International Food Research Journal 18(4): 1553-1557 (2011)

Preliminary study on the fortification of tofu with green tea catechins

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Abstract: Green tea catechins, especially epigallocatechin gallate (EGCG), have been linked with a number of health benefits. However, only drinking green tea may not provide a sufficient level of EGCG to achieve those health benefits. Incorporation of EGCG in tofu, which is a protein-rich food widely used in Asia, and by vegetarians in Western countries, is an alternative way of enhancing the intake of EGCG and its health promoting effects. The present study optimised the conditions necessary to obtain the optimum yield of tofu including concentration of soy protein isolate (SPI), coagulant and pressing time; it then further investigated the possibility of fortifying EGCG in tofu via the effect of EGCG on tofu production yield and the recovery of EGCG. It was possible to fortify both firm and silken tofu with EGCG. The results showed that optimum yield of firm tofu was obtained at 6% (w/w) SPI, 30 mM Nigari and 50 min pressure; and for the silken was at 6% (w/w) SPI, 30 mM GDL and 20 min pressure. EGCG recovery was higher in the silken tofu than the firm one. For both types of tofu, the EGCG addition was suggested at a level of 0.5% (w/w) to obtain the optimum yield of tofu, which resulted in 0.97 g and 0.95 g of EGCG in a serve of 100 g of firm and silken tofu, respectively.

Keywords: Catechin, functional food, tea extract, tofu, utilisation

Introduction

Tofu is a popular food in Asian countries and its consumption is recently increasing in Western countries (Oboh *et al*., 2007; Yoon and Kim, 2007). Tofu is a soy protein product, with homogeneous composition, cream-colour and mild flavour, which is produced by the coagulation of heated soymilk (Stanojević *et al*., 2010). Tofu has been considered as a particularly nutrititious product because it contains about 1% of dietary fiber, has a very low energetic value and does not contain cholesterol (Stanojević *et al*., 2010). Furthermore, it also contains high levels of isoflavones, vitamins and minerals, which contribute to both the health benefits and the nutritional value of the tofu (Stanojević *et al*., 2010).

There are several types of tofu; however, there are two main types of tofu including silken and firm tofu. The type or physical structure of tofu is influenced by the type of coagulant (Shih *et al*., 1997). There are three coagulants, which are more commonly used in making tofu, namely $CaSO_4$, Nigari and Glucono-Delta-Lactone (GDL) (Shih *et al*., 1997).

As tofu is made by coagulation of heated soy protein, followed by moulding and pressing the curd to remove the whey (Yoon and Kim, 2007); its quality and yield have been found to be affected by three main factors including the types and concentration of soybeans (Lim *et al*., 1990; Yoon and Kim, 2007); the types and concentration of coagulants (Sun and Breene, 1991; Hou *et al*., 1997; Obatolu, 2008); and processing conditions such as temperature, stirring and pressuring time (Shih *et al*., 1997).

Tea catechins, especially epigallocatechin gallate (EGCG), have been linked to the prevention of certain types of cancer, cardio-vascular diseases (CVD), microbial diseases, diabetes and obesity (Kao *et al*., 2006; Khan and Mukhtar, 2007; Vuong *et al*., 2010). The health benefits can be only obtained when several cups of tea are consumed daily. For example, an epidemiological study indicated that link with beneficial effect on CVD was achieved when five cups or more of green tea were consumed daily (Kuriyama, 2008). However, it can be challenging for many people to consume a large volume of tea daily. In addition, achievement of the health benefits from drinking tea is further limited because tea contains high content of caffeine, which may cause irritation of the gastrointestinal tract and sleeplessness in certain people (Chu and Juneja, 1997). Fortifying the tofu with catechin extracts is therefore an alternative way to supplement catechin consumption.

The present study studied the impact of: Soy Protein Isolate concentration, concentration of two types of coagulants, Nigari and GDL, and pressing time to maximise the yield of silken and firm tofu. Based on these optimal conditions determined, the study further investigated the fortification of tofu

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by a green tea catechin extract, which contains 94% EGCG, in terms of tofu yield as well the recovery of EGCG after tofu production.

Materials and Methods

Materials

Soy protein isolate (SPI) containing 85 to 90% pure soybean protein was purchased from Morlife (Pty) Ltd (Queensland, Australia). The coagulants including Nigari and Glucano-Delta-Lactone (GDL) were obtained from the Melbourne Food Ingredient Depot (Melbourne, Australia). Teavigo (green tea extract) containing approximately 94% EGCG (w/w) was obtained from RejuvaCare (Sydney, NSW, Australia).

Other chemicals were used for analysis: L-tryptophan (used as an internal standard during HPLC analysis) and EGCG (used as a standard curve) were obtained from Sigma Chem. Co. (Castle Hill, NSW, Australia); acetonitrile, orthophosphoric acid and tetrahydrofuran were purchased from Lomb Scientific (Taren Point, NSW, Australia). Ultra pure (type 1) de-ionised (DI) water was prepared by reverse osmosis and filtration using a Milli-Q Direct 16 system (Millipore Australia Pty Ltd, North Ryde, Australia).

Methods

All experiments were conducted at the University of Newcastle, Central Coast Campus, Australia.

Preparation of tofu

The tofu was produced by first heating the SPI solutions (at concentration 4, 5, 6% (w/w)) at 80°C for 10 min, followed by cooling at room temperature for 5 min. The coagulant (Nigari or GDL) at different concentration was then added to form a mixture. Nigari was used at concentrations of 15, 30 and 50 mM and GDL at concentrations of 20, 25 and 30 mM. The mixture was transferred to a perforated tray lined with cheese cloth to remove excess liquid. Pressure at various times was applied for obtaining firm and silken tofu.

Preparation of EGCG fortified tofu

Based on the conditions determined as providing the highest yield of tofu, the EGCG fortified tofu was produced by first heating the SPI and Teavigo (green tea extract containing approximately 94% EGCG (w/w)) at 80°C for 10 min followed by cooling at room temperature for 5 min. The coagulant was then added and the mixture transferred to a perforated tray lined with cheese cloth to remove excess liquid. Pressure was applied for 50 and 20 min to obtain firm and silken tofu, respectively.

Yields

The yield of tofu was the weight of tofu produced compared to the total weight of the input ingredients, expressed as a percentage as follows:

$$Yield\ (\%) = \frac{Weight\ of\ tofu \times 100}{Weight\ of\ total\ ingredients}$$

Analytical determination of EGCG

Triplicate 4 g samples of each tofu preparation were ground by mortar and pestle and the EGCG was extracted in 100 ml of 50% (v/v) aqueous acetone for 24 h at room temperature in the dark. The samples were then filtered through 0.45 μm cellulose syringe filters (Phenomenex Australia Pty. Ltd, Lane Cove, NSW, Australia). EGCG was measured by high performance liquid chromatography (HPLC) as mentioned in a previous study (Vuong *et al*., 2011) with some modification.

The samples (20 μL) were injected onto a Shimadzu HPLC system (Shimadzu Australia, Rydalmere, NSW, Australia) using UV detection at 280 nm, and a 250 x 4.6 mm Synergi 4μm Fusion-RP 80A reversed-phase column (Phenomenex Australia Pty. Ltd, Lane Cove, NSW, Australia) which was maintained at 35°C at a flow rate of 1mL/min.

The mobile phases consisted of solvent systems A and B; solvent A was 0.2% (v/v) orthophosphoric a cid:acetonitrile:tetrahydrofuran, 95.5:3:1.5% (v:v:v) and solvent B was 0.2% (v/v) orthophosphoric acid: acetonitrile:tetrahydrofuran, 73.5:25:1.5% (v:v:v). A gradient elution schedule was used: 100% A from 0-10 min; a linear gradient from 100% A to 100% B from 10-40 min; a linear gradient from 100% B to 100% A from 40-50 min, with a post-run re-equilibration time of 10 min with 100% A before the next injection.

EGCG in the samples was quantified by dividing its peak area by the peak area of the internal standard, L-tryptophan (Figure 1), and determining their concentration from a standard curve of the peak area ratios of increasing concentrations of pure EGCG external standard to 250 μM L-tryptophan. The recovery of EGCG in the tofu was expressed as a percentage of the amount added as an ingredient.

Statistical Analysis

The one-way ANOVA and the Bonferroni post-hoc test were used to determine statistical significance at $P<0.05$.

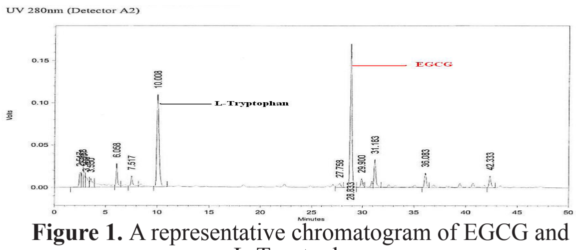

Figure 1. A representative chromatogram of EGCG and L-Tryptophan

Results and Discussion

Optimising conditions for obtaining high yield of tofu

As described previously, tofu is made by coagulation of heated soy protein, followed by molding and pressing the mixture to remove the liquid. The present study determined the conditions including the concentration of SPI, concentration of Nigari (for firm tofu) and GDL (for silken tofu) as coagulants and time of pressure for obtaining the highest yield of tofu.

The results revealed that concentration of SPI, types and concentration of coagulants and time of pressure had a significant influence on the yield of tofu (Figures 2 and 3). These findings were supported by results from previous studies, which reported that the yield of tofu was influenced by concentration of soy protein (Lim *et al*., 1990; Yoon and Kim, 2007), types and concentration of coagulants (Hou *et al*., 1997; Obatolu, 2008), and time of pressure (Shih *et al*., 1997).

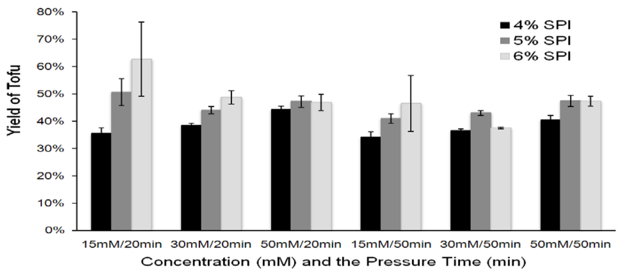

Figure 2. Impact of SPI (4%, 5%, 6%), Nigari (15 mM, 30 mM, 50 mM) and pressuring time (20 min and 50 min) on the yield of the firm tofu

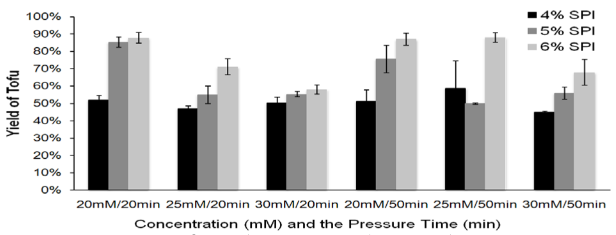

Figure 3. Impact of SPI (4%, 5%, 6%), GDL (20 mM, 25 mM, 30 mM) and pressuring time (20 min and 50 min) on the yield of the silken tofu

The results indicated that the optimal yield of the firm tofu was obtained at the conditions of 6% (w/w) concentration of SPI, 30 mM Nigari and 50 min pressure; the optimal yield of the silken tofu was achieved at SPI concentration 6% (w/w) SPI, 30 mM GDL and 20 min pressure. These conditions were subsequently applied for fortifying tofu with tea catechin extract.

Fortification of tofu with green tea catechin extract

Effect of EGCG on the yield of tofu

Based on the findings on the optimal conditions for obtaining the highest yield of tofu, Teavigo (green tea extract containing approximately 94% EGCG (w/w)) was incorporated into the tofu during the processing stage as described in the methods. The results showed that various concentrations of EGCG addition had a significant impact on the yield of both firm and silken tofu (Figure 4). The yield of firm and silken tofu increased when 2-folds of EGCG were added; however, the yield of both tofu types significantly decreased when 4-folds of EGCG were further added. Thus, the current results revealed that the highest yield of both firm and silken tofu was obtained when EGCG was added at the level of 0.5% (w/w).

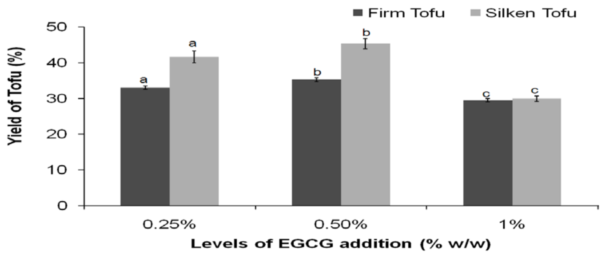

Figure 4. The effect of EGCG on the yield of tofu. The values are expressed as mean ± SD. For each type of tofu, values not sharing the same letter are significantly different (P<0.05)

The influence of EGCG on the yield of tofu can be explained by the interaction between EGCG and soy proteins (Vuong *et al*., 2010). The catechins including EGCG were found to interact with enzymes, such as lipoxygenase, α-amylase, pepsin, trypsin, and lipase to form precipitates and consequently inhibit the activity of these enzymes (Sekiya *et al*., 1984). The EGCG that have an ester bond had a greater ability to form precipitates with these enzymes than other catechins, such as EC and EGC (Sekiya *et al*., 1984). In addition, interaction between catechins and proteins like soybean lipoxygenase was found to occur mostly in the pH range from 4 to 7 (Sekiya *et al*., 1984; Huang *et al*., 2004), which encompasses the range of pH in tofu mixture (5.3-6). Therefore, the yield of tofu was affected by various quantities of added EGCG. However, the preliminary results only presented the impact of the three concentrations (0.25, 0.5 and 5% (w/w)) of EGCG; future study needs to cover a wider range of EGCG addition to obtain a higher yield of tofu.

The recovery of EGCG in tofu

As EGCG was associated with various health benefits (Khan and Mukhtar, 2007), the higher the EGCG recovery in tofu, the more likely the potential health contribution to the consumers when consuming EGCG fortified tofu. However, EGCG is very sensitive and unstable under several conditions such as high temperature and alkaline conditions (Vuong *et al*., 2010). As tofu is produced as a result of thermal processing, the current study also determined the recovery of EGCG for each type of tofu, silken or firm, after undergoing the various thermal process stages.

Results indicated that loss of EGCG was observed in both silken and firm tofu (Figure 4). The loss of EGCG could be partially caused by the heat treatment applied during the production process. Under high temperature, EGCG has been known to epimerise to Gallocatechin gallate (GCG) (Chen *et al*., 1998; Xu *et al*., 2003). In addition, pressure was also applied at the final stage of making tofu; therefore EGCG might be also lost along with the liquid drained out of the tofu before it has time to interact with the soy proteins.

The recovery of EGCG was found higher in silken tofu than in firm tofu. Interestingly, results showed that various concentrations of EGCG addition were associated with a significant difference on the recovery of EGCG in both firm and silken tofu. Except for when 1.0% (w/w) EGCG was added to the soft tofu, the percentage of EGCG recovered in both types of tofu generally increased as the level of EGCG addition increased (Figure 4). The highest EGCG recovery (83%) from firm tofu was observed when 1% (w/w) EGCG was added, while for silken tofu (89%) it was observed with 0.5% (w/w) EGCG addition. The differences in the recovery of EGCG at various concentrations can be explained by the interaction between EGCG and soy proteins (Sekiya *et al*., 1984); in this case EGCG might bind with soy protein so more EGCG can be trapped in tofu at higher concentration.

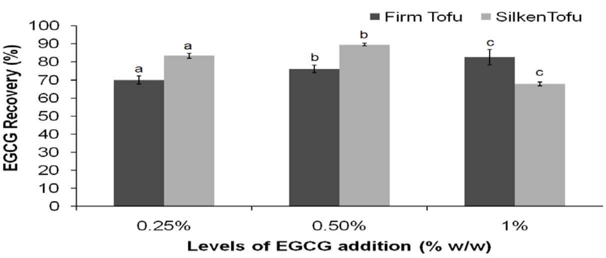

Figure 5. The recovery of EGCG in tofu. The values are expressed as mean ± SD. For each type of tofu, values not sharing the same letter are significantly different (P < 0.05)

Content of EGCG available in a typical serve of tofu

As mentioned previously, tea catechins, especially EGCG, have been associated with various health benefits (Khan and Mukhtar, 2007). However, their health potential attributes are limited because they require consuming a large amount of tea to achieve, thus, fortification of EGCG in tofu is an alternative way to supplement catechins to consumers. Findings from the current study showed that more than 85% of EGCG could be recovered after tofu production. However, to determine whether the amount of EGCG addition was adequate for achieving health benefits when consuming a serve of EGCG fortified tofu, the amount of EGCG available in a typical serve of tofu (100 g) was calculated and is shown in Table 1.

Table 1. Amount of EGCG (g) in a 100 g serve of tofu

Tofu type	EGCG addition (%)		
	0.25 %	0.5 %	1 %
Firm tofu	0.51 ± 0.02[a]	0.97 ± 0.12[b]	2.68 ± 0.13[c]
Silken tofu	0.48 ± 0.01[a]	0.95 ± 0.01[b]	2.16 ± 0.03[c]

The values are expressed as mean ± SD. Values in the same row not sharing the same superscript letter are significantly different (P < 0.05).

As can be seen from Table 1, the amount of EGCG in a typical 100 g serve of tofu increased when the levels of EGCG addition increased in both types of tofu. With the addition of 0.5% of EGCG, a serve of tofu can provide at least 950 mg of EGCG, which is equivalent to 5 or more cups of tea. Therefore, the health benefits can be achieved when people consume an average serve of tofu instead of drinking a large volume of tea. However, the amount of EGCG might be decreased because tofu may be cooked by steaming, boiling, stir-frying or deep-frying before consumption; thus, future study on the availability of tofu after cooking is required.

Conclusion

The current study confirmed that concentration of SPI, types and concentration of coagulants and time of pressure had a significant influence on the yield of tofu. The optimal yield of the firm tofu was obtained at the conditions of 6% (w/w) concentration of SPI, 30 mM Nigari and 50 min pressure; and the optimal yield of the silken tofu was achieved at SPI concentration 6% (w/w) SPI, 30 mM GDL and 20 min pressure. Tea catechins can be incorporated into tofu, thus creating a functional food. EGCG recovery was higher in the silken tofu than the firm one. For both types of tofu the suggested level of EGCG addition is at a level of 0.5% (w/w) allowing for an optimum yield, and providing a serve of 100 g firm tofu containing 0.97 g of EGCG and 100 g of silken tofu with 0.95 g of EGCG. However, future studies are needed to investigate a wider range of EGCG addition levels to maximise the yield and the bioavailability of EGCG incorporated in tofu. In addition, sensory and textural characteristics of EGCG-fortified tofu also need to be evaluated.

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