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Enzymatic Treatment of Spent Green Tea Leaves and Their Use in High-Fiber Cookie Production

Cookies with Enzymatically Treated Spent Green Tea Leaves

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SUMMARY

Research background. By-products of food industry have been studied as high fiber and antioxidant ingredients for healthy food products, because of their economic and environmental benefits. However, the soluble dietary fiber (SDF) content of these materials is usually lower than the recommended value that is claimed to bring great health effects. Enzymatic treatment could be an efficient method for modifying insoluble and soluble dietary fiber contents of these materials. The purpose of this study was to investigate the effects of enzymatic treatment conditions on SDF, insoluble dietary fiber (IDF), total dietary fiber (TDF) proportions of spent green tea leaves (STL) and evaluate the quality of dough and cookies, when different levels of untreated and treated STL were added to the recipe.

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Experimental approach. The contents of SDF, IDF and TDF of STL powder was evaluated after STL was treated by cellulase preparation with enzyme loadings ranged from 0 to 25 U/g and treatment times ranged from 0 to 2 h. Wheat flour was replaced by untreated and treated STL powder at 0, 10, 20, 30 and 40 % in cookie formulation. Textural properties of dough, proximate composition, physical properties and overall acceptability of cookies were analysed.

Results and conclusions. The appropriate condition of enzymatic treatment was enzyme loading of 20 U/g and biocatalytic time of 1.5 h, under which the SDF content of STL increased by 144.5 % in comparison with that of the control sample. The addition of STL led to the rise in dough hardness. Increase in STL level also enhanced fiber content, antioxidant activity and hardness of cookies but reduced their overall acceptability. Moreover, the enzymatic treatment of STL improved the SDF to TDF ratio of cookies, which relates to health benefits, and their textural property. Untreated and treated STL added cookies at 20 % received the overall acceptance.

Novelty and scientific contribution. For the first time, enzymatic treatment of STL was performed to improve its SDF to TDF ratio. The treated STL is a new promising high fiber and antioxidant ingredient for cookies making.

Keywords: antioxidant; cookies; dietary fiber; enzymatic treatment; spent green tea leaves

INTRODUCTION

Cookie is one of the most favoured bakery products all around the world thanks to its deliciousness, convenience and long shelf-life. This product is high in sugar, fat and starch, providing high energy intake but low in dietary fiber and antioxidants. Cookies were also reported to be among the most unhealthy foods that lead to weight gain (*1*). Therefore, the demand for cookies with high fiber content and antioxidative properties is becoming more and more essential. Various ingredients which are high in dietary fiber and antioxidants have been used in cookie formulation, such as oat flour (*2*), *Murraya koenigii* leaves (*3*) and beetroot leaves (*4*). Recently, by-products from agriculture and food manufacturing industry, such as watermelon rind waste (*5*) and spent coffee grounds (*6*), have also been studied as potential fiber and antioxidant ingredients for cookies. Among plant by-products, spent green tea leaves were discovered to be rich in dietary fiber and phenolic compounds, with the total fiber and total phenolic contents of 67.9 % and 13.0 % (by dry mass), respectively (*7*). It is reported that spent green tea leaves contained gallic acid, epicatechin, epigallocatechin and gallocatechingallate which had antioxidant behaviour (*8*). As a vast amount of spent tea leaves is exhausted from the beverage industry every year, this inexpensive and valuable by-product was considered as a promising ingredient for food production.

The potential of utilising spent green tea leave powder in cookie formulation was recently recognised. Soma *et al.* (*9*) investigated the impacts of spent green tea powder addition on the quality of biscuits and reported that 7.5 % of wheat flour could be replaced by spent tea powder to produce biscuits with enhanced nutritional value and acceptable sensory. The percentages of soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) of spent tea were 4.07 and 63.69 % (by dry mass) respectively (*9*), resulting in biscuit products, the SDF, IDF and TDF contents of which were 1.68, 7.73 and 9.41 % (by dry mass), respectively, making the ratio of SDF to TDF of nearly 18 %. The total polyphenol of the studied spent tea leaves was 357 mg gallic acid equivalent (GAE) and the total content of phenolic acids in blended cookies was 29.35 mg GAE (*9*).

Insoluble and soluble dietary fiber play different roles in human body. While IDF adsorbs water in the intestine which helps to increase fecal volume, reduce intestinal transit time and glucose absorption (*10*), SDF dissolves in water and forms gel, helps to attenuate blood cholesterol and glucose levels, supports digestive health and reduces antibiotic-related problems as a good source of prebiotics (*11*). Besides total dietary fiber (TDF) content, the ratio of SDF to TDF is an important index and well-balanced percentages of these fractions could improve health and well-being. As the SDF to TDF ratio was recommended to be 30-50 % (by mass) (*12*), the fiber fractions of spent tea added cookies studied by Soma *et al*. (*9*) needs to be improved in order to enhance the impacts of high fiber cookies on human nutrition. This leads to the necessity of treating spent tea leaves to increase its SDF content before adding to the cookie formulation.

The hydrolysis of IDF in order to enhance the SDF content using enzyme preparations has been reported in production of different food ingredients from Chinese cabbage waste (*13*) and rice husk (*14*). However, the enzymatic treatment of spent tea leaves has not been considered to improve the SDF content in order to incorporate in food products. Since cellulose made up the majority in tea fiber composition with 67.7 % by mass (lignin and hemicellulose contents were 20.0 and 4.5-7.2 % by mass, respectively) (*9*), the appropriate carbohydrase for this ingredient treatment was cellulase preparation. The purposes of our study were to investigate the effects of different enzymatic treating conditions on the dietary fiber fractions of spent green tea leaves (STL) and evaluate the impacts of untreated and treated STL powder on the characteristics of doughs and cookies.

MATERIALS AND METHODS

Materials

Spent green tea leaves (STL) was collected from the extracting process for green tea beverage manufacturing in a factory of Universal Robina Corporation Vietnam (Binh Duong, Vietnam), dried at 60 °C using a convectional dryer to reach a moisture content of 9-11 % (by mass), grinded into powder and sieved through a 40-mesh screen. Commercial wheat flour (Dai Phong Flour Milling Co., Ltd, Can Tho City, Vietnam), eggs (V.food Ltd, Ho Chi Minh City, Vietnam), unsalted butter (Anchor, Hamilton, New Zealand), table salt, baking powder (Alsa, Schirmeck, France), vanillin powder (Vianco Ltd., Ho Chi Minh City, Vietnam) and isomalt (Beneo, Mannheim, Germany) were procured from the local supermarket. Food grade acesulfame potassium was purchased from Vitasweet Co., Ltd. (Jiangsu, China). Celluclast 1.5 L preparation with endo-glucanase activity of 80 U/mL (produced by *Trichoderma reesei*) was originated from Novozymes A/S (Bagsværd, Denmark). One unit of cellulase activity was defined as reducing sugar content which was released from carboxymethylcellulose (CMC) as the substrate of hydrolysis and expressed by U/mL enzyme preparation. The optimal pH and temperature of this enzyme preparation are 4.5-6.0 and 50-60 °C, respectively.

Enzymatic treatment of spent tea leaves

An amount of 10 g STL powder was contained in a 250 mL Erlenmeyer flask. First, the enzyme preparation was mixed with deionised water. The quantity of deionised water was calculated so that the moisture content of treated mixture reached 7 mL/g dry STL. The quantity of enzyme preparation was calculated to achieve enzyme loadings of 0, 5, 10, 15, 20 and 25 U/g dry STL. The approximate pH value of treated mixture was 5.0. The Erlenmeyer flask was then covered with aluminum foil and brought into the incubator (Model WNB29, Memmert, Schwabach, Germany) for the enzymatic treatment at the temperature of 50 °C. The treatment time ranged from 0 to 2 h (0, 0.5, 1.0, 1.5 and 2.0 h). At the end of the treatment, the temperature was raised to 95 °C using a water bath and kept at this temperature for 10 min for enzyme inactivation. The enzyme-treated STL was dried at 60 °C in the convectional dryer to reach a moisture content of 10 %, then grinded and sieved through a 40-mesh screen. The proximate composition, antioxidant activity, water and oil holding capacity of treated and untreated STL powder were measured.

Cookie manufacturing process

The cookie formula used in this study included 100 g flour (wheat flour and STL powder), 38.8 g eggs (yolk and white), 58.3 g unsalted butter, 38.8 g isomalt, 0.5 g vanillin powder, 1.3 g baking powder, 0.6 g table salt, 10.8 g water and 0.15 g acesulfame potassium. Ingredients except for wheat flour and STL powder were mixed together at 200 rpm speed by a stand mixer (Model KitchenAid Artisan 5KSM175PSECA, Whirlpool Corporation, Ohio, USA) in the total of 5 min to obtain a cream mixture. STL powder was blended with wheat flour at 0 (control sample), 10, 20, 30 and 40 % (by mass). Powder blends were added to the cream mixture and kneaded at 100 rpm in 2 min to prepare dough. Dough was then split into halves, one half was immediately used for measuring textural properties and the other half was used for making cookies. Dough sheets with the thickness of 4 mm was then manually made and cut by a 36 mm diameter round cutter before being baked in an electric oven (Model VH-259S2D, Sanaky Vietnam Company Limited, Binh Duong, Vietnam). The baking process consisted two stages, the temperature was set at 175 °C in the first 15 min and lowered to 150 °C for the next 8 min. Baked cookies were then cooled to room temperature in 15 min. After that, the physical and textural properties were evaluated. Cookies were packed in sealed polyethylene pouches and stored in 24 h for analysing approximate composition and sensory quality. Polyethylene pouches were sealed by an impulse heat sealer (Model M17, Tan Thanh Service Trade Production Co., Ho Chi Minh City, Vietnam).

Proximate composition

Moisture content was measured using a moisture analyser (Model ML-50, A&D Company Limited, Tokyo, Japan). Total lipid content was quantified with Soxhlet extraction using diethyl ether solvent following AOAC 930.09 *(15)*. Protein content was determined using Kjeldahl method and the protein-nitrogen conversion factor was 5.7 for wheat flour and 6.25 for STL and cookies, following AOAC 979.09 (*16*). Samples were incinerated in an ashing furnace (Model AF 11/6B, Lenton Furnaces & Ovens, Hope Valley, England) at 600 °C for measuring ash content using AOAC 942.05 (*17*). Starch content was determined using starch digestion method described by Landhäusser *et al*. (*18*) and glucose content was measured using spectrophotometric method with dinitrocyclic acid reagent (*19*). Insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) contents were analysed according to AOAC 991.42 (*20*) and 993.19 (*21*) methods, respectively. Total dietary fiber (TDF) content was calculated as the sum of IDF and SDF contents, following AOAC 991.43 (*22*).

Total phenolics were determined using 50 % (by volume) acetone/water as the solvent for the ultrasound-assisted extraction (VCX 500 Sonicator, Sonics & Materials, Inc., Connecticut, United States), using 1 g sample in 10 mL solvent, following the method described by Bhebhe *et al*. (*23*) and expressed as mg gallic acid equivalent (GAE)/g dry mass. Antioxidant activity was measured with 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay following the method by Brand and Williams (*24*), and ferric reducing antioxidant power (FRAP) assay following the method by Benzie and Strain (*25*), expressed as mg Trolox equivalent (TE)/g dry mass.

Water and oil holding capacity of materials

Water holding capacity (WHC) of enzyme-treated STL powder, untreated STL powder and wheat flour were measured following AACC 88-04 method with a minor modification. An amount of 3 g sample was mixed with 30 mL of water using a vortex in 30 sec. After 2 h, the sample tube was centrifuged at 1,000 × *g* in 20 min. The supernatant was then decanted. The absorbed water was determined by the difference between the sediment and the initial sample weights. Oil holding capacity (OHC) were measured with the similar process in which samples were mixed with soybean oil (Tuong An Vegetable Oil Joint Stock Company, Ba Ria – Vung Tau Province, Vietnam).

Texture profile analysis (TPA) of dough

The texture of cookie dough was measured by TPA method using TA.XTplusC Texture Analyser (Stable Micro Systems, Godalming, UK). The dough was shaped by a 42 mm diameter and 40 mm height circular mold, compressed up to 25 % with the speed of 5 mm/s by a 25 mm diameter cylinder probe. Three recorded textural parameters of dough were hardness (g force), springiness and cohesiveness.

Physical properties of cookies

A set of six cookies was edge-to-edge laid on the platform to measure the average width (W, mm). The set was rotated 90° for replicated measurement. A stack of six cookies was measured for the average thickness (T, mm). The spread ratio (W/T) was then calculated. The L^*, a^* and b^* values of cookies were measured by Hunter Lab Colour Measuring system (CR-400, Konica Minolta, Inc., Tokyo, Japan) and the colour difference against the control sample (ΔE) was calculated as follows:

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \qquad /1/$$

where L_0^*, a_0^* and b_0^* are the colour values of the control sample; L^*, a^* and b^* are the colour values of the determined sample.

The hardness of cookies was evaluated by the breaking strength (g force) analysed with three-point break method, using the same instrument that was used to evaluate TPA of dough and a three-point break rig.

Overall acceptability of cookies

Cookies were evaluated for the overall acceptability on a 9-point hedonic scale (ranging from 1 as 'dislike extremely' to 9 as 'like extremely'), following the method described by Nguyen *et al*. (*26*). An untrained panel of 60 consumers who aged from 18 to 40 were selected among students and staffs in Ho Chi Minh City University of Technology (Ho Chi Minh City, Vietnam). Criteria for recruiting panelists were being regular with cookies and not being allergic to any food. Samples were presented in white plastic plates, coded with different sets of three digits, served in randomised order and simultaneous way.

Statistical analysis

All experiments were conducted in triplicate and data were reported as mean±SD. STATGRAPHICS Centurion 18 software (Statgraphics Technologies, Inc., Virginia, United States) (*27*) was employed to determine significant difference of means ($p<0.05$), using Analysis of variance (ANOVA) with Least Significant Difference (LSD) method. Correlation coefficient r and R^2 were determined using this software in order to evaluate the significant relationship between variables ($p<0.05$) when necessary.

RESULTS AND DISCUSSION

Effects of enzyme loadings on the fiber fractions of spent tea leaves

The impacts of different enzyme loadings on SDF, IDF and TDF contents of STL are illustrated in Fig. 1. After 1 h of the treatment, the SDF content increased by 139.2 % (by dry mass) (Fig. 1a) while the IDF content declined by 31.8 % (by dry mass) (Fig. 1b) as the enzyme loading rose from 0 to 20 U/g dry mass ($p<0.05$). This was due to the more vigorously cellulolysis of IDF at greater cellulase loadings. However, SDF and IDF contents both dropped as the enzyme loading gained from 20 to 25 U/g dry mass. Reduction in SDF content of STL at the high enzyme loading could be explained by the degradation of SDF into smaller molecules. Dietary fiber with degree of polymerisation from 3 to 9 monomers are hardly precipitated by alcohol and obtained after filtration in the measuring method (*28*). It was previously reported that the SDF yield extracted from pineapple pomace increased sharply when the cellulase content rose from 1 % to 5 % and decreased moderately when the enzyme content increased to 6 % (*29*).

The TDF content felt by 1.7 % when the enzyme loading increased from 0 to 5 U/g and remained unchanged as the enzyme loading increased from 5 to 20 U/g (Fig. 1c). Nevertheless, further increase in enzyme loading from 20 to 25 U/g reduced the TDF content by 8.1 %. The loss in TDF content could be due to intensive hydrolysis of SDF into smaller molecules at cellulase loading of 25 U/g.

Effects of treatment time on the fiber fractions of spent tea leaves

The impacts of treatment time on SDF, IDF and TDF contents of STL were also shown in Fig. 1. At enzyme loading of 20 U/g, when the treatment time increased from 0 to 1.5 h, the SDF content rose by 143.5 % (Fig. 1d) while the IDF content fell by 34.0 % (Fig. 1e). However, as the biocatalytic time was prolonged from 1.5 to 2.0 h, a decline in the SDF and IDF contents by 9.9 % and 4.3 %, respectively, were observed. This could be due to the prolonged hydrolysis of SDF to produce low molecular compounds (*28*). Nguyen *et al.* (*26*) also reported the improvement in SDF content of wheat bran when the incubation time rose from 0 to 150 min and its loss when the cellulase treatment lasted from 150 to 210 min.

Proximate composition, antioxidative and functional properties of the treated and untreated spent tea leaves and wheat flour used in the study

It is illustrated in Table 1 that the treatment of STL with the enzyme loading of 20 U/g in 1.5 h did not change its protein, lipid and ash contents. Both treated and untreated STL powder had greater contents of protein, lipid and ash than the wheat flour ($p<0.05$). The treatment enhanced the SDF content of STL by 145.2 %, decreased the IDF and TDF contents by 34.1 % and 3.0 %, respectively. Therefore, the SDF to TDF ratio of the treated STL was elevated by 1.5 times in comparison with that of the untreated STL. Decreases in the total phenolic compounds and antioxidant activity of STL were observed, owing to the oxidation of during the drying process (60 °C) of the treated STL. Sagrin and Chong *(30)* reported that drying processes (40-60 °C) could cause degradation of phenolic compounds in plant leaves. It is clear that both untreated and treated STL had significantly greater proportions of dietary fiber, phenolic compounds and antioxidant activity than the wheat flour ($p<0.05$).

Moreover, water and oil holding capacity of wheat flour were lower than those of untreated and treated STL. The enzymatic treatment reduced both WHC and OHC of STL by 4.4 % and 12.4 %, respectively, probably due to the decreased IDF and increased SDF contents. It was also claimed that the cellulase hydrolysis of dietary fiber destroyed the lamellar structure of IDF which had higher WHC and OHC compared to SDF *(31)*. These changes in functional properties of STL after the enzymatic treatment would probably affect dough and cookie quality.

Textural properties of dough

Hardness, cohesiveness and springiness are among the most concerned textural properties of solid foods *(32)*. The effects of different additions (0, 10, 20, 30 and 40 %) of untreated and enzyme-treated STL on these properties are shown in Table 2. Overall, increased levels of both untreated and treated STL powder added to the recipe significantly enhanced the hardness of cookie dough, but the untreated STL caused more dramatic increases ($p<0.05$). As the level of STL reached 40 %, the hardness of untreated and treated STL added dough were 3.0 and 2.5 times, respectively, greater than that of the control sample (0 %). The hardness of dough relates to its free water content and fiber fractions supplemented from STL. The incorporation of STL powder enhanced the total fiber content and the number of hydroxyl groups in dough system. The interaction between hydroxyl groups of fiber and free water molecules via hydrogen bonding could cause the increase in WHC of dough *(33,34)*. The reduction of free water amount due to the addition of fiber restricted the mobility of other components in the system and produced a more compact dough *(35)*. The enzymatic treatment reduced the IDF content and water holding capacity of STL. The availability of greater amount of free water molecules in dough system resulted in the lowered hardness of treated STL added dough.

On the other hand, the springiness and cohesiveness experienced downward trends as greater amounts of STL were added. The incorporation of untreated and treated STL at 40 % generated decreases in the springiness by 29.3 and 32.8 %, respectively, compared to the control sample, while the cohesiveness declined by 48.1 and 40.7 %, respectively. The treatment of STL produced a minor increase in the cohesiveness of dough at high STL levels like 40 %. Liu *et al*. (*36*) claimed that the presence of matcha tea fiber inhibited disulfide bonding and the formation of gluten network, led to the decrease in dough strength. The limitation of gluten network formation and disulfide bond regeneration after being broken by external forces could be an explanation for the decreased cohesiveness of dough and the ability to recover its initial form (springiness). Nevertheless, it was also reported that tea polyphenols caused the breakage of hydrogen bonds, increased the cross-linking of gluten network and interactively arranged gluten chains to build a “grid” structure, consequently enhanced the cohesiveness of dough (*36*). Different results were observed probably because of various conditions of dough kneading, including moisture content, kneading temperature and mixing rate. Combined effects of STL fiber and polyphenols on the formation of gluten network of cookie dough should be further investigated to clarify their impacts on dough textural properties.

Similar increase in hardness and reduction in springiness and cohesiveness of cookie dough were previously reported when the level of dried Moringa (*Moringa oleifera Lam.*) leaves powder was enhanced from 0 to 15 % (*37*). Armero and Collar (*38*) also claimed that dough cohesiveness was negatively correlated to its hardness and positively correlated with its springiness.

Chemical analysis of cookies

The proximate composition of STL incorporated cookies is shown in Table 3. The protein and ash contents of cookies slightly increased, while the starch content considerably decreased as greater amounts of STL powder were added ($p<0.05$). This could be due to the differences between proximate compositions of STL powder and wheat flour. Significant differences were not observed in protein, ash and starch contents between untreated and treated STL added cookies at similar STL levels ($p>0.05$). There were also substantial gains in TDF, SDF and IDF contents of cookies as the addition of STL increased. The enzymatic treatment of STL resulted in higher SDF, lower TDF and IDF contents of cookies. At the STL addition level of 30 %, the TDF, SDF and IDF contents of untreated STL added cookies (30 %) were 8.1, 3.3 and 12.5 times respectively as much as the control sample, while the contents of treated STL added cookies gained 7.4, 6.3 and 8.3 times, respectively ($p<0.05$). The SDF to TDF ratio of cookies incorporated with untreated STL did not reach the recommended value range (30-50 % (*12*)). Interestingly, the cellulase treatment of STL successfully improved the SDF to TDF ratio of cookies with levels of treated STL from 10 to 40 %, to accomplish the recommended ratio for good health impacts. As the level of STL increased from 10 to 40 %, the SDF to TDF ratio of treated STL added cookies were enhanced by 1.90, 2.04, 2.01 and 2.14 times compared to those of untreated STL added cookies, respectively. In the future, in-vivo tests should be carried out to confirm healthy impacts of both untreated and treated STL added cookies. However, the treated STL added cookies had lower total phenolic content and antioxidant activity than the untreated STL added samples. This could be explained by the degradation of antioxidant compounds in STL during the drying process after the enzymatic treatment.

Physical characteristics of cookies

Physical properties of cookies with different STL incorporations including width, thickness, spread ratio, hardness and instrumental colour are illustrated in Table 4. The thickness of cookies slightly grew as the STL proportion increased while the width slightly reduced, resulting in the decreased spread ratio (W/T). The spread of cookie during baking is caused by the expansion of dough with leavening and the gravitational flow of dough (*39*). As the dough flows during baking, protein undergoes the “apparent” glass transition which allows it to swell and form a network (*40*). The mobility of free water molecules is decreased and the dough viscosity is increased to a point when it is sufficient to stop the spreading of dough (*41*). It is suggested that addition of fiber into the cookie dough lessens the amount of free water to dissolve soluble components, increasing dough viscosity and reducing the spread of the product. Similar results were reported by Younis *et al*. (*42*); when the level of mosabi (*Citrus limetta*) peel powder added to cookie formula increased from 4 to 12 %, the thickness of cookies rose from 9.82 to 11.36 mm while their diameter decreased from 59.10 to 55.12 mm, resulting in the decreased spread ratio from 5.62 to 4.86. It can be noted that the cellulolytic treatment of STL did not affect spread ratio of the cookies.

As higher levels of wheat flour were replaced by STL powder, an upward trend in the hardness of cookies was witnessed which was positively correlated with dough hardness (The correlation coefficient r and coefficient of determination R^2 were 0.89 and 0.80, respectively, for the untreated STL added dough and cookies, while those were 0.92 and 0.85, respectively, for the treated STL added dough and cookies). Ajila *et al*. (*43*) investigated the impacts of mango peel powder on cookie characteristics and reported that the hardness of cookies increased from 0.88 to 1.97 kg as the mango peel powder level rose from 0 to 20 %. Another point worth mentioning is the hardness of treated STL cookies had less considerable difference against the control sample than the hardness of untreated STL cookies. This could be explained by the higher SDF content of cookies enriched with treated STL compared to ones enriched with untreated STL. Hydrolysed fiber had lower WHC and more free water was available in dough for the swelling of starch granules, in which the breakage of intramolecular hydrogen bondings and the mobility of macromolecules in dough system were increased, resulting in cookies with lower breaking forces (*35*). Our study confirmed that cookies produced from dough with greater hardness and cohesiveness tended to have harder texture and lower spreading (*44*).

The addition of STL powder from 10 to 30 % resulted in the darker colour (decreased *L** value) of cookies, probably because of dark coloured compounds such as thearubigins, theaflavins and theabrownines which were generated from the oxidation of STL catechins during baking process (*45*). The greenness of cookies was enhanced (lower negative value of a*) and the yellowness was reduced (decreased *b** value) as higher proportions of STL were incorporated. This could be explained by the increase in chlorophyll pigment content as STL was added to the formulation. As a result, the colour differences of STL added cookies against the control sample (ΔE) were enhanced when the STL level rose from 10 to 30 %. However, ΔE values of 30 % and 40 % STL added cookies differed insignificantly (p>0.05). Ahmad *et al*. (*46*) observed decreased lightness of the cookie samples with 4 % green tea powder. Moreover, the enzymatic treatment of STL also reduced brightness (*L**) of cookies, resulted in the greater colour difference against the control sample compared to untreated STL added cookies. This might be due to the degradation of chlorophyll into colourless compounds (*47*) and the oxidation of phenolic compounds (*48*) of the enzyme treated STL during the drying process.

Overall acceptability of cookies

The overall acceptability of cookies with different STL levels is illustrated in Table 4. The overall score fell with the rise in STL level and the enzymatic treatment of STL did not significantly affect the acceptability of cookies (p>0.05). The control cookies received the highest score (6.6±1.4) and the least accepted samples were cookies added 40 % untreated and treated STL (3.3±1.5 and 3.7±1.6, respectively). The highest level of both types of STL accepted by the panelists (score ≥ 5.0) was recorded to be 20 %. The reason for this downward trend in the acceptability could be the increase in cookie astringency and hardness contributed by STL. Astringency was considered as a negative contributor to the acceptability of various food products and a major reason for plant products being rejected by consumers, despite the health benefits from phytonutrients (*49*). Despite the textural improvement of cookies as STL was treated with cellulase, significant difference between the overall acceptance of untreated and treated STL added cookies at similar STL levels was not observed. The responses to cookie textural preference were reported to be non-persistent, there were consumers who preferred hard crunchy cookies, while others preferred soft chewy cookies since they were less dry and “fresher” than hard ones (*50*). Therefore, the increased hardness of cookies could be an unfavourable attribute for panelists who prefer soft cookies, but it could also be a positive characteristic for people who prefer hard cookies.

CONCLUSIONS

The treatment of STL using Celluclast 1.5 L with the enzyme loading of 20 U/g for 1.5 hour successfully improved the SDF to TDF ratio of cookies incorporated with the treated STL powder. The SDF to TDF ratio of untreated STL added cookies fell short of the advisable value for efficiently providing health benefits. The enzymatic treatment of STL elevated the SDF to TDF ratio of treated STL added cookies by 1.90, 2.04, 2.01 and 2.14 times compared to these of untreated STL added samples as the STL level rose from 10 to 20, 30 and 40 %, respectively, achieving the recommended value of 30-50 %. Despite some negative effects of STL powder on the quality of cookies such as increased hardness, limited spreading and darker colour, the nutritional value of cookies was enhanced with the supplementation of dietary fiber and antioxidant compounds. The cellulase treatment of STL decreased the differences in physical properties of dough and cookies between 100 % wheat flour cookies and STL added ones. The greatest level of untreated and treated STL powder in cookie formulation which achieved the overall sensory acceptance was up to 20 %. Thus, enzymatic treatment was an efficient method for modifying dietary fiber composition of spent green tea leaves. Enzymatic treated spent green tea leaves could be considered as a promising source of dietary fiber and antioxidants that can be utilised in manufacturing value-added food products.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS’ CONTRIBUTION

N.D.T. Nguyen designed and performed the experiments, analysed data and wrote the manuscript. T.T.H. Phan contributed with the experiment performance, data collection and discussion.

T.T.T. Tran, N.M.N. Ton, D.L.T. Vo contributed with the data interpretation and revision. V.V.M. Le performed the supervision and final revision of the manuscript to be published.

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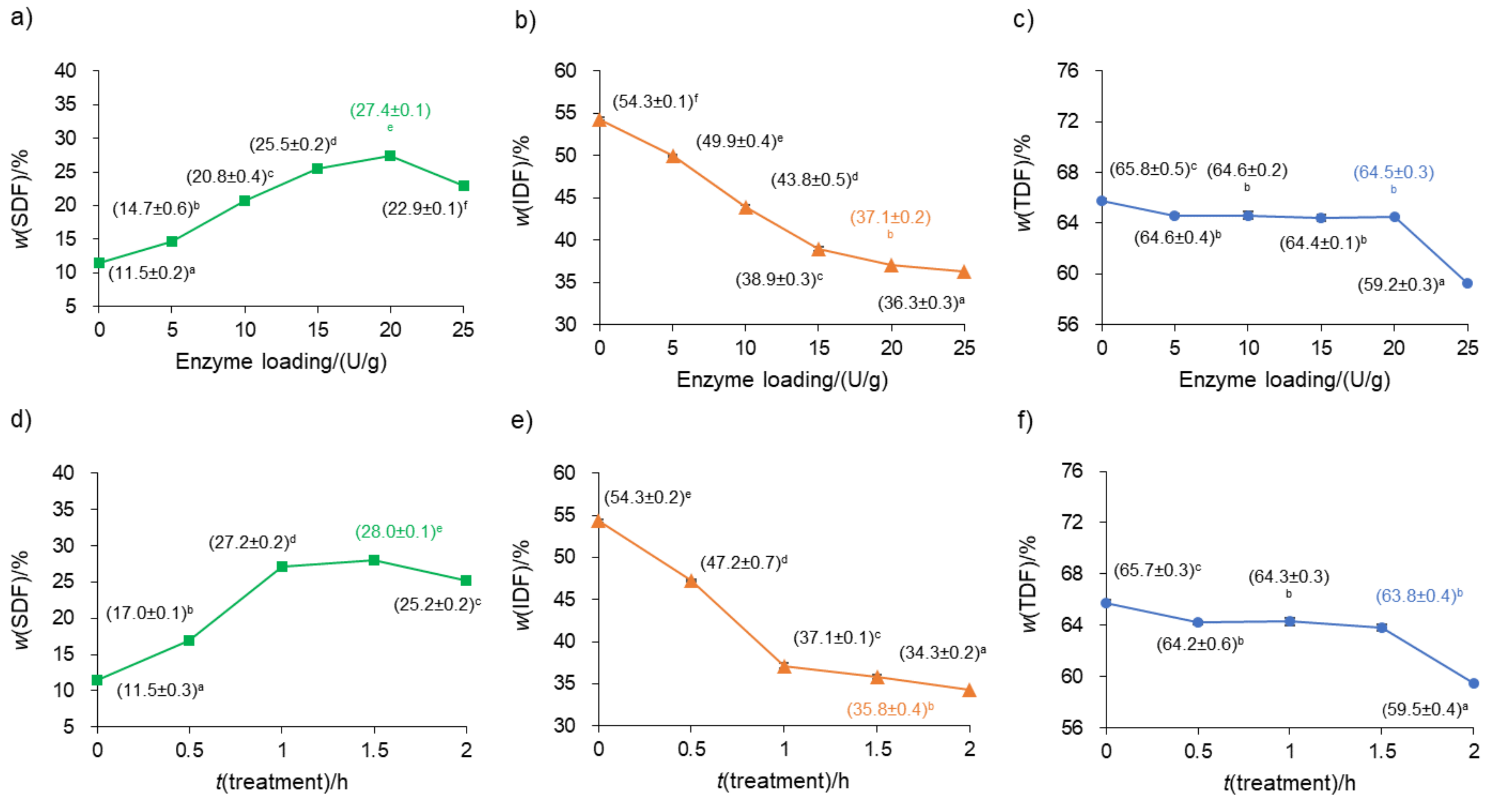

Fig. 1. Effect of enzyme loadings (a-c) and treatment times (d-f) on soluble dietary fiber (SDF), insoluble dietary fiber (IDF) and total dietary fiber (TDF) contents of spent tea leaves; a,b,c-fixed level: water content of 7 mL/g dry mass STL and time of 1 h; d,e,f-fixed level: water content of 7

mL/g dry mass STL and enzyme loading of 20 U/g dry mass STL. Values followed by different letters for each component differ significantly ($p<0.05$).

Table 1. Proximate composition, antioxidative and functional properties of treated, untreated spent tea leaves (STL) and wheat flour

Component	Treated STL	Untreated STL	Wheat flour
w(protein)/%*	$(12.34\pm0.09)^{b}$	$(12.23\pm0.11)^{b}$	$(9.64\pm0.06)^{a}$
w(lipid)/%*	$(1.74\pm0.05)^{b}$	$(1.75\pm0.08)^{b}$	$(1.46\pm0.14)^{a}$
w(starch)/%*	-	-	(84.75 ± 0.44)
w(ash)/%*	$(3.49\pm0.03)^{b}$	$(3.47\pm0.01)^{b}$	$(0.77\pm0.06)^{a}$
w(TDF)/%*	$(63.82\pm0.41)^{b}$	$(65.81\pm0.66)^{c}$	$(2.76\pm0.06)^{a}$
w(SDF)/%*	$(28.00\pm0.05)^{c}$	$(11.42\pm0.21)^{b}$	$(1.34\pm0.04)^{a}$
w(IDF)/%*	$(35.82\pm0.39)^{b}$	$(54.39\pm0.54)^{c}$	$(1.42\pm0.02)^{a}$
ζ(SDF/TDF)/(g/g)	$(43.87\pm0.26)^{b}$	$(17.36\pm0.25)^{a}$	$(48.49\pm0.49)^{c}$
w(TP)/(mg GAE/g)*	$(84.74\pm2.83)^{b}$	$(95.90\pm2.97)^{c}$	$(2.58\pm0.08)^{a}$
DPPH scavenging activity/(µmol TE/g)*	$(2037.64\pm48.46)^{b}$	$(2488.48\pm69.27)^{a}$	$(10.18\pm0.47)^{c}$
Ferric reducing power/(µmol TE/g)*	$(964.01\pm14.21)^{b}$	$(1108.52\pm14.83)^{c}$	$(3.14\pm0.08)^{a}$
WHC/(g water/g)*	$(4.30\pm0.04)^{b}$	$(4.50\pm0.10)^{c}$	$(0.82\pm0.02)^{a}$
OHC/(g oil/g)*	$(1.76\pm0.07)^{b}$	$(2.01\pm0.03)^{c}$	$(1.18\pm0.05)^{a}$

*Results are reported on dry basis. Values are presented as means±standard deviation. Values followed by different letters in the same row differ significantly ($p<0.05$). TDF=total dietary fiber, SDF=soluble dietary fiber, IDF=insoluble dietary fiber, TP=total phenolic compounds, GAE=gallic acid equivalents, TE=Trolox equivalents, WHC=water holding capacity, OHC=oil holding capacity.

Table 2. Effects of spent tea leaves (STL) addition on cookie dough properties

Parameter	0 % (Control)	*x*(untreated STL)/%				*x*(treated STL)/%			
		10 %	20 %	30 %	40 %	10 %	20 %	30 %	40 %
Hardness (g force)	(614±15)[a]	(685±22)[b]	(767±15)[c]	(964±40)[e]	(1846±11)[g]	(636±17)[a]	(739±24)[c]	(879±16)[d]	(1534±30)[f]
Springiness	(0.58±0.01)[f]	(0.47±0.01)[d]	(0.45±0.01)[c]	(0.43±0.01)[bc]	(0.41±0.01)[b]	(0.53±0.03)[e]	(0.45±0.02)[cd]	(0.41±0.02)[ab]	(0.39±0.01)[a]
Cohesiveness	(0.27±0.01)[g]	(0.20±0.01)[d]	(0.18±0.01)[c]	(0.17±0.01)[bc]	(0.14±0.00)[a]	(0.25±0.01)[f]	(0.21±0.02)[e]	(0.18±0.01)[c]	(0.16±0.00)[b]

Values are presented as means±standard deviation. Values followed by different letters in the same row differ significantly ($p<0.05$).

Table 3. Effects of spent tea leaves (STL) addition on proximate composition, total phenolic content and antioxidant activity of cookies

Component	0 % (Control)	*x*(untreated STL)/%				*x*(treated STL)/%			
		10 %	20 %	30 %	40 %	10 %	20 %	30 %	40 %
w(protein)/%*	(8.20±0.20)^{a}	(8.69±0.16)^{b}	(8.96±0.13)^{bc}	(9.49±0.25)^{de}	(9.87±0.22)^{f}	(8.68±0.32)^{b}	(9.15±0.23)^{cd}	(9.59±0.19)^{ef}	(9.92±0.2)^{f}
w(lipid)/%*	(24.11±0.29)^{a}	(24.33±0.08)^{ab}	(24.58±0.11)^{bc}	(24.85±0.07)^{cd}	(25.13±0.13)^{de}	(24.52±0.09)^{abc}	(24.81±0.46)^{cd}	(25.15±0.49)^{de}	(25.37±0.27)^{e}
w(starch)/%*	(55.56±0.31)^{e}	(49.87±0.17)^{d}	(45.07±0.24)^{c}	(39.16±0.18)^{b}	(32.76±0.14)^{a}	(49.37±0.23)^{d}	(45.26±0.72)^{c}	(39.05±0.5)^{b}	(32.87±0.47)^{a}
w(ash)/%*	(1.25±0.03)^{a}	(1.35±0.03)^{b}	(1.49±0.06)^{c}	(1.63±0.05)^{d}	(1.72±0.03)^{ef}	(1.38±0.02)^{b}	(1.52±0.02)^{c}	(1.67±0.03)^{de}	(1.75±0.05)^{f}
w(TDF)/%*	(1.42±0.04)^{a}	(6.09±0.09)^{c}	(8.27±0.17)^{e}	(11.51±0.03)^{g}	(14.71±0.08)^{i}	(4.75±0.08)^{b}	(7.49±0.23)^{d}	(10.48±0.11)^{f}	(14.33±0.31)^{h}
w(SDF)/%*	(0.68±0.03)^{a}	(1.36±0.04)^{b}	(1.72±0.03)^{c}	(2.25±0.06)^{e}	(2.67±0.03)^{f}	(2.01±0.08)^{d}	(3.18±0.05)^{g}	(4.31±0.07)^{h}	(5.56±0.11)^{i}
w(IDF)/%*	(0.74±0.02)^{a}	(4.73±0.09)^{d}	(6.55±0.14)^{f}	(9.25±0.07)^{h}	(12.04±0.06)^{i}	(2.74±0.05)^{b}	(4.31±0.19)^{c}	(6.17±0.13)^{e}	(8.77±0.2)^{g}
ζ(SDF/TDF)/(g/g)	(47.63±0.5)^{h}	(22.32±0.60)^{d}	(20.80±0.16)^{c}	(19.59±0.56)^{b}	(18.13±0.15)^{a}	(42.34±1.22)^{g}	(42.45±0.92)^{g}	(41.15±0.78)^{f}	(38.79±0.12)^{e}
w(TPC)/ (mg GAE/g)*	(2.63±0.08)^{a}	(6.04±0.06)^{b}	(13.04±0.16)^{d}	(22.17±0.54)^{f}	(25.94±0.42)^{g}	(5.58±0.06)^{b}	(7.92±0.17)^{c}	(13.11±0.4)^{d}	(15.07±0.19)^{e}
DPPH scavenging activity/ (µmol TE/g)*	(2.14±0.03)^{a}	(46.94±0.97)^{b}	(74.46±0.87)^{e}	(85.93±0.50)^{f}	(87.63±0.19)^{f}	(47.27±0.60)^{b}	(58.61±1.11)^{c}	(62.94±1.89)^{d}	(72.56±2.33)^{e}
Fe^{3+} reducing power / (µmol TE/g)*	(14.44±0.4)^{a}	(76.29±1.61)^{d}	(129.25±1.35)^{g}	(215.43±1.18)^{h}	(246.88±2.30)^{i}	(30.17±0.64)^{b}	(68.06±2.85)^{c}	(94.63±0.99)^{e}	(119.44±1.23)^{f}

*Results are reported on dry basis. Values are presented as means±standard deviation. Values followed by different letters in the same row differ significantly ($p<0.05$). TDF=total dietary fiber, SDF=soluble dietary fiber, IDF=insoluble dietary fiber, TPC=total phenolic compounds, GAE=gallic acid equivalents, TE=Trolox equivalents.

Table 4. Effects of spent tea leaves (STL) addition on physical properties and sensory acceptability of cookies

Parameter	0 % (Control)	*x*(untreated STL)/%				*x*(treated STL)/%			
		10 %	20 %	30 %	40 %	10 %	20 %	30 %	40 %
Width (*W*, mm)	(35.10±0.32)^{c}	(35.15±0.35)^{c}	(34.47±0.29)^{ab}	(34.33±0.25)^{ab}	(34.10±0.24)^{a}	(35.25±0.39)^{c}	(35.05±0.44)^{c}	(34.96±0.35)^{c}	(34.53±0.32)^{b}
Thickness (*T*, mm)	(5.75±0.06)^{a}	(5.82±0.09)^{a}	(6.09±0.06)^{c}	(6.25±0.05)^{d}	(6.38±0.02)^{e}	(5.92±0.08)^{b}	(6.12±0.11)^{c}	(6.28±0.08)^{d}	(6.44±0.05)^{e}
Spread ratio (*W/T*)	(6.10±0.05)^{f}	(6.04±0.10)^{ef}	(5.66±0.08)^{cd}	(5.49±0.06)^{b}	(5.34±0.04)^{a}	(5.96±0.12)^{e}	(5.73±0.11)^{d}	(5.56±0.09)^{bc}	(5.36±0.04)^{a}
Hardness (g force)	(998±56)^{a}	(1219±175)^{bc}	(1468±43)^{d}	(1760±76)^{e}	(2130±120)^{g}	(1114±89)^{ab}	(1286±114)^{c}	(1582±184)^{d}	(1928±200)^{f}
*L**	(70.67±0.03)^{f}	(48.6±0.13)^{e}	(44.47±0.43)^{d}	(43.5±0.09)^{c}	(43.45±0.19)^{c}	(48.74±0.39)^{e}	(41.23±0.2)^{b}	(40.75±0.05)^{a}	(40.58±0.22)^{a}
*a**	(-0.42±0.01)^{g}	(-0.92±0.03)^{e}	(-1.37±0.02)^{c}	(-1.43±0.06)^{bc}	(-1.75±0.04)^{a}	(-0.83±0.02)^{f}	(-0.93±0.02)^{e}	(-1.07±0.04)^{d}	(-1.47±0.07)^{b}
*b**	(35.74±0.32)^{f}	(19.47±0.36)^{e}	(14.94±0.17)^{d}	(13.96±0.12)^{c}	(13.51±0.09)^{b}	(19.71±0.24)^{e}	(14.28±0.16)^{c}	(13.31±0.37)^{b}	(12.87±0.13)^{a}
ΔE	(0.00±0.00)^{a}	(27.42±0.28)^{b}	(33.48±0.26)^{c}	(34.84±0.04)^{d}	(35.18±0.34)^{d}	(27.17±0.35)^{b}	(36.44±0.38)^{e}	(37.4±0.4)^{f}	(37.81±0.06)^{f}
Acceptability	(6.6±1.4)^{e}	(5.9±1.3)^{d}	(5.7±1.4)^{cd}	(4.3±1.4)^{b}	(3.3±1.5)^{a}	(6.1±1.5)^{d}	(5.4±1.6)^{c}	(4.5±1.5)^{b}	(3.7±1.6)^{a}

Values are presented as means±standard deviation. Values followed by different letters in the same row differ significantly ($p<0.05$). *L**=brightness, ±*a**=red/green, ±*b**=yellow/blue, *ΔE*: colour difference against the control sample.