*, 2016; Ranadheera *et al.*, 2013; Da Silva *et al.*, 2015; Cruz *et al.*, 2009; Akin *et al.*, 2007) [1, 15, 8, 9, 4], prebiotics (Akalin *et al.*, 2008) [2], and dietary fibers (Akbari *et al.*, 2016; Hashemi *et al.*, 2015; dos Santos *et al.*, 2013; Comas *et al.*, 2013; Soukoulis *et al.*, 2009) [3, 11, 9, 6, 17]. 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 ice cream by incorporating spices and to evaluate their glycemic index and antidiabetic properties by in-vitro studies.

### 2. Materials and Methods

#### 2.1 Materials

Fresh cow milk is procured from the community cattle care center, College Food and Dairy Technology, Alamathi. Butter and skim milk powder were purchased from Aavin milk parlour, Madhavaram milk colony. Carboxymethylcellulose and glycerol monostearate were purchased from Venus Essence Pvt. Ltd., Chennai. Spices *viz.*, coriander, fenugreek, black cumin, cinnamon and sugar were purchased from Sri MRV supermarket, Redhills. The present research was carried out in the College of Food and Dairy Technology, Alamathi, a constituent college of Tamil Nadu Veterinary and Animal Sciences University, Chennai.

#### 2.2 Estimation of antidiabetic activity

##### 2.2.1 $\alpha$ -glucosidase inhibition

$\alpha$ -glucosidase inhibitory effects in samples were determined according to the method given by Watanabe *et al.*, (1997) [18]. Yeast  $\alpha$ -glucosidase (0.7 U) dissolved in 100mM phosphate buffer (pH 7.0) containing 2g/l bovine serum albumin and 0.2g/l sodium azide (NaN<sub>3</sub>) which was used as enzyme source. Paranitrophenyl- $\alpha$ -D-glucopyranoside (PNPG) was used as substrate. The enzyme solution (1000 $\mu$ l) and 100 $\mu$ l of the test sample at various concentrations (50, 100 and 150 $\mu$ l) were mixed and incubated for 5 min.

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After incubation, 50 $\mu$ 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

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

### 2.2.2 $\alpha$ -amylase inhibition

$\alpha$ -amylase inhibitory effects in samples were determined according to the method described by Jung *et al.*, (2006). The assay mixture containing 200 $\mu$ l of 0.02M sodium phosphate buffer, 20 $\mu$ l of enzyme and the sample extracts in concentration range 50-150 $\mu$ l/ml were incubated for 10

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

### 2.2.3 Sucrase inhibition

The effect of the sample on sucrase activity was analyzed according to the method described by Honda and Hara, (1993)<sup>[12]</sup>. The enzyme solution (10 $\mu$ l) and varying concentrations (50, 100 and 150 $\mu$ l) of the samples were incubated together for 10 minutes at 37 $^{\circ}$ C, and the volume is made up of 200 $\mu$ l

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

### 2.3 Estimation of glycemic index

The glycemic index of samples was determined according to the method given by Goni *et al.*, (1997)<sup>[10]</sup>. 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  $^{\circ}$ C for 1 hr in a shaking water bath. Volume was make up to 25ml with Tris-Maleate buffer (pH = 6.9). A 5ml of a solution of  $\alpha$ -amylase in Tris-Maleate buffer containing 2.6 UI were added to each sample. Then, the samples were incubated at 37 $^{\circ}$ 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 $^{\circ}$ 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 $\mu$ l of amyloglucosidase were used to hydrolyse the digested starch into glucose after 45 minutes at 60 $^{\circ}$ 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 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* GI was determined by using the following equation as described by Goni *et al.*, (1997)<sup>[10]</sup>.

$$GI = 39.71 + 0.549 HI$$

### 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<sup>®</sup>20.0 software for windows (Snedecor and Cochran, 1994)<sup>[16]</sup>.

is monitored by an increase in absorption at 405nm. Control was taken without the extract.

minutes at room temperature. Then, add 200 $\mu$ l of starch in all test tubes. The reaction was terminated with the addition of 400 $\mu$ l dinitrosalicylic acid (DNS) reagent 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.

with maleate buffer (pH 6.0). The enzyme reaction is started by adding a 100 $\mu$ l sucrose solution (60mM). After 30 minutes, the reaction is stopped by adding 200 $\mu$ l of 3, 5-dinitrosalicylic 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.

## 3. Results and Discussion

### 3.1 Antidiabetic activities of spices incorporated ice cream

The antidiabetic activities of spice powders incorporated ice cream (SPII) by  $\alpha$ -glucosidase,  $\alpha$ -amylase and sucrase inhibition was illustrated in Fig 1, 2 and 3 respectively.

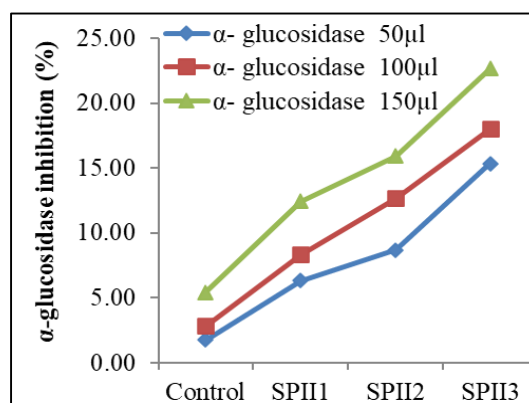


Fig 1:  $\alpha$ -glucosidase inhibition of spice powders incorporated ice cream

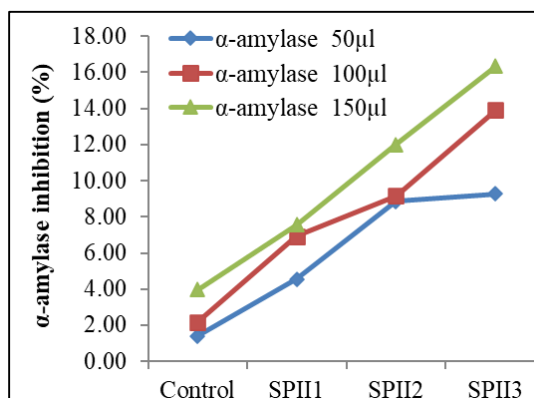
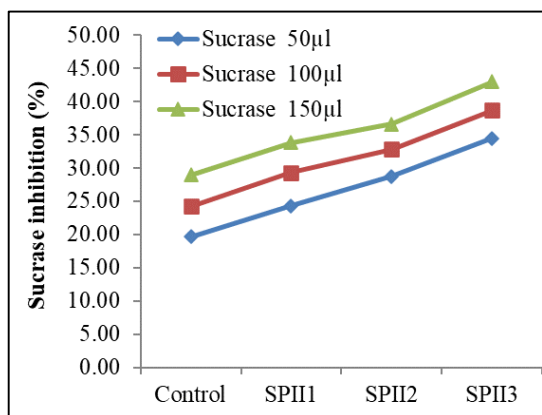


Fig 2:  $\alpha$ -amylase inhibition of spice powders incorporated ice cream



**Fig 3:** Sucrase inhibition of spice powders incorporated ice cream

On analysis, there was a highly significant ( $P \leq 0.01$ ) difference observed in the control and developed ice cream. From the Fig 1, it was observed that the  $\alpha$ -glucosidase inhibition was found to be in the range of 6.34-15.36%, 8.36-

18.04% and 12.47-22.69% at 50µl, 100µl and 150µl respectively. The  $\alpha$ -amylase inhibition was found to be in the range of 4.56-9.29%, 6.92-13.93% and 7.55-16.35% at 50µl, 100µl and 150µl respectively (Fig 2). The sucrase inhibition was found to be in the range of 24.30-34.49%, 29.31-38.68% and 33.82-43.01% at 50µl, 100µl and 150µl respectively (Fig 3). The variations found in the inhibition of  $\alpha$ -glucosidase,  $\alpha$ -amylase and sucrase in developed ice cream might be attributed to the addition of spices in different levels of substitution.

### 3.2 Glycemic index of spices incorporated ice cream

The glycemic index of spice powders incorporated ice cream (SPII) was shown in Table 1. The glycemic index of developed ice cream was found to be ranged from 43.32 to 45.18, 42.10 to 44.01 and 41.08 to 43.54 at 0, 90 and 180 minutes respectively. The glycemic index of control ice cream was found to be higher than the developed ice cream. The low GI of developed ice cream might be attributed to the inclusion of spices as they are low GI in nature.

**Table 1:** Glycemic index of spice powders incorporated ice cream

Glycemic index	0 min	90 min	180 min	F-value
Control	44.71±0.389 <sup>ba</sup>	46.29±0.338 <sup>cb</sup>	48.01±0.296 <sup>cc</sup>	19.000**
SPII1	45.18±0.258 <sup>bb</sup>	44.01±0.227 <sup>bb</sup>	43.54±0.345 <sup>ba</sup>	7.982**
SPII2	44.61±0.332 <sup>bb</sup>	43.72±0.398 <sup>bb</sup>	42.04±0.244 <sup>aa</sup>	18.571**
SPII3	43.32±0.343 <sup>ab</sup>	42.10±0.172 <sup>aa</sup>	41.08±0.225 <sup>aa</sup>	18.140**
F-value	4.000*	34.617**	78.804**	-

Data expressed as Mean ± SE; n=6; \* - Significant difference ( $0.01 < P \leq 0.05$ );

\*\* - Highly significant difference ( $P \leq 0.01$ );

Different superscripts within the same column (lowercase) and row (uppercase) differ significantly ( $P \leq 0.01$ )

### 4. Conclusion

The results reported in the present study indicated that the  $\alpha$ -glucosidase showed increased inhibition as the inclusion of spices increased. The glycemic index values reported in the present study suggested that the developed ice cream falls in the category of low GI foods as the GI value is less than 55. Further studies were carried out to confirm the health benefits of developed ice cream.

### 5. Acknowledgements

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