World Academy of Science, Engineering and Technology
International Journal of Nutrition and Food Engineering
Vol:8, No:1, 2014

Effect of adding Supercritical Carbon Dioxide Extracts of *Cinnamomum tamala* (Bay Leaf) on Nutraceutical Property of Tofu

Sudip Ghosh, Probir Kumar Ghosh, Paramita Bhattacharjee

Open Science Index, Nutrition and Food Engineering Vol:8, No:1, 2014 publications.waset.org/9997143/pdf

***Abstract*—**Supercritical carbon dioxide extracts of *Cinnamomum tamala* (bay) leaves obtained at 55°C, 512 bar was found to have appreciable nutraceutical properties and was successfully employed as value-added ingredients in preparation of tofu. The bay leaf formulated tofu sample was evaluated for physicochemical properties (pH, texture analysis and lipid peroxidation), proximate analysis, phytochemical properties (total phenol content, antioxidant properties and total reducing sugar), microbial load and sensory profile analysis for a storage period of ten days, *vis-à-vis* an experimental control sample. These assays established the superiority of the tofu sample formulated with supercritical carbon dioxide extract of bay leaf over the control sample. Bay leaf extract formulated tofu is a new green functional food with promising nutraceutical benefits.

Keywords*—Cinnamomum tamala,* Physicochemical properties Phytochemical properties, Supercritical carbon dioxide extraction, Tofu.

I. Introduction

TOFU is a soybean derived non-fermented food product widely consumed for its nutritional benefits. It is popular globally especially in the Asian countries [1]. It is a protein gel like product and is consumed as soft, regular or packed tofu [2]. Tofu being an alternative inexpensive source of protein could possibly redress malnutrition problem. Several factors affect quality of tofu, such as cultivar of soybeans [3], [4], its processing methods [2], [5]–[7] and type of coagulants used in its preparation [8]–[10].

Tofu is a favorable medium for microorganisms owing to its high protein and moisture content. Polyunsaturated fatty acids (PUFA) in tofu are particularly susceptible to oxidation by free radicals and ROS [1]. Shelf life of tofu can be extended by smoking, by natural coagulants such as fruit juices and acetic acid, by addition of chitosan and by use of preservatives [11]–[13].

Cinnamomum tamala Nees, commonly known as bay leaf, is one of the commonly used ingredients in Indian cookery. It's free radical scavenging activity has been explored by several researchers [14], [15]. It is also known to have other therapeutic properties such as antimicrobial [16] and anti-inflammatory [17]. Eugenol is one of the main chemical constituents of bay leaves among several others such as β-caryophyllene, linalool, cinnamic aldehyde, and cinnamyl acetate [18]. However, report of bay leaf extracts in food processing and preservation are scanty. It has been reported to be used as a preservative in pineapple juice [19]. We envisage designing a new green tofu product using bay leaf extract without compromising its sensory attributes.

The extracts from natural sources are commonly obtained by hydro distillation and solvent extraction. The green technology of solvent-less supercritical carbon dioxide (SC-CO_2) extraction is a better alternative over these conventional extraction methods, since these latter methods pose problems of thermal degradation, hydrolysis and water solubilization of desirable constituents, besides environment and health hazards, owing to the presence of residual solvents in the extracts [20]. SC-CO_2 extraction circumvents all these problems and additionally offers the advantage of selective extraction of desired active ingredients from natural matrices simply by altering the extraction parameters, such as temperature, pressure, extracting time and flow rate of CO_2.

We have investigated SC-CO_2 extraction of *Cinnamomum tamala* Nees and assayed the extract for its phytochemical properties. The SC-CO_2 extract with maximum eugenol content (0.721±0.101 mg/ g dry bay leaf powder) was obtained at 55°C, 512 bar after 1h extracting time. Comparative evaluation of its phytochemical properties with extracts obtained by the conventional techniques, established that this extract had the best combination of eugenol and total phenols; along with reducing power, anti-inflammatory, antimicrobial and antioxidant activities. Further GC-MS analysis was performed to identify other active components along with eugenol in the extract.

We then aimed in examining the feasibility of formulating a new tofu product with enhanced nutraceutical properties, using this extract of SC-CO_2. Two sets of tofu samples – one with bay leaf extract (B) and a control sample (C), were prepared in our laboratory. The samples were compared for their physicochemical properties (moisture, pH, and texture profile analysis and lipid peroxidation), proximate analysis, phytochemical properties (total phenol content, antioxidant property and reducing power), microbial load and sensory properties for a storage period of ten days. This study reports for the first time on 'green product design' of tofu using SC-CO_2 extract of bay leaves and their physicochemical, phytochemical, microbial and sensory properties.

Sudip Ghosh, Probir Kumar Ghosh, and Paramita Bhattacharjee (corresponding author) are with Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata 700 032, India (phone: +91 33 2414 6822; fax: 91 33 2414 6822; e-mail: pb@ ftbe.jdvu.ac.in).

Open Science Index, Nutrition and Food Engineering Vol:8, No:1, 2014 publications.waset.org/9997143/pdf

II. Materials and Methods

A. Materials

Soybeans and *Cinamomum tamala* (bay leaves) were purchased from a local market of Jadavpur (West Bengal region of India). Specialty chemicals such as eugenol (99% pure), 1,1-diphenly-2-picrylhydrazyl (DPPH), sodium nitroprusside and gallic acid were procured from M/s Sigma, India. Folin-Ciocalteu's reagent (FCR), chloroform, methanol, dichloromethane, *n*-hexane, sodium carbonate, aluminium chloride, potassium acetate and sodium sulfate were procured from M/s E-Merck, India. All chemicals, solvents and buffers used in the work were of AR grade.

B. SC-CO_2 Extraction of Bay Leaf Extract

For SC-CO_2 extractions, a SPE-ED SFE 2 model of M/s Applied Separations, Allentown, USA, was used. It comprises of a modifier pump (Speed MAX P/N 7025) fitted with refrigerated cooling bath to chill the pump head at -2°C. 10g bay leaf powder was charged into a 50ml SS 316 extraction vessel. The flow rate of CO_2 (food grade) was maintained constant at 25.0cm^3/s for extraction. A central composite rotatory design (CCRD) was used to study the supercritical extraction processes. Liquid CO_2 was compressed to desired pressure and then continuously pumped into the extractor. A static time of 30 min and dynamic time of 30 min were kept constant throughout the experimental trials since variation of these had insignificant effect on the extract yield. The extracts obtained were waxy, semi-solid in nature, were gravimetrically weighed and successively stored in amber colored screw capped glass vials at 4°C (post dilution in minimum amount of food grade ethanol), until further analyses. All experiments were conducted in triplicate in each extraction mode.

C. Evaluation of Phytochemical Properties of the SC-CO_2 Bay Leaf Extract

Total phenolic compounds of the bay leaf extracts was estimated using Folin-Ciocalteu method [21] and expressed as g gallic acid equivalent/g dry bay leaf powder and its reducing power was assayed in accordance to the method of Oyaizu [22] and reported as mg BHT equivalent/g of dry bay leaf powder, from their respective standard curves. The antioxidant activity was determined by DPPH method and expressed as IC_{50} values (defined as the concentration of the test material which brings about a 50% decrease in initial DPPH concentration) [23], [24].

D. GC-MS Analysis of SC-CO_2 Extract of Bay Leaves

The SC-CO_2 extract obtained at 55°C and 512 bar having the best combination of phytochemical properties was analyzed by GC-MS for identification of its chemical constituents. A Polaris Q Mass Spectrometer coupled with Trace GC Ultra Gas Chromatography and DB-5 MS fused silica capillary column (30 x 10^3cm × 0.025cm i.d; 0.25 x 10^{-3} cm film thickness) was employed. The oven module was programmed as follows: it was held isothermally at 85°C for 3 min, then increased at the rate of 2°C/min to 200°C with holding time of 1min; then further increased to 250°C at the rate of 3°C/min with holding time of 5min and finally increased to 300°C at the rate of 10°C/min and held for 15min. Helium was used as the carrier gas at a flow rate of 0.02cm^3/s. 1µl (1 x $10^{-3}cm^3$) of the sample was injected in split less mode through the injection port held at 280°C. The ionization of the sample was achieved in the EI mode (70eV) and the acquisition mass range was set in the range of 35 to 350amu. The chemical compounds in the extracts were identified by computer matching of the chromatogram peak profiles with the NIST (2007) library and with literature reports [25]–[27].

E. Preparation of Tofu

Among all SC-CO_2 extracts obtained, 0.467g of bay leaf extract obtained by a single extraction run of SC-CO_2 at 55°C, 512bar had the maximum eugenol content (0.721mg/g dry bay leaf) with best combination of the above phytochemical properties. The whole bay leaf extract was added to the tofu preparation without any health concerns, since bay leaves reportedly have insignificant toxicity [28].

Tofu was prepared as follows- soybeans (100g) were soaked overnight in water at room temperature (26ºC), drained, rinsed and ground in 1000cm^3 water using a mixer grinder (Phillips Mixer Grinder, Model-HL 1618, Phillips India Ltd, Chennai, India) at high speed. The resulting slurry was heated for 50min to eliminate the characteristic 'beany' flavor. The soymilk was obtained from the slurry by filtering it through double layered cheesecloth. The solid content of the soymilk was measured using a hand held refractometer (ERMA Hand Refractometer, Erma Inc, Tokyo, Japan) and was found to be of 10-11ºBrix. Bay leaf extract (0.467mg/ 100g soybean) obtained by SC-CO_2 along with citric acid (2% w/v) coagulant was added to the bulk soymilk maintained at 80ºC-90ºC. The ingredients were stirred well to obtain a homogeneous mixture and allowed to coagulate at room temperature (26ºC) for 15min. The curd thus obtained was gently transferred to a perforated SS container (10cm × 6cm × 30cm) lined with double layered cheese cloth and pressed for 30min using a brick weighing 500g. The prepared tofu was cut into pieces of 2cm × 2cm × 2cm dimensions and stored in drinking water in screw capped glass jars under refrigerated conditions at 4ºC prior to analyses (Fig. 1).



Fig. 1 Laboratory prepared tofu samples without and with SC-CO_2 extracts of bay leaf

Open Science Index, Nutrition and Food Engineering Vol:8, No:1, 2014 publications.waset.org/9997143/pdf

F. Proximate Composition Analyses

The proximate composition analyses of the tofu samples were performed according to AOAC method 2000 for moisture, protein, crude fat, crude fibre, ash and carbohydrates by difference [29].

G. Determination of Physicochemical Properties of Tofu Samples

1. pH

The pH of the tofu sample was measured using pH meter (Model-PC 510 pH, M/s Eutech Instruments, Singapore). 5g of tofu samples were homogenized with 25ml deionized water using mortar and pestle and then filtered through single layered cheese cloth to measure pH of the sample [13].

2. Texture Profile Analysis

The texture profile analysis (TPA) of tofu samples was conducted using Instron texture analyzer (M/s Instron Inc., Buckinghamshire, UK, model number 4301) with a 20 x 10^5 dynes load cell and a 4cm diameter cylindrical plunger and 0.25cm/s velocity of the head. The tofu samples were cut into cubes of dimension 2cm × 2cm × 2cm from central portion of the tofu cake and compressed to 50% of original height. The texture parameters of tofu such as hardness, springiness, cohesiveness, gumminess and chewiness, were determined on 0, 2, 5, 7 and 10^{th} days of storage. The experiments were conducted in triplicates and values were reported as mean ± SD of three experimental analyses of each sample.

3. Lipid Peroxidation in Tofu Samples

Peroxidation of PUFA present in tofu causes rancidity and was estimated by malondialdehyde formation during storage. The percentage of malondialdehyde formed was measured according to the method of Ohkawa et al. [30].

H. Determination of Phytochemical Properties of the Tofu Samples

For determination of phytochemical properties, 5g tofu samples were extracted with 50cm^3 methanol under constant rotatory shaking (190rpm) at 26ºC for 4h. Appropriate dilution of the extracted solution was used for different assays and the experimental samples were centrifuged at 1,677g for 10min before recording OD in a UV-Visible spectrophotometer (Hitachi U-2000 spectrophotometer, Tokyo, Japan). Total phenol content, antioxidant property and reducing power of the tofu samples were determined as described above in Section II.C.

I. Microbiological Analysis of Tofu Samples

1. Total Aerobic Plate Count

1g of tofu sample was mixed with 4cm^3 of 0.1% peptone water (10^{-1} dilution) and serially diluted up to 10^{-5} dilution with peptone water (0.1%). 100µl (100 x $10^{-3}cm^3$)of the sample solution was cultured on plate count agar medium (PCA, Himedia) and incubated at 37°C for 24h. The following day, the total number of colonies were counted and expressed as CFU/g [11].

J. Sensory Evaluation of Tofu Samples

Sensory evaluation of tofu was carried out by a semi-trained panel of 10 members consisting of University staff and students. The panel was first trained on attributes of definition and scaling procedure by a professional tester. On each sampling date, the coded samples were randomly presented to each panelist, with a rest period between sample presentations to minimize sensory fatigue. Sensorial sessions were conducted in an air-ventilated room under white light. The tofu samples were evaluated for appearance, texture, odor, color, after taste and overall acceptability using standard 9-point Hedonic scale (1-extremly dislike; 9-extremely dislike), according to the method described by Ranganna [31].

K. Statistical Analysis

In this experiment, one-way ANOVA was performed to observe for significant differences among the physicochemical and phytochemical properties among different samples of tofu. A p-value of 0.05 was used to verify the significance of all tests. All statistical tests were performed by STATISTICA Software version 8.0 (Statsoft, OK, USA).

III. Results and Discussion

A. Phytochemical Properties of Bay Leaf Extracts

Maximum amount of eugenol (0.721mg/g dry bay leaf) was obtained by SC-CO_2 extraction at 55°C, 512 bar and this extract was evaluated for its phytochemical properties. Total phenolic content of this extract was found to be 1.77mg of gallic acid equivalent/g dry bay leaf powder. The reducing power was found to be 0.80mg BHT equivalent/g dry bay leaf powder and the IC_{50} values for DPPH radical scavenging activity was found to be 0.20 mg/ml. The extract was found to be nutraceutically enriched with these phytochemical properties and further formulation of tofu was performed employing this extract.

B. GC-MS Analysis of the SC-CO_2 Extracts of Bay Leaf

The compounds in the eugenol-rich fraction of SC-CO_2 extract (55°C, 512 bar) of bay leaves were identified by GC-MS (Fig. 2) and have been reported in Table I. It is observed that eugenol is one of the major compounds in the bay leaf extract; besides β-sitosterol, α- pinene, β- elemene, β-caryophyllene, spathulenol, caryophyllene oxide and cinnamayl acetate, all of which are reported to have nutraceutical properties [19].

C. Proximate Composition

The percentage moisture, protein, crude fat, crude fiber, ash and carbohydrate content of the prepared tofu samples were found to be 75, 14, 3.9, 0.6, 0.6 and 4.9 respectively. No significant change in proximate composition of tofu samples were observed with addition of bay leaf extracts in the same.

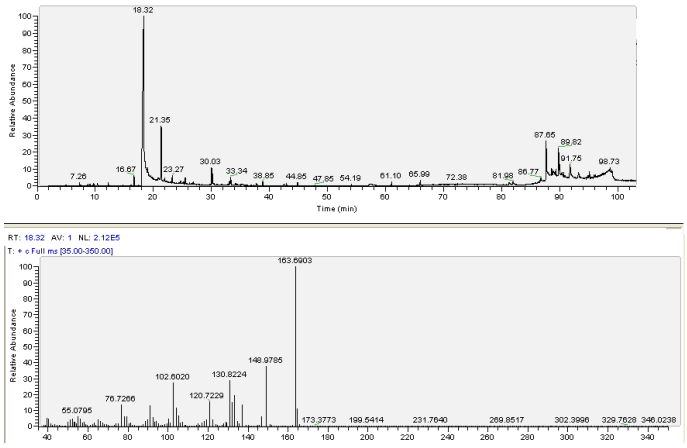


Fig. 2 GC-MS chromatogram of SC-CO_2 extract of bay leaves obtained at 55°C, 512 bar and 60 min extraction time

D. Effect of Adding SC-CO_2 Extracts of Bay Leaf on Physicochemical Properties of the Tofu Sample

1. Effect of Adding SC-CO_2 Extracts of Bay Leaf on pH on Tofu Samples

The pH values of tofu samples have been tabulated in Table II. It is observed that there is a significant change ($p = 0.000$) in pH values of both samples (B and C) over the period of ten days. A decrease in pH values of both samples was observed on the second day of storage. Similar effect on variation of pH in tofu samples was observed due to rapid growth of microorganisms and decomposition of protein therein in agreement with Tuitemwong and Fung [32]. The logarithmic phase of bacterial growth in the tofu samples during the first two days (discussed in Section III.*F*) could have resulted in effective utilization of nutrients, acid production and decrease in pH values in agreement with Anbarasu and Vijayalakshmi [1]. Although the pH of sample B increased slightly after two days of storage, sample C registered a decrease in pH indicating formation of acidic compounds in it consequent to microbial proliferation which is in agreement with our microbial study (Section III.*F*).

TABLE I
LIST OF MAJOR COMPOUNDS IDENTIFIED IN GC-MS

Peak No.	R.T. (min)	[M]+ (m/z)	Base Peak (m/z)	Peak Area (AU)	Identified Compounds[x]
1	7.26	136	93	18599	α-pinene
2	16.67	N.A	81	295765	β-elemene
3	18.32	164	163.69	10550974	Eugenol
4	21.35	204	93	1875318	β-caryophyllene
5	30.03	220	43	585813	Spathulenol
6	38.85	204	93	45624	Bicyclogermacrene
7	44.85	176	43	36259	Cinnamyl acetate
8	54.19	N.A	62	15169	NI
9	65.99	220	43	97429	Caryophyllene oxide
10	86.77	N.A	71	240903	NI
11	87.65	486	357	2158860	β-sitosterol
12	98.73	426	43	509307	Lupeol

[x] Identifications were carried out using NIST 2007 and R.P. Adams [27], Identification of essential oil components by gas chromatography/mass spectrometry.

AU, N.A and NI stand for arbitrary unit, not available and not identified respectively.

TABLE II
PHYSICO-CHEMICAL PROPERTY OF THE TOFU SAMPLES OVER THE TEN DAYS STORAGE PERIOD

	pH[k]		Moisture content (%)[k]		Malondialdehyde content (mg/100g tofu sample) [k]	
Days	Sample C	Sample B	Sample C	Sample B	Sample C	Sample B
0	6.78±0.95a	6.83±0.83a	75.12±1.21a	74.93±1.24a	5.61±0.79a	4.72±0.74a
2	6.31±0.83b	6.61±0.84b	78.14±1.72b	75.39±1.31b	5.79±0.78b	4.73±0.63a
5	6.22±0.82c	6.65±0.83c	79.67±1.81c	75.68±1.33c	5.93±0.79c	4.74±0.52b
7	6.21±0.80c	6.66±0.81c	81.89±1.92d	75.89±1.43d	6.17±0.81d	4.75±0.42b
10	6.18±0.79d	6.72±0.84d	83.13±1.98e	75.97±1.44d	6.37±0.83e	4.75±0.31b

[k] IC_{50} of DPPH, Total phenolic content and reducing power of tofu samples are mean ±SD of three independent experiment performed at 23±2°C. Different letters in a column indicates significant differences at $p < 0.05$ level.

2. Effect of Adding SC-CO_2 Extracts of Bay Leaf on Moisture Content of the Tofu Samples

The moisture content of the tofu samples (Table II) changed significantly ($p = 0.000$) with storage time. There was a significant difference between the moisture content of C and B ($p = 0.009$). The moisture content of C increased relatively more than B. At the end of ten days, structure of C was less compact than B. This loosening of structure could have been consequent to the disintegration of proteins and oxidation of lipid globules therein, as also observed by Anbarasu and Vijayalakshmi [1]. The addition of bay leaf extract in tofu maintained the structural integrity of tofu which showed less increase in moisture content.

3. Effect of Adding SC-CO_2 Extracts of Bay Leaf on Lipid Peroxidation of Tofu Samples

Lipid peroxidation of the tofu samples was assessed by measuring the malondialdehyde content in tofu samples (Table II). It was observed that the malondialdehyde content increased for C while it remained almost constant for B, with storage. The peroxidation in B was less compared to that in C. This slow peroxidation in the former is attributed to the antioxidant property of the bay leaf extract (Section III.*A*).

4. Texture Profile Analysis of Tofu Samples

The texture analyses data of both C and B samples are shown in Table III. It was observed that on day zero, B was softer than C, which reversed on day 5 of storage with increased moisture content of C. Structure of C deteriorated

Open Science Index, Nutrition and Food Engineering Vol:8, No:1, 2014 publications.waset.org/9997143/pdf

more than B, with a concomitant increase in malondialdehyde and moisture content of this sample (Table II). After seven days, C deformed drastically compared to sample B. Cohesiveness of B was less compared to C on day zero, which may be attributed to weak internal bonding in the former caused by intervention of the administered leaf extract during coagulation. However, cohesiveness in B did not decrease significantly with time as was true for C, which showed increased deterioration in cohesiveness with storage time. Springiness of sample C was more compared to B over the entire storage period of ten days. Chewiness of C was more compared to B in the initial period of storage, but decreased rapidly indicating comparatively increased deformation of the former. Therefore, textural attributes were better retained in the bay formulated tofu sample B compared to the control sample.

TABLE III
TEXTURE ANALYSIS OF THE TOFU SAMPLES OVER THE TEN DAYS STORAGE PERIOD

	Hardness (N) [k]		Cohesiveness[k]		Springiness (mm) [k]		Gumminess (N) [k]		Chewiness (Nmm) [k]	
Days	Sample C	Sample B	Sample C	Sample B	Sample C	Sample B	Sample C	Sample B	Sample C	Sample B
0	15.13±1.10a	10.21±1.03a	0.46±0.04a	0.47±0.03a	5.67±1.04a	5.37±1.03a	7.006±1.07 a	4.89±1.02 a	39.73±1.41 a	26.29±1.29 a
2	10.27±1.04b	10.14±1.07b	0.43±0.05b	0.40±0.05b	5.43±1.03b	5.20±1.05b	4.50±1.03b	4.57±1.02 b	24.51±1.32 b	23.81±1.26 b
5	9.014±1.03c	10.09±1.04a	0.38±0.03c	0.34±0.02c	5.38±1.03c	5.17±1.03c	3.48±1.046 c	3.59±1.01 c	18.78±1.21 c	18.59±1.21 c
7	8.082±1.02d	9.89±1.02c	0.36±0.03d	0.30±0.02d	5.27±1.02d	5.14±1.04d	2.96±1.03d	2.97±1.01 c	15.63±1.18 d	15.32±1.12 d
10	4.671±1.01e	9.09±1.01d	0.32±0.03e	0.28±0.01e	5.20±1.03d	5.10±1.03e	1.527±1.02 e	2.32±1.01 d	7.946±1.16 e	11.88±1.11 e

[k]Hardness, Cohesiveness, Springiness, Gumminess and Chewiness of tofu samples are mean ±SD of three independent experimental data.
Different letters in a column indicates significant differences at $p < 0.05$ level.

E. Effect of Adding SC-CO_2 Extracts of Bay Leaf on Phytochemical Properties of Tofu

Addition of bay leaf extract increased the total phenol content (Table IV) of B significantly ($p = 0.000$), compared to C. With storage period, there is a rapid decrease in phenol content of C while there was no significant change ($p = 0.101$) in B. The antioxidant property (Table IV) gradually deteriorated in C; whereas the antioxidant property of the bay treated sample remained almost constant over the entire storage period of ten days. The reducing power of B was higher than C (Table IV) and remained almost constant, while it deteriorated in C over time. To conclude, all phytochemical properties of the bay formulated tofu were higher than the control sample.

F. Microbiological Analysis of Tofu Samples

There was a significant change ($p = 0.024$) in the microbial load in either sample, over the storage period of ten days (Table V). The microbial count of B was lesser than C. Rapid growth of microorganisms was observed in both the samples in the first 24 to 48h; however, a decrease in the same was observed with storage period in B, contrary to that in C. The antimicrobial property of the bay leaf extract contributed to reduced microbial growth in sample B.

TABLE IV
PHYTOCHEMICAL PROPERTIES OF THE TOFU SAMPLES OVER THE TEN DAYS STORAGE PERIOD

	Antioxidant activity (IC_{50} of DPPH radical scavenging activity) (mg/ml) [k]		Total phenolic content (mg equivalent GA/g tofu) [k]		Reducing Power (mg equivalent BHT/g tofu) [k]	
Days	Sample C	Sample B	Sample C	Sample B	Sample C	Sample B
0	8.91±1.17a	0.76±0.08a	0.12±0.05a	0.27±0.02a	0.08±0.04a	1.08±0.09a
2	9.91±1.18b	0.80±0.08a	0.10±0.03a	0.26±0.03a	0.06±0.04a	0.98±0.08b
5	10.52±1.19c	0.77±0.07a	0.08±0.02a	0.25±0.04a	0.05±0.03a	0.96±0.08b
7	13.82±1.20d	0.78±0.07a	0.07±0.01a	0.24±0.03a	0.03±0.02a	0.95±0.09b
10	18.86±1.24e	0.79±0.06a	0.05±0.01b	0.24±0.01a	0.01±0.01a	0.94±0.09b

[k]IC_{50} of DPPH, Total phenolic content and reducing power of tofu samples are mean ±SD of three independent experimental data.
Different letters in a column indicates significant differences at $p < 0.05$ level.

TABLE V
MICROBIOLOGICAL STUDY OF THE TOFU SAMPLES OVER THE TEN DAYS STORAGE PERIOD

	Total plate counts (CFU/g)	
Days	Sample C[k]	Sample B[k]
0	2×10^4	1×10^4
2	4×10^4	3×10^4
5	4×10^4	2×10^4
7	5×10^4	2×10^4
10	6×10^4	3×10^4

[k]Different letters in a column indicates significant differences at $p < 0.05$ level

Open Science Index, Nutrition and Food Engineering Vol:8, No:1, 2014 publications.waset.org/9997143/pdf

Open Science Index, Nutrition and Food Engineering Vol:8, No:1, 2014 publications.waset.org/9997143/pdf

G. Sensory Analysis of the Tofu Samples

The results of sensory studies have been presented in a radar plot (Figs. 3 (a), (b)). The appearance of both tofu samples i.e., their color and block geometry were sensorically approved. With time, appearance of sample C deteriorated faster than sample B. Texture was much better retained in B than in C (as shown by texture profile analysis data in Table III) in accordance to the trend in moisture content of the samples (Table II). The aroma of sample B was preferred over sample C. The herbal aroma of B sample masked the beany flavor of soybean. With increase of storage time, the aroma of sample B improved and was preferred by the sensory panel; whereas for sample C, the aroma deteriorated significantly and was not approved by the panel. This could have been due to increase in lipid peroxidation and subsequent increase in MDA content (Table II) in C causing typical rancidity aroma, not detected in sample B. With increased storage time, the color of sample C faded which was not observed in B. The after-taste is one of the important parameters of sensory analysis. A distinct aftertaste of herbal extract was detected in B on the first day which was more appealing sensorically than the control sample. Over storage time, the after-taste of C deteriorated rapidly and bitter taste was observed after seven days; however, there was no disagreeable aftertaste of sample B over the entire storage period. The hedonic scale score of overall acceptability at the end of ten days for sample B was 6, *vis-à-vis* 4 for the control sample. These studies concluded that the addition of bay leaf extract in the tofu formulation improved its acceptability over the control sample.

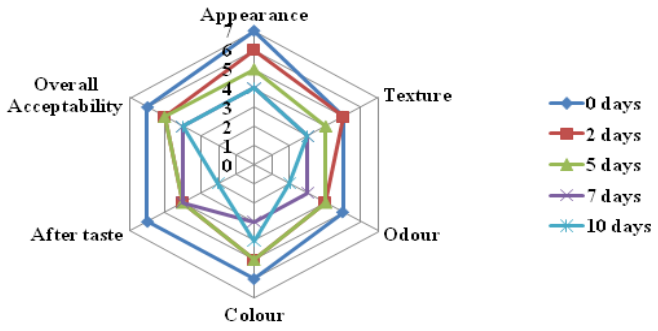


Fig. 3 (a) Radar plot of hedonic scores of control tofu sample

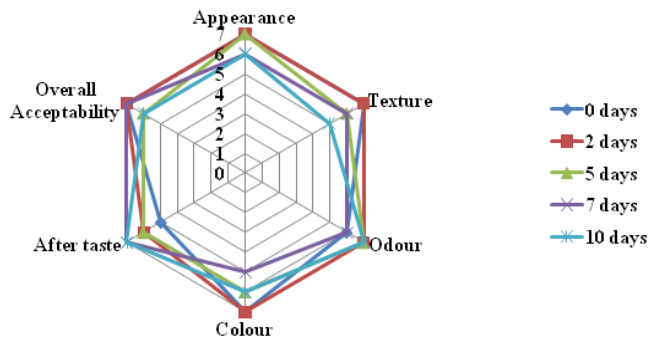


Fig. 3 (b) Radar plot of hedonic scores of bay leaf formulated tofu sample

IV. Conclusions

The addition of SC-CO_2 extract of bay leaves improves the phytochemical and the physicochemical properties of the tofu sample over the storage period of ten days. The texture profile analysis confirmed the structural stability of the bay leaf treated sample over the control sample throughout the storage period of ten days. The microbial analysis along with the sensorical studies affirms the acceptability of the tofu sample with enhanced nutraceutical property. We advocate the tofu sample with SC-CO_2 extract (55°C and 512 bar) of bay as a new green functional food with appreciable nutraceutical properties.

Acknowledgment

The authors are thankful to University Grant Commission (UGC), New Delhi, Government of India for the financial support and Mr. Shyama Prasad Das of Tara Food Products, Ichapur, West Bengal, India for his technical support in tofu preparation.

References

[1] K. Anbarasu, and G. Vijayalakshmi, "Improved shelf life of protein-rich tofu using *Ocimum sanctum* (tulsi) extracts to benefit Indian rural population," *J. Food Sci*., vol. 72, pp. M 300–305, Oct. 2007.

[2] K. Saio, "Tofu- relationships between texture and fine structure," Cereal Foods World, vol. 24, pp. 342–354, Aug. 1979.

[3] C. F. Shen, L. De Man, R. I. Buzzel, and J. M. De Man, "Yield and quality of tofu as affected by soybean and soymilk characteristics: Glucono-delta-lactone coagulant," *J. Food Sci*., vol. 56, pp. 109–112, Jan. 1991.

[4] N. Sun, and W. M. Breene, "Calcium sulfate concentration influence on yield and quality of tofu from five soybean varieties," *J. Food Sci*., vol. 56, pp. 1604–1607, Nov. 1991.

[5] C. G. Beddows, and J. Wong, "Optimization of yield and properties of silken tofu from soybean. I. The water: bean ratio," *Int. J. Food Sci*. Technol., vol. 22, pp. 15–21, Feb. 1987a.

[6] C. G. Beddows, and J. Wong, "Optimization of yield and properties of silken tofu from soybean. II. Heat processing," *Int. J. Food Sci*. Technol., vol. 22, pp. 23–27, Feb. 1987b.

[7] C. G. Beddows, and J. Wong, "Optimization of yield and properties of silken tofu from soybean. III. Coagulant concentration, mixing and filtration pressure," *Int. J. Food Sci. Technol*., vol. 22, pp. 29–34, Feb. 1987c.

[8] S. J. Tsai, C. Y. Lan, C. S. Kao, S. C. Chen, "Studies on the yield and quality characteristics of tofu," *J. Food Sci*., vol. 46, pp. 1734–1737, Nov. 1981.

[9] J. M. deMam, L. deMan, and S. Gupta, "Texture and microstructure of soybean curd (tofu) as affected by different coagulants," *Food Microstruct*., vol. 5, pp. 83–89, 1986.

[10] B. T. Lim, J. M. DeMan, L. DeMan, and R. I. Buzzell, "Yield and quality of tofu as affected by soybean and a soymilk characteristic. Calcium sulfate coagulant," *J. Food Sci*., vol. 55, pp. 1088–1092, July 1990.

[11] A. J. Pontecorvo, and M. C. Bourne, "Simple methods for extending the shelf life of soy curd (tofu) in tropical areas," *J. Food Sci*., vol. 43, pp. 969–72, May 1978.

[12] A. Miskovsky, and M. B. Stone, "Effects of chemical preservatives on storage and nutrient composition of soybean curd," *J. Food Sci*., vol. 52, pp. 1535–1537, Nov. 1987.

[13] H. K. No, and S. P. Meyers, "Preparation of tofu using chitosan as a coagulant for improved shelf–life," *Int. J. Food Sci. Technol*., vol. 39, pp. 133–141, Feb. 2004.

[14] L. S. Devi, S. Kannappan, and C. V. Anuradha, "Evaluation of in vitro antioxidant activity of Indian bay leaf, *Cinnamomum tamala* (Buch.-Ham.) T. Nees & Eberm using rat brain synaptosomes as model system," *Indian. J. Exp. Biol*., vol. 45, pp. 778–784, Sep. 2007.

[15] U. Chakraborty, and H. Das, "Antidiabetic and antioxidant activities of *Cinnamomum tamala* leaf extracts in stz-treated diabetic rats," *Global J. Biotech & Biochem*., vol. 5, pp.12–18, 2010.
[16] P. Goyal, A. Chauhan, and P. Kaushik, "Laboratory evaluation of crude extracts of *Cinnamomum tamala* for potential antibacterial activity," *Electron. J. Biol*., vol. 5, pp. 75–79, 2009.
[17] M. N. Gambhire, A. R. Juvekar, and S. S. Wankhede, "Anti-inflammatory activity of aqueous extract of *Cinnamomum tamala* leaf by in vivo and in vitro methods," *J. Pharm. Res*., vol. 2, pp. 1521–1524, 2009.
[18] V. V. Dighe, A. A. Gursale, R. T. Sane, S. Menon, and P. H. Patel, "Quantitative determination of eugenol from *Cinnamomum tamala* Nees and Eberm. Leaf powder and polyherbal formulation using reverse phase liquid chromatography," *Chromatographia*, vol. 61, pp. 443–446, May 2000.
[19] I. P. S. Kapoor, B. Singh, G. Singh, "Essential oil and oleoresins of *Cinnamomum tamala* (Tejpat) as natural food preservatives for pineapple fruit juice," *J. Food Process Pres*., vol. 32, pp. 719–728, Oct. 2008.
[20] W. Guan, S. Li, R. Yan, S. Tang, and C. Quan, "Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods," *Food Chem*., vol. 101, pp. 1558–1564, 2007.
[21] G. A. Spanos, and R. E. Wrolstad, "Influence of processing and storage on the phenolic composition of Thompson Seedless grape juice", *J. Agric. Food. Chem*., vol. 38, pp. 1565–1571, July 1990.
[22] M. Oyaizu, "Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine", *Jpn. J. Nutr*., vol. 44, pp. 307–315, 1986.
[23] O. A. Aiyegoro, and A. I. Okoh, "Preliminary added screening and in vitro antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC", *BMC Complement Altern. Med*., vol. 10, pp. 2–8, May 2010.
[24] S. Ghosh, D. Chatterjee, S. Das, and P. Bhattacharjee, "Supercritical carbon dioxide extraction of eugenol-rich fraction from *Ocimum sanctum* Linn and a comparative evaluation with other extraction techniques: Process optimization and phytochemical characterization," *Ind. Crop. Prod*., vol. 47, pp. 78– 85, May 2013.
[25] A. Ahmed, M. I. Choudhary, A. Farooq, B. Demirci, F. Demirci, and K. H. C. Başer, "Essential oil constituents of the spice *Cinnamomum tamala* (Ham.) Nees & Eberm," *Flavour Frag. J*., vol. 15, pp. 388–390, Dec. 2000.
[26] S. R. Mir, M. Ali, and R. Kapoor, "Chemical composition of essential oil of *Cinnamomum tamala* Nees et Eberm. leaves," *Flavour Frag. J*., vol. 19, pp. 112–114, Feb. 2004.
[27] R. P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*, 4th ed. Illinois: Allured Publishing Corporation, 2007.
[28] S. Kumar, "Bay leaves", in *Handbook of Herbs and Spices*, K. V. Peter Ed. Cambridge: Woodhead Publishing Ltd., 2001, pp. 59.
[29] A. O. A. C., *Official Methods of Analysis*, 15th ed., Washington, D. C.: Association of Official Analytical Chemists', 2000.
[30] H. Ohkawa, N. Ohishi, and K. Yagi, "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction," *Anal. Biochem*., vol. 95, pp. 351–358, June 1979.
[31] S. Ranganna, *Manual Analysis of Fruits and Vegetable Products*. New Delhi: McGraw Hill, 1987.
[32] K. Tuitemwong and D.C. Fung, "Microbiological study of tofu," J. *Food Protec*., vol. 54, pp. 212-215, 1991.

Open Science Index, Nutrition and Food Engineering Vol:8, No:1, 2014 publications.waset.org/9997143/pdf