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Research Article

Selected Health Benefits of Spices (Garlic, Ginger, Turmeric)

Dawn Nash, Rajwinder Kaur, Vanessa Ward, Fredreana Hester, Shantrell Willis and Martha Verghese*

Department of Food and Animal Sciences, Alabama Agricultural and Mechanical University, Normal, Alabama, USA

Abstract

The use of spices has been dated back many centuries ago due to their health-promoting properties and their ability to preserve food. Research suggests that spices have the ability to prevent chronic diseases such as type 2 diabetes and obesity. The objectives of this study were to determine the selected health benefits of spices: garlic, ginger, and turmeric. Total Phenolic Content (TPC), Total Flavonoid Content (TFC), free radical scavenging activity by 1,1-Diphenyl-2Picrylhydrazyll (DPPH), Trolox Equivalent Antioxidant Capacity (TEAC), Ferric Reducing Antioxidant Power (FRAP) and inhibition of α- glucosidase, α-amylase, and lipase were evaluated. The results suggest that spices have great potential in decreasing chronic diseases due to their bioactive compounds. A product named Spiceola Bites was formulated along with the chemical analysis being conducted on the product. Total Phenolic Content (TPC), Total Flavonoid Content (TFC), free radical scavenging activity by 1,1-Diphenyl-2-Picrylhydrazyl (DPPH), Trolox Equivalent Antioxidant Capacity (TEAC), Ferric Reducing Antioxidant Power (FRAP) and inhibition of lipase and α-glucosidase and were evaluated on the Spiceola Bites extracted with water and ethanol. Results suggest incorporation of garlic, turmeric, and ginger into a functional food product (Spiceola Bite) may improve consumers' antioxidant status. Recently, spices have gained a lot of attention in the food industry due to their health benefits.

Keywords: Enzymes, Obesity; Spices; Type 2 diabetes

Abbreviations

TPC: Total Phenolic Content

TFC: Total Flavonoid Content

DPPH: 1,1-Diphenyl-2Picrylhydrazyl

TEAC: Trolox Equivalent Antioxidant Capacity

FRAP: Ferric Reducing Antioxidant Power

GAE: Gallic Acid Equivalent

DW: Dry Weight

CE: Catechin Equivalent

TE: Trolox Equivalent

SBE: Spiecola Bites Extracted with Ethanol

SBW: Spiceola Bites Extracted with Water

Introduction

It has been suggested from research that spices have multiple bioactive compounds that have a positive effect on the human body along with some cancer-fighting abilities [1]. It has been reported that spices have been used for many centuries due to their known health benefits and antimicrobial activities. Spices are retrieved from the plant's bulb, stem, and root which are usually found at the bottom of the plant. In addition to phytochemicals, spices also consist of antioxidants, minerals, vitamins, and essential oils that have many medicinal properties. Spices are known as a functional food. The compounds found in spices provide additional health benefits, beyond their nutritional value [2]. The health benefits attributed to spices are stemmed from medicinal properties including hypocholesterolemia, anti-atherogenic, and anti-obesity/thermogenic influence. Garlic, ginger, and turmeric specifically have anti-inflammatory, cardioprotective effects, and anti-diabetic properties through their blood cholesterol-lowering abilities [3].

Phytochemicals are secondary metabolites of plants. They provide them with a defense mechanism against outside influences such as predators and microorganisms. Common phytochemicals include polyphenols, flavonoids, anthocyanidins, and carotenoids [4]. It has been reported that the phytochemicals found within spices including gingerol, allicin, and curcumin are responsible for spices' color and many health benefits [5]. It has been suggested that the phytochemicals found within spices may regulate the detoxification system of enzymes, and improve the immune system. Beyond spices' medicinal properties, spices have antibacterial and antiviral capacities. Consumers that incorporate spices within their diet regularly may be able to improve their chemopreventive activities [6].

Functional food is a term that has gained popularity throughout the past couple of decades. They are used within the food industry to provide consumers with additional health benefits beyond their nutrition such as providing sources of protein, fats, and carbohydrates. Functional food products that have gained a significant amount of popularity are spices, probiotics/prebiotics, and polyunsaturated fatty acids [7]. The terminology of functional foods originates from Japan in the 1980s. This term is commonly misused because not regulated in many countries [8,7]. It has been suggested that functional foods aid in improving one's health by reducing the risk of dyslipidemia, type 2 diabetes, cancer, and cardiovascular diseases [7,9-11].

***Corresponding author:** Martha Verghese, Department of Food and Animal Sciences, Alabama Agricultural and Mechanical University, Normal, Alabama, USA, Tel: +1 2563724175; E-mail: Martha.verghese@aamu.edu

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Objectives

The objectives of this study were to develop a functional food product utilizing spices. Determination of the antioxidant potential of spices and the product developed from the spices. Determining selected health benefits of spices: Garlic, ginger, and turmeric and the product developed by evaluating the inhibition of selected metabolizing enzymes (α-glucosidase, α-amylase, and lipase).

Materials and Methods

All chemicals were obtained from Sigma Chemical Company, St. Louis, Mo. and Fisher Scientific Company, Waltham, Mass.

Determination of phytochemicals in spices and spiceola bites

Sample preparation and extraction of phenolics in spices: The three spices: Garlic, turmeric, and ginger were extracted with deionized distilled water or 80% ethanol. Triplicates were made for each spice during the aqueous and ethanolic extractions. The spices were stirred for 2 hours at 23°C. The samples were centrifuged using the Thermo Scientific ST8 Benchtop centrifuge (Waltham, MA) at 3000Xg at 4°C for 20 minutes. The samples were evaporated using a Buchi Rotavapor R-100 rotary evaporator (New Castle, DE) and filtered with Whatman no. 4 filter paper. The extracts were stored using a -20°C walk-in until analysis.

Sample preparation and extraction of phenolics in spiceola bites

Spiceola Bites are a granola-like treat that incorporated garlic, turmeric, and ginger at 2% of the total formulation and targets adolescents as a primary market. Spiceola Bites were formulated, freeze-dried, and powdered. Total phenolic and flavonoid content, antioxidant, and metabolizing enzyme inhibition assays were conducted on this product.

Spiceola Bite powder was extracted with water or ethanol. Spiceola Bite was grounded and put into 50mL of 25ºC H20 or 80% ethanol. It was then stirred for 2 hours using an orbital shaker and sonicated for 30 minutes. The sample was centrifuged for 20 minutes at 3000Xg for 20 minutes and was filtered. The samples were evaporated to dryness (BUCHI, Rotavapor); reconstituted. The samples were stored using a -20°C until analysis.

Determination of total phenolic and flavonoid contents in spice extracts and spiceola bite extracts

The total phenolic content was determined using the Folin-Ciocalteu method [12]. Slight changes were made to the method while using gallic acid as the standard. The extracts' total phenolic content is expressed as mg Gallic Acid Equivalent (GAE) per 100mg dry weight.

The total flavonoid content was determined utilizing the colorimetric assay method [12]. Catechin was used as the standard. The extracts' total flavonoid content is expressed as mg CE/100g dry weight.

Antioxidant potential of spice extracts and spiceola bite extracts

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging activity: The samples' inhibition to scavenge the free radical was measured by the method published by [12]. The absorbance was read in a microplate reader (Synergy HT, Bio Tek Instruments, Winooski, VT, USA) at 517 nm with 30-minute intervals: 0, 30, 60, and 90 minutes.

Determination of Ferric Reducing Antioxidative Potential (FRAP): The FRAP assay was determined using the method developed by [12]. The standard for this assay was made using ferrous sulfate. The absorbance was read at 593nm for 4 minutes at 1-minute intervals. The results of the sample extracts' antioxidant potential are expressed as mg Fe^{+2}/g dry weight.

Trolox Equivalent Antioxidant Capacity (TEAC): The sample extracts' TEAC was measured by using the method by [13], where Trolox vitamin E analog was used as the standard. The absorbance was read at 734nm for 6 minutes at 1-minute intervals.

Determination of lipid and carbohydrate metabolizing enzyme inhibition

Determination of α-amylase inhibition activity: The α-amylase inhibition activity of the sample extracts was determined using the method by Mutai et al. [14].

Determination of α-glucosidase activity: α-glucosidase enzyme inhibition was evaluated from using the method Mutai et al., (14). The absorbance was read at 405nm. The data was reported in percentages using the calculation below.

$$\text{Inhibition (\%)} = (Abs_{control} – Abs_{sample} / Abs_{control}) * 100$$

Determination of lipase inhibition activity

The lipase enzyme inhibition was evaluated using the modified version of the method from Mutai et al. [14], where 2,4 *p,* nitrophenyl butyrate (2,4 *p*-NPB) as a substrate.

Functional food product development

A functional food product, named Spiceola Bites, was reformulated using garlic, ginger, and turmeric.

Statistical analysis of spice extracts

All experiments were performed in triplicates and the data was recorded as means ± standard deviation. The factorial design for the chemical analysis portion of this experiment was 3 (garlic, ginger, and turmeric) ×2 (solvent extractions). One-way ANOVA was performed using SAS 9.2 version. Significant differences between means were determined using Tukey's studentized range test, and $p \leq 0.05$ was regarded as significant.

Statistical analysis of spiceola bite extracts

All experiments were performed in triplicates and the data was recorded as means ± standard deviation. One-way ANOVA was performed using SAS 9.2 version. Significant differences between means were determined using Tukey's studentized range test, and $p \leq 0.05$ was regarded as significant.

Results and Discussion

Figure 1, displays the total phenolic content of spices to determine the spices' reducing ability with the presence of the FC reagent. Among the aqueous extracts, a significantly higher amount of phenolic compounds was found in the garlic and ginger extracts compared to the turmeric extract. The opposite trend was found among the ethanolic extracts where both ethanolic ginger and turmeric extracts yielded a significantly higher total phenolic content compared to the ethanolic garlic extract. There was over a three-fold increase in total phenolic content found in the ethanolic turmeric extract compared to the garlic extract. Many studies [15-17], have demonstrated that a lot of the antioxidative potential of plants stems from phenolics along with other vitamins and minerals. The results from figure 1, showed that the ethanolic extraction resulted in higher phenolic compounds with the exception of garlic. Due to spices containing mainly essential oils that are non-polar, utilizing an ethanolic extraction was more sufficient. The findings from figure 1, are in accordance with a study [18], where the ethanoic turmeric extracts had the highest total phenolic followed by turmeric and gingerol. This may be because the structure of alliin is a lot less stable compared to the structures of curcumin and gingerol [18].

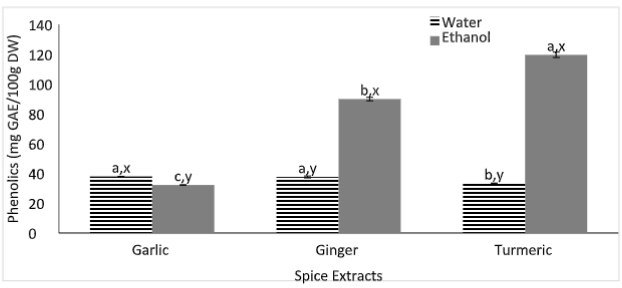

Figure 1: Total phenolic content of spices (garlic, ginger, and turmeric).

Note: Values (n=3) are means ± SEM; Bars with superscripts ([ab]) without a common letter have a significant difference between spices ($p \leq 0.05$). Bars with superscripts ([xy]) without a common letter have a significant difference between solvents ($p \leq 0.05$). GAE: Gallic Acid Equivalent; DW: Dry Weight.

Figure 2, displays the total flavonoid content of spices. Among the aqueous extracts, the aqueous ginger extract yielded a significantly higher TFC compared to both aqueous turmeric and garlic extracts. Although, the aqueous turmeric extract extracted more flavonoid compounds compared to the aqueous garlic extract. Among the ethanoic extracts, the ethanoic ginger extract yielded over a 4-fold increase in TFC compared to both turmeric and garlic extracts. The same trend from figure 1, was found in figure 2, where the ethanolic ginger extract resulted in a significantly higher total flavonoid content while the aqueous garlic extracted yielded in a significantly higher total flavonoid content compared to its aqueous extract. The hydroxyl groups found within spices are responsible for their total flavonoid content and part of their antioxidant content [19]. Due to spices being incorporated into dishes, they are usually consumed with heat treatment. It has been reported that heat treatment of spices along with their combination results in a higher phytochemical content [20].

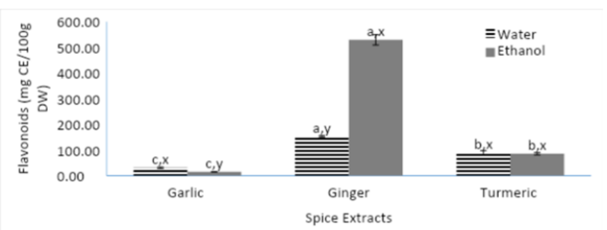

Figure 2: Total flavonoid content of spices (garlic, ginger, and turmeric).

Note: Values (n=3) are means ± SEM; Bars with superscripts ([abc]) without a common letter have a significant difference between spices ($p \leq 0.05$). Bars with superscripts ([xy]) without a common letter have a significant difference between solvents ($p \leq 0.05$). CE: Catechin Equivalent; DW: Dry weight.

Figure 3, displays the 2,2 Diphenyl-1-dicrahydrazyl radical scavenging (DPPH) of the spices (garlic, ginger, and turmeric). DPPH is a stable free radical whereupon reduction the dark color purple is changed to a pale-yellow color [21]. Overall all of the spice extracts exhibited DPPH percent inhibition. The same trend from figure 2, was found in figure 3 where both ginger extracts resulted in a significantly higher DPPH percent inhibition. Among the aqueous extracts, a 30% increase of DPPH percent inhibition was found between the aqueous ginger extract and turmeric extract. Among the ethanol extracts, the ethanol ginger extract had a significantly higher DPPH inhibition of 87% compared to both ethanolic turmeric and garlic extracts. All ethanoic extracts yielded a higher DPPH percent inhibition compared to their respective aqueous extracts. The finding of this study from the figure is in accordance with another study [22]. In that study the same trend was found among the ethanolic extracts of the spices where the DPPH percent inhibition are in the following order from least to greatest: Ginger > turmeric > garlic.

Figure 3: DPPH inhibition by spices (garlic, ginger, and turmeric).

Note: Values (n=3) are means ± SEM; Bars with superscripts ([abc]) without a common letter have a significant difference between spices ($p \leq 0.05$). Bars with superscripts ([xy]) without a common letter have a significant difference between solvents ($p \leq 0.05$). DPPH: 2,2 Diphenyl-1-dicrahydrazyl radical scavenging.

Figure 4, displays the ferric reducing antioxidant power of spices (garlic, ginger, and turmeric). The FRAP assays determine the spices' ability to reduce the oxidized form of iron (ferric) into the reduced form (ferrous) through the transfer of electrons. The aqueous garlic extract had a significantly higher ferric reducing ability compared to both aqueous ginger and turmeric extracts. The aqueous ginger extract had over a 3-fold higher FRAP value compared the aqueous turmeric extract. Both ethanolic turmeric and ginger extract yielded over a 70% significantly higher ferric reducing ability compared to the garlic extract. Out of the ginger and turmeric extracts, their ethanolic extracts were more successful in reducing the oxidized form of iron into its reduced form of ferrous while no significant difference was found between the garlic extracts. The findings from figure 4, are not in accordance with a study [23], which may have been attributed to the origin of the spices, storage, processing, and method of extraction. These factors have a significant impact on spices' reducing ability and antioxidant content.

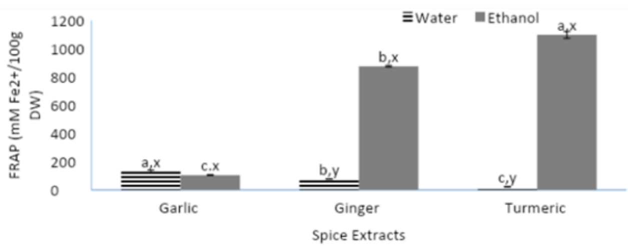

Figure 4: Ferric reducing antioxidant power of spices (garlic, ginger, and turmeric).

Note: Values (n=3) are means ± SEM; Bars with superscripts ([abc]) without a common letter have a significant difference between spices ($p \leq 0.05$). Bars with superscripts ([xy]) without a common letter have a significant difference between solvents ($p \leq 0.05$). FRAP- Ferric reducing antioxidant power. Fe (II): Ferrous Iron; DW: Dry Weight.

Figure 5, displays the Trolox equivalent antioxidant capacity of spices to evaluate the spice's ability to scavenge the ABTS radical. Starting with the first comparison, among the aqueous extracts, the ginger extract had a significantly higher Trolox equivalence antioxidant capacity as compared to both garlic and turmeric aqueous extracts. The aqueous garlic extract had over a 30% higher Trolox equivalence compared to the turmeric extract. Among the ethanolic extracts, both ethanoic turmeric and ginger extracts had over a 24-fold higher reducing ability compared to the garlic ethanolic extract. With the exception of garlic, all ethanolic extracts (ginger and turmeric) had a significantly higher TEAC compared to their respective aqueous extracts. The results from figure 5, show that the ethanolic extracts of turmeric and ginger have a high reducing ability through scavenging the ABTS radical. This may be attributed to the complex structures of the phytochemicals, curcumin, and gingerol, found within turmeric and ginger.

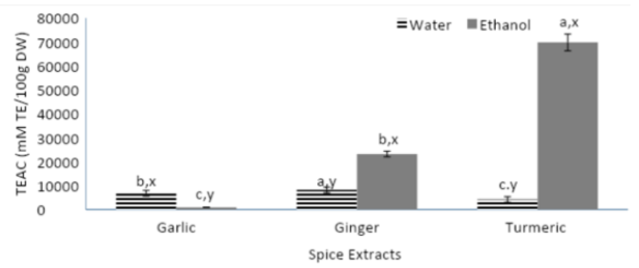

Figure 5: Trolox equivalent antioxidant capacity of spices (garlic, ginger, and turmeric).

Note: Values (n=3) are means ± SEM; Bars with superscripts (^{abc}) without a common letter have a significant difference between spices ($p \leq 0.05$). Bars with superscripts (^{xy}) without a common letter have a significant difference between solvents ($p \leq 0.05$). Trolox Equivalent Antioxidant Capacity (TEAC).TE: Trolox Equivalence; DW: Dry Weight.

The graph for α-amylase inhibition by spices (garlic, ginger, and turmeric) is not shown. α-amylase is an enzyme that is induced upon consumption of carbohydrates. Due to the western diet being high in simple carbohydrates, possible inhibition of this enzyme may be beneficial in preventing chronic diseases such as type 2 diabetes and obesity [24]. No inhibition was reported among the aqueous and ethanolic extracts. The presence of the spices may have not interfered with the binding of the substrate to the enzyme in this assay. The results from figure 6 show that non-competitive enzyme inhibition may have taken place, while competitive inhibition was desired [25].

Figure 6, displays the α-glucosidase inhibition by spices (garlic, ginger, and turmeric). Among the ethanolic extracts α-glucosidase inhibition was reported, while no α-glucosidase inhibition was reported among the aqueous extracts. The highest α-glucosidase percent inhibition was found within the ginger extract. The ethanolic ginger extract had over an 18% higher enzyme percent inhibition compared to the ethanolic turmeric extract. The ethanolic turmeric extract had a significantly higher enzyme percent inhibition of 61% compared to the garlic extract. The results from figure 6, show possible implications of spices being able to reduce blood glucose levels, which may be beneficial in preventing chronic diseases.

Figure 7, displays the lipase inhibition by spices (garlic, ginger, and turmeric). The lipase enzyme is responsible for the hydrolysis of triglycerides into free fatty acids. Inhibition of the lipase enzyme may be beneficial towards preventing obesity within individuals. Lipase percent inhibition was reported among both aqueous and ethanolic

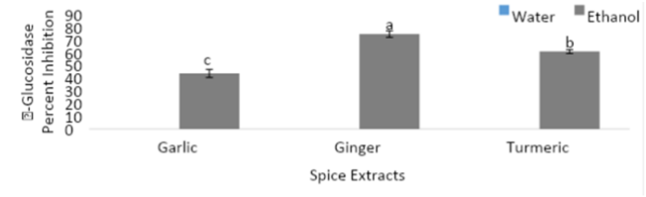

Figure 6: α-glucosidase inhibition by spices (garlic, ginger, and turmeric).

Note: Values (n=3) are means ± SEM; Bars with superscripts (^{abc}) without a common letter have a significant difference between spices ($p \leq 0.05$).

extracts. In figure 7, the opposite trend was found compared to the previous figures where the aqueous extracts of the spices resulted in a higher lipase percent inhibition. Overall the aqueous garlic and ginger extracts resulted in a significantly higher lipase percent inhibition followed by the aqueous turmeric extracts.

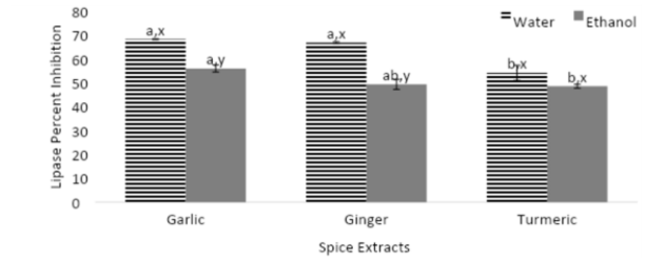

Figure 7: Lipase inhibition by spices (garlic, ginger, and turmeric).

Note: Values (n=3) are means ± SEM; Bars with superscripts (^{abc}) without a common letter have a significant difference between spices ($p \leq 0.05$). Bars with superscripts (^{xy}) without a common letter have a significant difference between solvents ($p \leq 0.05$).

Figures 8-13, display the chemical analysis that was conducted on the Spiceola Bites, a granola-like product formulated with garlic, ginger, and turmeric. In each of the assays conducted, the spciecola bite extracted with ethanol results in higher phytochemical content, antioxidant capacity, reducing ability, and enzyme inhibition. The Spiceola Bites were formulated to be marketed toward adolescents. The results show that the Spiceola Bites have a great potential in decreasing the risk and prevalence of obesity and type 2 diabetes in individuals, especially adolescents.

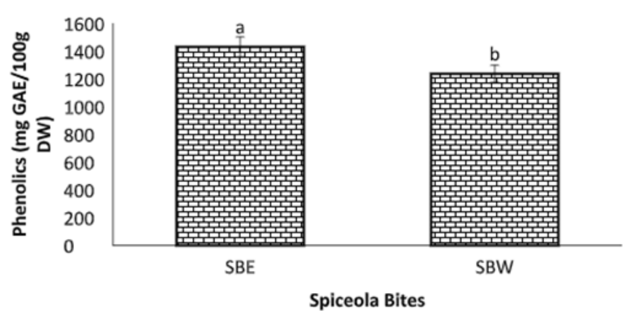

Figure 8: Total Phenolic Content of Spiceola Bites.

Note: Values (n=3) are means ± SEM; Bars with superscripts (abc) without a common letter have a significant difference between spiceola bite extracts ($p \leq 0.05$). Abbreviations: GAE: Gallic Acid Equivalents; SBE: Spiecola Bites extracted with Ethanol; SBW: Spiceola Bites Extracted with water.

Discussion

The Total Phenolic Content (TPC), Total Flavonoid Content (TFC), 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical

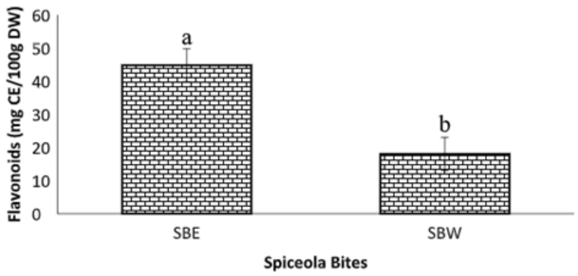

Figure 9: Total flavonoid content of spiceola bites.

Note: Values (n=3) are means ± SEM; Bars with superscripts (^{abc}) without a common letter have a significant difference between spiceola bite extracts ($p \leq 0.05$). Abbreviations: CE: Catechin Equivalents; SBE: Spiecola Bites extracted with ethanol; SBW: Spiceola Bites Extracted with water; DW: Dry weight

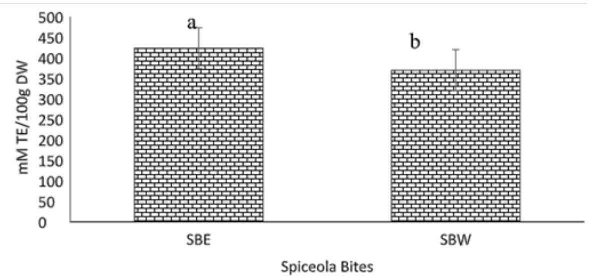

Figure 10: Trolox equivalent antioxidant capacity of spiceola bites.

Note: Values (n=3) are means ± SEM; Bars with superscripts (^{abc}) without a common letter have a significant difference between spiceola bite extracts ($p \leq 0.05$). Abbreviations: TE: Trolox Equivalents; SBE: Spiecola Bites extracted with ethanol; SBW: Spiceola Bites Extracted with water; DW: Dry Weight.

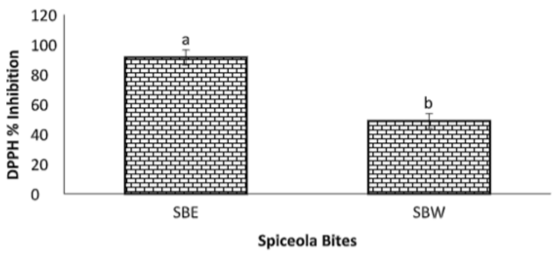

Figure 11: DPPH percent inhibition by spiceola bites.

Note: Values (n=3) are means ± SEM; Bars with superscripts (^{abc}) without a common letter have a significant difference between spiceola bite extracts ($p \leq 0.05$). Abbreviations: DPPH: 2,2-Diphenyl-1-Picrylhydrazyl; SBE: Spiecola Bites extracted with ethanol; SBW: Spiceola Bites Extracted with water.

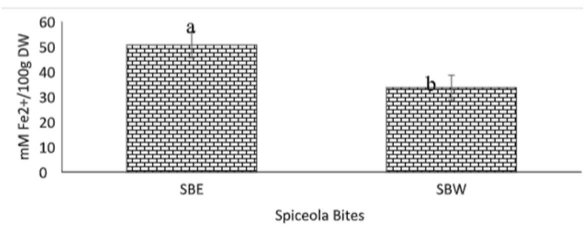

Figure 12: Ferric reducing antioxidant power of spiceola bites.

Note: Values (n=3) are means ± SEM; Bars with superscripts (^{abc}) without a common letter have a significant difference between spiceola bite extracts ($p \leq 0.05$). Abbreviations: FE2+- Ferrous Sulfate; SBE: Spiecola Bites extracted with Ethanol. SBW: Spiceola Bites Extracted with water; DW: Dry Weight.

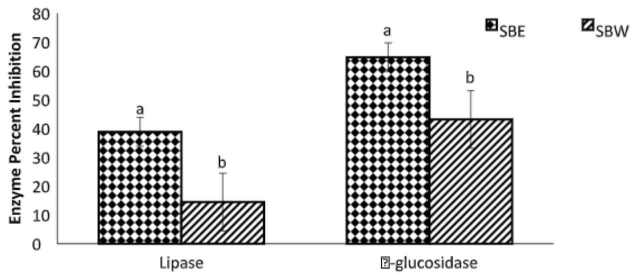

Figure 13: Inhibition of lipase and α-glucosidase activities by spiceola bites.

Note: Values (n=3) are means ± SEM Bars with superscripts (^{abc}) without a common letter have a significant difference between spiceola bite extracts ($p \leq 0.05$). SBE: Spiceola Bites extracted with ethanol; SBW: Spiceola Bites Extracted with water.

scavenging, Trolox Equivalent Antioxidant Capacity (TEAC), Ferric Reducing Antioxidant Power (FRAP), α-amylase, α-glucosidase, and lipase enzyme inhibition was measured from the spice extracts and Spiceola Bite extracts. The extraction method of both extracts had a great impact on the results, which demonstrated that the use of 80% ethanol was more sufficient in extracting the phytochemical compounds in the extracts. The findings of this study are in accordance with a study [26], where the ethanolic extractions of ginger and turmeric resulted in higher bioactivity compared to the aqueous extracts. The phytochemicals found in ginger and turmeric are more stable than the phytochemicals found in garlic due to their presence of benzoic rings, double bonds, and hydroxyl groups.

Conclusion

To conclude, the results from this study, demonstrate that spices and the Spiceola Bites functional food product has great potential in preventing chronic diseases within individuals. Overall, the ginger extracts exhibited a higher antioxidant content while the opposite trend was found among the garlic extracts. The results from the assays performed on the Spiceola Bites also demonstrate that spices combined in a food matrix have a great synergistic effect in regards to phytochemical content and antioxidant capacity. Due to blood glucose levels and free fatty acids being significantly elevated within diabetic and obese individuals, this study investigated the spices' and Spiceola Bites' ability to inhibit the metabolizing enzymes: α-amylase, α-glucosidase, and lipase. The results showed promising results towards possibly decreasing blood glucose and free fatty acid levels within individuals. Spices should be incorporated into more functional foods within the food industry to improve the prevalence of chronic diseases.

Future Work or Recommendations

Future work will involve determining the anti-diabetic potential of spices using a cell culture model.

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