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Food Chemistry 93 (2005) 141–148
Food Chemistry
www.elsevier.com/locate/foodchem

Extractability of tea catechins as a function of manufacture procedure and temperature of infusion

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Received 28 June 2004; received in revised form 13 October 2004; accepted 13 October 2004

Abstract

Green teas were made by different inactivation procedures ranging from steaming, thermal inactivation by heating in an oven or microwave-mediated inactivation, followed by either unidirectional or bi-directional rolling and drying. Teas were infused at different temperatures (80 and 100 °C) and the tea ceremony way of infusing, and analyzed for tea catechins and xanthine alkaloids by HPLC. Teas manufactured following microwave inactivation, bi-directional rolling and drying showed higher catechins and methyl xanthines by HPLC, and extracted higher catechins and caffeine in infusions. Catechins, especially EGCG, EGC, and EC, showed marked differences when extracted at different temperatures. Considerable amounts of catechins and caffeine can be extracted each time when the same leaf is infused repeatedly 4–5 times, as done in Japan for making ceremonial teas.

Keywords: Green tea; Catechins; Xanthine alkaloids; Infusion; Rolling; Microwave

1. Introduction

Tea is a processed product of the leaves of *Camellia sinensis*. The shoots, consisting of tender apical bud and subtending three leaves, are processed to give the tea, which upon infusion with hot/cold water gives a non-alcoholic beverage. Tea shoots are rich in catechins, and the ratio and quantity of these determine the taste and quality of the teas. Tea is manufactured as non-fermented green tea, semi fermented oolong tea and fermented black tea (Lin, Lin, Liang, Lin-Shiau, & Juan, 1998). Green teas or non-fermented teas are characterized by inactivation of the enzyme polyphenol oxidase immediately after plucking of the tea shoots. This enzyme is responsible for oxidizing the catechins to theaflavins and thearubigins, the tea pigments responsible for the colour and taste of black teas. The inactivation can be achieved by either parching, roasting or steaming the tea shoots. Traditionally, Chinese people roast the tea shoots in a metal roaster and process the tea shoots by using a unidirectional rotatory roller. This type of rolling gives a twist to the leaf and compacts the particles. The Chinese green tea is characterized by a roast odour. On the other hand, the Japanese inactivate the tea shoots by steaming, followed by bi-directional rolling. This rolling makes the shoot surface flat with leaf juice spread over the entire surface. Green tea is gaining popularity because of its medicinal properties that range from anticancer to antiulcer (Jankun, Selman, Swiercz, & Skrzypczak-Jankun, 1997; Sakanaka, Kim, Taniguchi, & Yamamoto, 1989; Yang, 1997). These pharmacological properties are mainly due to the presence of catechins which constitute up to 30% on a dry weight basis, are water-soluble and can be easily extracted in infusions. The amount of catechins extracted depends upon the genetic characters, the manufacture style and

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0308-8146/$ - see front matter
doi:10.1016/j.foodchem.2004.10.016

the temperature of infusion. This paper presents the effect of different styles of manufacture on infusion characteristics, mainly extractability of catechins into the infusion, the importance of repeated extraction on the amount of catechins extracted into the infusion at different temperatures and amount of catechins extracted into successive cups of extracts consumed in the traditional ceremonial teas.

2. Material and methods

2.1. Green tea

Commercial Japanese green tea was purchased from a local market, Shizuoka, Japan.

2.2. Plant material

China hybrid tea shoots, comprised of apical bud and subtending three leaves, were harvested from plots under regular 7 day plucking at Banoori Tea Experimental Farm of the Institute. About 5 kg of tea shoots were plucked and divided into five equal portions of 1 kg each.

2.3. Manufacture process

2.3.1. Process 1

A portion of 1 kg of leaf was subjected to inactivation by exposing the tea shoots in a glass container with lid to microwave energy (600 W full power) for one minute, during which the tea shoots generated enough steam, that was trapped within the container and was sufficient to inactivate the shoots. The inactivated shoots were uni-directionally rolled in an orthodox piezy roller wherein the shoots were twisted under pressure. During the process, the shoots became coated with squeezed juice from within the shoots. The rolled shoots were then dried in a locally fabricated lab tray dryer. The dryer was fabricated locally and standardized for performance. This manufacture matched the China type of green tea manufacture.

2.3.2. Process 2

A second portion of plucked shoots was microwave-inactivated twice at 60% power for 3 min. The inactivated shoots were rolled by to and fro bi-directional rolling on a wire mesh. With the increase in pressure, the juice is squeezed out and coats the shoots. The rolled teas were dried as in process 1.

2.3.3. Process 3

A third portion of the plucked tea shoots was manufactured as in (2) except that they were covered with glass and microwave-inactivated at 600 W full power for 1 min.

2.3.4. Process 4

The remaining 2 kg of tea shoots were processed following inactivation by steaming/thermal inactivation by heating in an oven and bi-directionally rolled in a piezy roller as described by Gulati, Rawat, Singh, and Ravindranath (2003).

2.4. Tea infusions

Teas were infused at 80 and 100 °C and following the south-east Asian countries.

2.4.1. Ceremonial infusions

Hot distilled water at 100 °C was kept in a porcelain pot. Three small porcelain bowls with lids measuring 80 ml were serially used. Each one was rinsed with hot water. Forty ml of water were poured into the first bowl and kept open for 2 min and the temperature of the water was checked with a laboratory thermometer. The water from the first bowl was poured into the second bowl containing 3 g of green tea and covered with a lid. The first bowl was again filled with hot water from the pot. After 2 min, the temperature of infusion was measured with a thermometer and the infusion from the second bowl was decanted into the third bowl. The water from the first bowl was poured over the infused leaf in the second bowl and the first bowl was again filled with hot water. The infusion in the third bowl was analysed for catechins and xanthine alkaloids. The cycle was repeated 4–5 times in sequence of first bowl–second bowl–third bowl, using the same infused leaf.

2.4.2. Double infusions

For double infusion at 100 °C, 2 g of tea, as described under manufacture process 2, were added to 100 ml hot distilled water at 100 °C and covered with a lid for 3 min and filtered. This was the first infusion. A second infusion was prepared by adding 2 g of tea to the hot distilled water containing the infused leaf of the first infusion and covered for 3 min. The infusion was filtered into a separate cup. This second infusion served as the double infusion.

For double infusion at 80 °C, Distilled water was heated to 80 °C in a pot and temperature of the water was measured by a thermometer. As soon as the first bubble appeared (80 °C), 2 g of tea, as described under manufacture process 2, were added to it and covered for 3 min and decanted. This was the first infusion. The second infusion was made as described in double infusion at 100 °C.

2.4.3. Repeat infusions at 100 and 80 °C

Distilled water was heated to 100 °C/80 °C and the temperature of the water was measured by thermometer. To the hot water (125 ml), 3 g of tea as described under manufacture process 2, were added and covered for 3 min and the infusion was decanted into the second cup. This was the first infusion. Hot distilled water at 100 °C/80 °C was added to the same infused leaf in the container and covered for 3 min. This was the second infusion. The third infusion was also prepared following the same procedure as for second cup.

2.5. Estimation of catechins

Tea infusions were analyzed for catechins and caffeine according to the method described by Sharma, Gulati, Ravindranath, and Kumar (2004) on a Merck–Hitachi model D-7000 HPLC using a C-18 reverse phase 250 × 4.0 mm, 5 μm column, fitted with a C-18 guard column.

3. Results and discussion

3.1. Effect of manufacture procedure on the extraction of catechins

Tea blend, manufacturing particles, and methods of beverage preparation influence the composition of a tea brew (Graham, 1992). Qualitative and quantitative differences were observed in catechins and xanthine alkaloids when teas with different manufacture styles were analyzed. The effect of manufacture procedure on the extraction of catechins is given in Table 1A–1F. The green tea from Japan showed the highest levels of catechins, followed by green tea manufactured by microwave inactivation and bi-directional rolling (Table 1A–1F). This may be attributed to the flatness of particles, that have higher open surface which leads to more extraction of catechins whereas unidirectional rolling compacts the particles and needs more time for extraction of catechins (Table 1A–1F). Higher levels of catechins were extracted by microwave treatment and steam-inactivation than by oven inactivation, whereas the caffeine shows a different pattern (Table 3). Caffeine is extracted in higher amounts in green teas made by microwave inactivation and oven inactivation than by steam inactivation.

3.2. Effect of temperature of infusing water on the extraction of catechins

The main factor determining the taste and aroma of tea is the method of infusion preparation. Like the man-

Table 1A
Biochemicals extracted (mg/ml)[a] in first infusion of different green teas (Tea ceremony way of infusing)

Manufacture process	Total catechins	Gallic acid	EGC	Cat	EC	EGCG	ECG	Caffeine	Theobromine
Japanese green tea	2.90 ± 0.001	nd[b]	1.54 ± 0.004	0.04 ± 0.001	0.4 ± 0.030	0.86 ± 0.050	0.06 ± 0.006	0.58 ± 0.010	0.137 ± 0.04
Process 1	1.86 ± 0.007	0.008 ± 0.0002	0.78 ± 0.02	0.04 ± 0.001	0.2 ± 0.002	0.78 ± 0.02	0.06 ± 0.004	0.54 ± 0.006	0.127 ± 0.01
Process 2	2.12 ± 0.01	0.008 ± 0.0	0.78 ± 0.037	0.04 ± 0.001	0.2 ± 0.005	1.00 ± 0.045	0.08 ± 0.009	0.62 ± 0.041	0.137 ± 0.01
Process 3	2.11 ± 0.02	0.012 ± 0.001	0.80 ± 0.006	0.04 ± 0.003	0.2 ± 0.002	0.98 ± 0.004	0.08 ± 0.001	0.62 ± 0.002	0.12 ± 0.002
Process 4	1.71 ± 0.01	0.012 ± 0.0001	0.63 ± 0.05	0.05 ± 0.001	0.18 ± 0.02	0.76 ± 0.020	0.078 ± 0.009	0.52 ± 0.020	0.12 ± 0.002

[a] Data for content of biochemicals extracted in the third cup are average values of triplicate analyses, and are given with SD.
[b] Not detected.

Table 1B
Biochemicals extracted (mg/ml)[a] in second infusion of different green teas (Tea ceremony way of infusing)

Manufacture process	Total catechins	Gallic acid	EGC	Cat	EC	EGCG	ECG	Caffeine	Theobromine
Japanese green tea	4.21 ± 0.010	0.004 ± 0.0	1.92 ± 0.04	0.054 ± 0.001	0.70 ± 0.05	1.46 ± 0.014	0.078 ± 0.005	0.84 ± 0.05	0.21 ± 0.006
Process 1	2.10 ± 0.008	0.002 ± 0.001	0.82 ± 0.028	0.04 ± 0.004	0.20 ± 0.002	0.96 ± 0.012	0.08 ± 0.006	0.62 ± 0.003	0.127 ± 0.012
Process 2	2.99 ± 0.017	0.01 ± 0.0	1.12 ± 0.029	0.05 ± 0.001	0.32 ± 0.031	1.41 ± 0.033	0.08 ± 0.007	0.78 ± 0.02	0.187 ± 0.01
Process 3	2.19 ± 0.003	0.013 ± 0.002	0.80 ± 0.004	0.04 ± 0.002	0.22 ± 0.001	1.04 ± 0.012	0.08 ± 0.003	0.66 ± 0.01	0.12 ± 0.002
Process 4	1.80 ± 0.019	0.012 ± 0.001	0.68 ± 0.04	0.05 ± 0.004	0.18 ± 0.048	0.80 ± 0.02	0.078 ± 0.004	0.56 ± 0.02	0.12 ± 0.002

[a] Data for content of biochemicals extracted in the third cup are average values of triplicate analyses, and are given with SD.

Table 1C
Biochemicals extracted (mg/ml)[a] in third infusion of different green teas (Tea ceremony way of infusing)

Manufacture process	Total catechins	Gallic acid	EGC	Cat	EC	EGCG	ECG	Caffeine	Theobromine
Japanese green tea	3.51 ± 0.02	nd[b]	1.68 ± 0.06	0.052 ± 0.009	0.50 ± 0.03	1.22 ± 0.04	0.06 ± 0.009	0.68 ± 0.06	0.14 ± 0.03
Process 1	1.90 ± 0.02	0.002 ± 0.001	0.76 ± 0.005	0.04 ± 0.004	0.18 ± 0.003	0.86 ± 0.004	0.06 ± 0.003	0.58 ± 0.01	0.127 ± 0.005
Process 2	1.95 ± 0.017	0.008 ± 0.001	0.69 ± 0.03	0.05 ± 0.003	0.19 ± 0.031	0.94 ± 0.035	0.08 ± 0.008	0.60 ± 0.03	0.12 ± 0.015
Process 3	1.70 ± 0.04	0.008 ± 0.001	0.60 ± 0.00	0.05 ± 0.0	0.16 ± 0.001	0.82 ± 0.012	0.08 ± 0.003	0.56 ± 0.01	0.12 ± 0.008
Process 4	1.26 ± 0.019	0.016 ± 0.001	0.42 ± 0.04	0.04 ± 0.004	0.12 ± 0.048	0.58 ± 0.02	0.06 ± 0.004	0.52 ± 0.06	0.10 ± 0.008

[a] Data for content of biochemicals are average values of triplicate analyses, and are given with SD.
[b] Not detected.

Table 1D
Biochemicals extracted (mg/ml)[a] in fourth infusion of different green teas (Tea ceremony way of infusing)

Manufacture process	Total catechins	Gallic acid	EGC	Cat	EC	EGCG	ECG	Caffeine	Theobromine
Japanese green tea	2.77 ± 0.011	nd[b]	1.40 ± 0.004	0.052 ± 0.002	0.34 ± 0.04	0.92 ± 0.004	0.06 ± 0.009	0.54 ± 0.01	0.10 ± 0.02
Process 1	1.56 ± 0.011	0.002 ± 0.001	0.56 ± 0.013	0.040 ± 0.005	0.16 ± 0.01	0.74 ± 0.03	0.06 ± 0.007	0.56 ± 0.02	0.127 ± 0.02
Process 2	1.80 ± 0.007	0.008 ± 0.001	0.66 ± 0.015	0.05 ± 0.002	0.16 ± 0.01	0.90 ± 0.012	0.08 ± 0.004	0.58 ± 0.006	0.12 ± 0.01
Process 3	1.59 ± 0.004	0.001 ± 0.0002	0.58 ± 0.005	0.05 ± 0.003	0.14 ± 0.008	0.74 ± 0.004	0.078 ± 0.006	0.54 ± 0.003	0.10 ± 0.02
Process 4	1.7 ± 0.010	0.008 ± 0.001	0.36 ± 0.02	0.04 ± 0.003	0.10 ± 0.02	0.60 ± 0.02	0.06 ± 0.003	0.50 ± 0.01	0.08 ± 0.02

[a] Data for content of biochemicals are average values of triplicate analyses, and are given with SD.
[b] Not detected.

Table 1E
Biochemicals extracted (mg/ml)[a] in fifth infusion of different green teas (Tea ceremony way of infusing)

Manufacture process	Total catechins	Gallic acid	EGC	Cat	EC	EGCG	ECG	Caffeine	Theobromine
Japanese green tea	2.38 ± 0.011	nd[b]	1.20 ± 0.004	0.04 ± 0.001	0.24 ± 0.03	0.78 ± 0.014	0.012 ± 0.006	0.46 ± 0.06	0.10 ± 0.008
Process 1	1.02 ± 0.010	0.002 ± 0.04	0.32 ± 0.004	0.04 ± 0.001	0.12 ± 0.01	0.48 ± 0.04	0.06 ± 0.005	0.52 ± 0.03	0.127 ± 0.009
Process 2	1.23 ± 0.008	nd[b]	0.40 ± 0.008	0.05 ± 0.002	0.11 ± 0.015	0.60 ± 0.009	0.07 ± 0.009	0.52 ± 0.012	0.120 ± 0.01
Process 3	1.22 ± 0.014	0.006 ± 0.04	0.42 ± 0.006	0.04 ± 0.002	0.10 ± 0.020	0.60 ± 0.012	0.06 ± 0.006	0.48 ± 0.01	0.10 ± 0.015
Process 4	0.96 ± 0.009	0.002 ± 0.04	0.30 ± 0.003	0.04 ± 0.001	0.06 ± 0.009	0.50 ± 0.01	0.06 ± 0.004	0.42 ± 0.01	0.06 ± 0.01

[a] Data for content of biochemicals are average values of triplicate analyses, and are given with SD.
[b] Not detected.

Table 1F
Taster's evaluation of ceremonial infusions

Infusion number	Third cup infusion[a]		
	Sweet	Astringent	Bitter
Infusion 1	+++	+	–
Infusion 2	+	+++	+
Infusion 3	+	++	+
Infusion 4	–	++	–
Infusion 5	–	+	–

[a] Values are averages of three replicates.

ufacture process, temperature of the infusing water has a bearing on the extraction of catechins in tea (Khokhar & Magnusdottir, 2002). Tea infused at 100 °C showed higher levels of catechins (specially EGCG and EGC) and methyl xanthines (especially caffeine) than did tea infusions made at 80 °C (Tables 2A and 3). Infusions made at lower temperatures (80 °C) were sweet in taste and appreciated more by tea tasters than were infusions made at higher temperatures (100 °C) (Table 2B). Significant levels of catechins can be extracted when the same leaf is infused three times at 100 °C/80 °C in repeated infusions made at 100 or 80 °C, respectively (Table 2A). No major differences were observed in the levels of catechins in the first and second infusion but the third infusion showed a decline in the levels of catechins when the same leaf was infused thrice at 100 °C/80 °C. An increase of 10–15% in the levels of catechins was observed in the second infusion when double infusions were made (Table 3).

In Japan and some other Southeast Asian countries, people are accustomed to making tea infusions three or four times with the same tea leaves and this ceremony is popularly known as the Tea Ceremony. In these types of ceremonies, people appreciate the changing taste of infusions. These infusions showed higher levels of catechins especially EGCG, EGC and methyl xanthines, in the second infusion with all the biochemicals (catechins and methyl xanthines) gradually decreasing in later infusions (Table 1A–1E). Gradation in taste was also observed from the first infusion to the fifth infusion (Table 1F). The first infusion was sweet due to the presence of carbohydrates which are water-soluble and are easily extracted in the first infusion. Decrease in temperature of infusing water was also observed in the ceremonial tea infusions from 60 °C in the first cup to 48 °C in the second cup and 40 °C in the third cup infusion.

The study therefore shows that teas can be re-extracted, thereby extracting considerable amounts of antioxidants, the catechins, each time with the same leaf infused repeatedly as in ceremonial infusions and repeated infusions. The manufacture procedure and temperature of infusion also influence the extraction of catechins in the infusions.

Table 2A
Biochemicals extracted (mg/ml)[a] in repeat infusions

Infusion		Catechins (mg/ml)							Xanthine alkaloids (mg/ml)	
Temperature (°C)	Number	Total catechins	Gallic acid	EGC	Cat	EC	EGCG	ECG	Caffeine	Theobromine
100	1st	3.16 ± 0.01	0.08 ± 0.004	0.90 ± 0.04	0.03 ± 0.004	0.38 ± 0.01	1.71 ± 0.004	0.06 ± 0.04	1.08 ± 0.06	0.35 ± 0.06
	2nd	3.12 ± 0.05	0.07 ± 0.005	0.91 ± 0.01	0.028 ± 0.004	0.37 ± 0.01	1.68 ± 0.03	0.06 ± 0.02	0.87 ± 0.04	0.31 ± 0.02
	3rd	2.14 ± 0.06	0.04 ± 0.004	0.60 ± 0.01	0.2 ± 0.003	0.15 ± 0.03	1.30 ± 0.01	0.03 ± 0.02	0.44 ± 0.01	0.14 ± 0.01
80	1st	2.33 ± 0.02	0.02 ± 0.005	0.75 ± 0.01	0.028 ± 0.003	0.20 ± 0.01	1.29 ± 0.02	0.05 ± 0.03	0.60 ± 0.01	0.17 ± 0.01
	2nd	2.36 ± 0.02	0.01 ± 0.005	0.75 ± 0.03	0.028 ± 0.002	0.20 ± 0.009	1.32 ± 0.003	0.05 ± 0.004	0.67 ± 0.02	0.17 ± 0.01
	3rd	1.59 ± 0.04	nd[b]	0.40 ± 0.01	0.02 ± 0.001	0.08 ± 0.006	1.04 ± 0.01	0.05 ± 0.009	0.36 ± 0.008	0.08 ± 0.01

[a] Data for content of biochemicals are average values of triplicate analyses, and are given with SD.
[b] Not detected.

Table 2B
Taster's evaluation of repeat infusions

Infusions		Sweetness[a]	Astringency[a]	Bitterness[a]
Temperature (°C)	Number			
100	1	+	+++	+++
	2	+	+++	++
	3	–	++	+
80	1	+++	++	+
	2	++	+	+
	3	+	+	–

[a] Values are averages of three replicates.

Table 3
Biochemicals extracted in double infusions

Manufacture process	Infusion condition	Catechin (mg/ml)[a]							Xanthine alkaloids (mg/cup)[a]	
		Total catechins	Gallic acid	EGC	Cat	EC	EGCG	ECG	Caffeine	Theobromine
MW covered (3 min)-inactivated, bi-directional rolling and drying	Infusion 1 (100 °C)	3.10 ± 0.006	0.03 ± 0.001	0.91 ± 0.006	0.03 ± 0.010	0.53 ± 0.006	1.54 ± 0.01	0.06 ± 0.005	0.42 ± 0.008	0.27 ± 0.01
	Infusion 2 (100 °C)	3.41 ± 0.005	0.01 ± 0.002	0.98 ± 0.003	0.03 ± 0.004	0.62 ± 0.008	1.71 ± 0.009	0.06 ± 0.006	0.44 ± 0.009	0.35 ± 0.01
	Infusion 1 (80 °C)	2.53 ± 0.006	0.05 ± 0.006	0.80 ± 0.004	0.02 ± 0.005	0.33 ± 0.007	1.28 ± 0.01	0.05 ± 0.004	0.40 ± 0.01	0.17 ± 0.01
	Infusion 2 (80 °C)	2.82 ± 0.006	0.04 ± 0.004	0.85 ± 0.006	0.02 ± 0.005	0.42 ± 0.004	1.44 ± 0.01	0.05 ± 0.008	0.46 ± 0.009	0.24 ± 0.01
Oven (60 °C, 10 min)-inactivated, bi-directional rolling and drying	Infusion 1 (100 °C)	0.60 ± 0.005	0.02 ± 0.002	0.15 ± 0.009	0.02 ± 0.004	0.03 ± 0.005	0.35 ± 0.008	0.03 ± 0.007	0.41 ± 0.007	0.04 ± 0.01
	Infusion 2 (100 °C)	0.72 ± 0.007	0.02 ± 0.003	0.20 ± 0.009	0.02 ± 0.010	0.04 ± 0.010	0.40 ± 0.007	0.04 ± 0.008	0.41 ± 0.015	0.04 ± 0.01
	Infusion 1 (80 °C)	0.46 ± 0.005	0.02 ± 0.002	0.14 ± 0.014	0.02 ± 0.009	0.03 ± 0.010	0.22 ± 0.009	0.03 ± 0.009	0.40 ± 0.009	0.03 ± 0.01
	Infusion 2 (80 °C)	0.58 ± 0.01	0.02 ± 0.003	0.18 ± 0.021	0.02 ± 0.010	0.03 ± 0.009	0.30 ± 0.009	0.03 ± 0.001	0.41 ± 0.01	0.045 ± 0.02
Steam-inactivated, bi-directional rolling and drying	Infusion 1 (100 °C)	2.88 ± 0.01	0.04 ± 0.005	0.83 ± 0.024	0.03 ± 0.009	0.45 ± 0.010	1.47 ± 0.004	0.06 ± 0.009	0.12 ± 0.015	0.32 ± 0.01
	Infusion 2 (100 °C)	3.39 ± 0.01	0.02 ± 0.007	0.92 ± 0.022	0.03 ± 0.010	0.63 ± 0.007	1.73 ± 0.02	0.06 ± 0.009	0.14 ± 0.020	0.40 ± 0.01
	Infusion 1 (80 °C)	2.28 ± 0.01	0.06 ± 0.006	0.68 ± 0.014	0.02 ± 0.020	0.27 ± 0.020	1.20 ± 0.015	0.05 ± 0.008	0.12 ± 0.010	0.29 ± 0.01
	Infusion 2 (80 °C)	3.04 ± 0.01	0.03 ± 0.002	0.87 ± 0.009	0.03 ± 0.012	0.53 ± 0.010	1.52 ± 0.009	0.06 ± 0.006	0.13 ± 0.010	0.30 ± 0.02

[a] Data for content of biochemicals are average values of triplicate analyses, and are given with SD.

Acknowledgements

The authors are thankful to the Director, Institute of Himalayan Bioresource Technology, Palampur for taking keen interest in the work.

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