

The Fruit Liquor Prepared from Everbearing Strawberry (*Fragaria X Ananassa* Duch.) Cultivar, ‘Summertiera’ Berries with Superior Health Promoting Abilities

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Abstract

The fruit liquor was prepared from everbearing strawberry cultivar, ‘Summertiera’ berries and was characterized. It gave a mellow rich and full-flavored, and was sweet to the taste, because the sugar-acid ratio was low to add a large quantity of crystal sugar. The color of the liquor was Koji color tinged red color like fruit of evergreen shrubs in the rue family, Rutaceae. The contents of total phenols and total flavonoids were fairly high, although most of soluble vitamins such as B₁, B₂, and C destroyed during brewing. A considerable amount of anthocyanins remained in the liquor. The liquor possessed strong antioxidative activity and reactive oxygen species scavenging activities, and these activities increased the liquor concentration-dependently. Moreover, the liquor almost entirely inhibited angiotensin I-converting enzyme and hyaluronidase activities. Except for high sugar content, therefore, the fruit liquor made from everbearing strawberry cultivar, ‘Summertiera’ berries may be a drink with superior health promoting potential.

Keywords

‘Summertiera’ berry; Fruit liquor; Chemical analysis; Anti-oxidative activity; Anti-hypertensive activity; Anti-allergic activity

Introduction

In all parts of the world, strawberry (*Fragaria x ananassa* Duch.) is cultivated, and is also consumed as a fresh fruit and a processing ingredient of products such as jams, cakes, ice cream, yogurts, preserves, juices, and liquors and so on. Strawberry is most popular fruit for consumers because of the favorable appearances and desirable flavor among berry fruits [1]. It is known that strawberry is abundant in antioxidants as ascorbic acid and phenol compounds (anthocyanins and flavonoids) [2]. These compounds act as one of scavengers against

reactive oxygen species and unstable free radicals such as superoxide anion radicals, hydroxyl radicals, hydrogen peroxide, and singlet oxygen [3]. The formation and increase of these radical species in the human body directly

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or indirectly cause degenerative diseases and chronic diseases to damage cellular bio molecules such as amines, carbohydrates, DNA, lipids, and proteins [4]. That is, the oxidation and anti-oxidation balance plays an important role for sustenance of a healthy biological system. Strawberry is an excellent source of phytochemicals and contains some nutrients. Especially, phenol compounds possess remarkable antioxidant activity [5]. So, the consumption of diet rich in fresh fruits as strawberry is believed to have the ability to protect the human body from these diseases [6, 7].

In Japan most of strawberry species on the commercial sales and distribution are June-bearing strawberry cultivars, such as ‘Tochiotome’, ‘Amaou’, and ‘Toyonoka’. However, since Ohishi had succeeded to cultivate an everbearing strawberry cultivar, ‘Ohishi-shikinari 1’ in 1954, many everbearing strawberry cultivars have been cultivated in Japan one after another [8]. Recently, an everbearing strawberry cultivar, ‘Summertiarā’ was cultivated in Yamagata Prefecture, Tohoku region of Japan. Nagai et al. [9] was to characterize the fresh berries, with relation to chemical parameters and phenol components. Moreover, they have proved to possess excellent antioxidant and antihypertensive properties in the berries [9]. However, as far as we know, there is still lacking of scientific information about nutritional values and medicinal properties in the processed foods using the berries to encourage more and more production and consumption of the berries. Therefore, the main objectives of the present study aims (1) to investigate the chemical parameters, total phenols, flavonoids, anthocyanins, and vitamins B₁, B₂, and C, and (2) to clarify the functional properties as anti-oxidative activity, anti-hypertensive activity, and anti-allergic activity of the fruit liquor prepared from everbearing strawberry cultivar, ‘Summertiarā’ berries.

Materials and Methods

Samples

The fresh everbearing strawberry cultivar, ‘Summertiarā’ berries (weight 3.6-7.4 g, average weight 4.9 g) were gifted from the Farm Village Industry Federation of JA Kushibiki Agricultural Cooperatives, Yamagata, Japan, and used in this study.

Preparation of The Liquor from The Berries

The berries were washed with flowing water and were wiped of the water with a paper towel. Next, the hull was removed by a fruit knife, and then the berries were weighed. The berries were added 62.5% (w/w) of crystal sugar and 225% (w/w) of commercially available white liquor (white liquor contained 35% ethanol is one of Shochu; that is commonly used to make fruit liquor). After the brewing for 4 years at room temperature in the dark condition, the liquor was used in the following investigation.

Chemical Analysis of The Liquor

Chemical analysis of the liquor was performed as described by Nagai et al. [9]. The pH was measured using a pH meter (PHL-40; DKK Co. Ltd., Tokyo, Japan). Sugar content was determined using a refractometer (PAL-Pâtissier; Atago Co. Ltd., Tokyo, Japan). Alcohol content was measured using a refractometer (PAL-34S; Atago Co. Ltd., Tokyo, Japan) after distillation. Total vitamin B₁ was determined by the *p*-aminoacetophenone method [10]. Total vitamin B₂ was measured by the lumiflavin fluorescence method [10]. Total vitamin C was determined by the α,α' -dipyridyl method [11]. Total phenols analysis was performed using ellagic acid as standard [12]. Total flavonoids contents were expressed as (+)-catechin equivalent [13]. Total anthocyanins were measured after an extraction by methanolic hydrochloric solution (1.0% HCl-methanol) [14]. The content was expressed as pelargonidin 3-*o*-glucoside (P3G) equivalents.

Color Measurements of The Liquor

Color analysis was performed using a colorimeter (NR-11A; Nippon Denshoku Industries Co. Ltd., Tokyo, Japan) with illuminant D65 calibrated to black and white standards. The tristimulus $L^* a^* b^*$ measurement mode was used as the relation to human eye response to color. The L^* , a^* , and b^* scales represents lightness, the red-green dimension, and the yellow-blue dimension, respectively. The results were shown as the mean of ten measurements.

Antioxidative Activity of The Liquor

The antioxidative activity of the liquor prepared from everbearing strawberry cultivar, ‘Summertiarā’ berries was investigated using a linoleic acid oxidation system described by Nagai et al. [9]. Ascorbic acid, *tert*-butyl-4-hydroxyanisole (BHA), 2,6-di-*t*-butyl-4-

methylphenol (BHT), α -tocopherol, and trolox were used as positive control, and distilled water was used as negative one.

Superoxide Anion Radical Scavenging Activity of The Liquor

The superoxide anion radical scavenging activity of the liquor prepared from the berries was measured using xanthine/xanthine oxidase system as described by Nagai et al. [9]. Ascorbic acid, BHA, BHT, α -tocopherol, and trolox were used as positive control, and distilled water was used as negative one. The IC_{50} value was defined as the concentration of the liquor required to inhibit 50% of the superoxide anion radical activity. Moreover, the activity was also expressed as moles of trolox equivalents per kg of the liquor [trolox equivalents antioxidant capacity (TEAC); $\mu\text{mol TE/kg}$].

Hydroxyl Radical Scavenging Activity of The Liquor

The hydroxyl radical scavenging activity of the liquor prepared from the berries was evaluated using Fenton reaction system as described by Nagai et al. [9]. Ascorbic acid, BHA, BHT, α -tocopherol, and trolox were used as positive control, and distilled water was used as negative one. The IC_{50} value was defined as the concentration of the liquor required to inhibit 50% of the hydroxyl radical activity. Moreover, the activity was also expressed as TEAC ($\mu\text{mol TE/kg}$).

1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Activity of The Liquor

The DPPH radical scavenging activity of the liquor prepared from the berries was performed as described by Nagai et al. [9]. Ascorbic acid, BHA, BHT, α -tocopherol, and trolox were used as positive control, and distilled water was used as negative one. The IC_{50} value was defined as the concentration of the liquor required to inhibit 50% of the DPPH radical activity. Moreover, the activity was also expressed as TEAC ($\mu\text{mol TE/kg}$).

Angiotensin I-Converting Enzyme (ACE) Inhibitory Activity of The Liquor

The ACE inhibitory activity of the liquor prepared from the berries was measured as described by Nagai et al. [9]. The IC_{50} value was defined as the concentration of the liquor required to inhibit 50% of the ACE activity.

Hyaluronidase Inhibitory Activity of The Liquor

The hyaluronidase inhibitory activity of the liquor prepared from the berries was investigated by the Morgan-Elson method [15] with some modifications. A 0.02 ml of the extracts and 0.01 ml of hyaluronidase from Ovine testes (1,000U/ml; Wako Pure Chemicals Industries, Ltd., Osaka, Japan) were mixed in an Eppendorf tube and were pre-incubated at 37°C for 20 min. The mixture was added 0.02 ml of compound 48/80 solution, and was incubated at the same condition. The enzyme reaction was started by the addition of 0.05 ml of hyaluronic acid solution. After incubation at 37°C for 40 min, the reaction was stopped by addition of 0.02 ml of 1.77 N NaOH and 0.02 ml of 0.8 M boric acid-0.4 N NaOH. The mixture was boiled for 3 min, and then was cooled in water. The mixture was added 0.6 ml of *p*-dimethylaminobenzaldehyde solution. After incubation at 37°C for 20 min, the absorbance of the mixture was measured at 585 nm. The inhibition rate was calculated by the measuring the amount of *N*-acetyl glucosamine released. Test samples were replaced by the buffer solution for the control. The enzyme solution was replaced by the buffer solution for the blank. Percent inhibition was calculated as the following equation.

$$\text{Inhibition (\%)} = [(A - B) - (C - D)] / (A - B) \times 100$$

A : control OD_{585} ; B : control blank OD_{585} ; C : sample OD_{585} ; D : sample blank OD_{585}

Moreover, the IC_{50} value was defined as the concentration of the liquor required to inhibit 50% of the hyaluronidase activity.

Statistical Analysis

Except for color analysis, each assay was repeated 3 times independently and the results were reported as means \pm standard deviation (SD).

Results and Discussion

The liquor was prepared successfully from everbearing strawberry cultivar, 'Summertiera' berries (Fig. 1). It gave a mellow rich and full-flavored. The liquor was sweet to the taste, because of addition of a large quantity of crystal sugar.

Chemical Analysis

Chemical parameters of the liquor were investigated. The specific gravity and the pH at 20°C

were 1.047 and 3.65, respectively (Table 1). The Brix% at 20°C was 25.7%. The titratable acidity of the liquor was estimated to 0.38% as citric acid equivalent; the sugar-acid ratio was calculated to 67.6. The sugar-acid ratio of the liquor was extremely high to add a large quantity of crystal sugar, although the Brix% of the berries as the ingredients was low (7.3%) and the titratable acidity was very high (1.19%) [9]. The alcohol content was low about 6.1%, compared with the commercially available wine. Total vitamins B₁, B₂, and C contents were determined and were about 3.54 (µg/100 ml), 0.03 (µg/100 ml), and 344.8 (µg/100 ml), respectively (Table 1). Preservation and processing of the fruits cause significant changes of their nutritional properties and composition [16]. Generally, it is known that the strawberry berries are the richest source of vitamin C [17]. In fact, the content of total vitamin C of fresh ‘Summertiar’ berries was high (62.2 mg/100 g FW) [9]. However, it suggested that not only vitamin C, but also vitamins B₁ and B₂ destroyed during brewing for over 4 years. It may be maintain the contents of vitamins B₁, B₂, and C by low temperature fermentation and under light-blocking condition. The contents of total phenols, total flavonoids, and total anthocyanins of the liquor were measured, and the results were as follows: 51.7 mg ellagic acid equivalent/100 ml, 17.0 mg (+)-catechin equivalent/100 ml, 2.0 mg P3G equivalent/100 ml, respectively (Table 1). Häkkinen et al. [18] reported the content of ellagic acid in strawberry cultivars, ‘Jonsok’ and ‘Senga’ berries in the storage conditions (a domestic freezer or refrigerator). The contents in these cultivars berries reduced to about 40% during nine months of storage at -20°C. They also reported the content in the jam maintained about 80% of that in fresh berries. In any case, the content of ellagic acid decreased in the storage condition or in the processing procedure [18].

Color Measurement

The color of the liquor was Koji color tinged red color like fruit of evergreen shrubs in the rue family, Rutaceae (Fig. 1). The color of the liquor was measured by a colorimeter, and the parameter was as follows: $L^* = 12.15$, $a^* = 6.26$, $b^* = 12.04$ (Table 1). The characteristic of the berries is to have a predominately red color, either in the peels ($L^* = 26.57$, $a^* = 22.14$, $b^* = 15.51$) or in the central portion ($L^* = 40.05$, $a^* = 17.44$, $b^* = 10.01$) of the berries [9]. After the red pigment of the berries was almost extracted to 24 hours from brewing start, the color

gradually discolored during the brewing period for over 4 years.

Figure 1. The fruit liquor prepared from everbearing strawberry, cultivar, ‘Summertiar’ berries



Table 1. Chemical parameters of the fruit liquor prepared from everbearing strawberry cultivar, ‘Summertiar’ berries

Parameters	
Specific gravity	1.047±0.008
pH at 20°C	3.65±0.02
Brix% at 20°C	25.7±0.6
Titratable acid content	0.38±0.02 (%)
Sugar-acid ratio	67.6±0.08
Alcohol at 20°C	6.1±0.1%
Total vitamin B ₁	3.54±0.23(µg/100 ml)
Total vitamin B ₂	0.03±0.005(µg/100 ml)
Total vitamin C	344.8±9.7(µg/100 ml)
Total phenols	51.7±1.02*(mg/100 ml)
Total flavonoids	17.0±0.25**(mg/100 ml)
Total anthocyanins	2.0±0.02*** (mg/100 ml)
Color parameter	
L*	12.15±1.30
a*	6.26±0.83
b*	12.04±1.53

*ellagic acid equivalent, **(+)-catechin equivalent, ***P3G equivalent

Antioxidative Activity

Antioxidative activity of the liquor prepared from the berries was determined to evaluate the inhibition effect at the initiation stage of linoleic acid peroxidation. The liquor showed the antioxidative activities and the activities increased with an increasing the concentration of the liquor (Table 2). The activities of 0.1 and 1.0% liquor were fairly low and the same as that of 1.0 mM ascorbic acid. The

activity of 10% liquor was below the levels of 0.01 mM BHA, BHT, and trolox after 200 min. The activity of 100% liquor was almost equivalent to that of 5.0 mM ascorbic acid (Table 2). As the remaining total vitamin C content of the liquor was considerable low, it suggested that most of the effects were attributed to its high phenolic contents in the liquor.

Table 2. Antioxidative activity of the fruit liquor prepared from everbearing strawberry cultivar, ‘Summertiarra’ berries

Absorbance (500 nm)			
Samples	Time (min)		
	50	100	200
Liquor conc. (%)			
0.1	0.342±0.013	0.422±0.019	0.458±0.024
1.0	0.319±0.014	0.371±0.016	0.445±0.022
10	0.086±0.009	0.188±0.012	0.363±0.015
100	0.021±0.003	0.032±0.007	0.096±0.009
Ascorbic acid (mM)			
1.0	0.022±0.001	0.135±0.006	0.469±0.027
5.0	0.016±0.001	0.032±0.003	0.090±0.008
BHA (mM)			
0.01	0.084±0.005	0.120±0.008	0.245±0.012
0.1	0.056±0.003	0.090±0.006	0.165±0.010
1.0	0.054±0.002	0.057±0.003	0.100±0.006
BHT (mM)			
0.01	0.082±0.003	0.112±0.009	0.248±0.011
0.1	0.058±0.004	0.108±0.005	0.173±0.008
1.0	0.044±0.002	0.051±0.003	0.093±0.005
α-Tocopherol (mM)			
1.0	0.006	0.025±0.001	0.028±0.002
Trolox (mM)			
0.01	0.084±0.005	0.094±0.006	0.262±0.013
0.1	0.038±0.002	0.051±0.003	0.123±0.008
1.0	0.011±0.001	0.031±0.002	0.032±0.002
Control	0.379±0.008	0.715±0.025	1.406±0.041

Superoxide Anion Radical Scavenging Activity

Superoxide anion radical scavenging activity of the liquor prepared from the berries was investigated. The activities tended to increase with an increasing degree of the concentration of the liquor, although 0.1% liquor was not detected the activity at all (Table 3). The 1.0% liquor was the same activity as 0.01% BHT, and the 10%

liquor showed slightly lower activity than 1.0 mM BHA and α-tocopherol. On the contrary, the activity for 100% liquor was extremely high; it was higher than that of 1.0 mM trolox, but was not up to that of 5.0 mM ascorbic acid (Table 3). The IC₅₀ value was calculated about 9.9% as the concentration of the liquor (data not shown). Also, the TEAC against this radical was estimated to 7.47 x 10⁴

μmol TE/kg. It has been widely known that vitamin C and bilirubin showed greater potential to scavenge superoxide anion radicals. The characteristics may be associated with total vitamin C contained in the liquor, even though the remaining total vitamin C content of the liquor was considerable low.

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity of the liquor prepared from the berries was measured. The 0.1 and 1.0% liquor did not show the activity (Table 3). For 10% liquor, the activity was fairly low about 3.1% compared with 1.0 or 5.0 mM ascorbic acid. Even for 100% liquor the activity was not up to those of BHA, BHT, α-tocopherol, and trolox tested. The IC₅₀ value was calculated to about 497.4% as the concentration of the liquor (data not shown). The TEAC

against the radical was 6.86 x 10⁴ μmol TE/kg. Hydroxyl radical is scavenged by many species of compounds such as vitamin E, flavonoids, α- and β-carotene, cysteine, reduced glutathione, and so on. A great amount of total phenols, total flavonoids, and total anthocyanins were obtained in the liquor, nevertheless the scavenging activity against hydroxyl radicals were low.

DPPH Radical Scavenging Activity

DPPH radical scavenging activity of the liquor prepared from the berries was determined. As a result, each sample exhibited the activity and these activities increased with increasing the concentration of the liquor (Table 3). The liquor for 0.1% did not show the activity. The 1.0% liquor showed the same activity as 1.0 mM ascorbic acid and 0.01 mM BHA and BHT. The activity

Table 3. Superoxide anion radical, hydroxyl radical, and DPPH radical scavenging activities of the fruit liquor prepared from everbearing strawberry cultivar, ‘Summertiarar’ berries

Scavenging activity (%)			
Samples	Superoxide anion radical	Hydroxyl radical	DPPH radical
Liquor conc. (%)			
0.1	0.0	0.0	1.5±0.06
1.0	10.8±0.22	0.0	4.2±0.10
10	50.1±1.03	3.1±0.05	35.4±0.81
100	79.8±2.59	31.6±0.83	84.5±2.03
Ascorbic acid (mM)			
1.0	14.7±0.20	13.2±0.21	3.1±0.04*
5.0	89.9±5.31	17.6±0.71	34.1±2.01**
BHA (mM)			
0.01	29.3±0.52	59.1±0.78	5.5±0.04
0.1	36.4±0.91	93.3±1.39	17.5±0.36
1.0	51.9±1.36	95.2±1.44	72.7±3.58
BHT (mM)			
0.01	11.7±0.19	82.8±0.91	3.9±0.03
0.1	46.6±1.02	97.6±1.55	7.9±0.08
1.0	48.4±1.17	>100.0	31.7±0.76
α-Tocopherol (mM)			
1.0	52.6±4.18	67.6±4.34	87.6±2.75
Trolox (mM)			
0.01	46.4±0.98	81.5±0.63	0.1±0.01
0.1	58.1±1.12	91.8±1.17	17.9±0.20
1.0	76.1±1.89	>100.0	86.3±3.27

*0.1 mM ascorbic acid; **1.0 mM ascorbic acid

for 10% liquor was slightly higher than those of 5 mM ascorbic acid and 1.0 mM BHT. On the other hand, 100% liquor possessed remarkable scavenging activity as well as 1.0 mM α -tocopherol and trolox (Table 3). The IC_{50} value was calculated to about 14.2% as the concentration of the liquor (data not shown). The TEAC against the radical was $1.28 \times 10^7 \mu\text{mol TE/kg}$. The phenol compounds are reported to scavenge DPPH radicals, and its scavenging activity is correlated with the total phenol contents. As shown in Table 1, the liquor contained a great quantity of phenolic compounds. It seems that the higher scavenging activity of the liquor attributed to these phenolic compounds.

ACE Inhibitory Activity

ACE inhibitory activity of the liquor prepared from the berries was measured. Particularly, the liquor for

100% almost inhibited the activity about 94.1% (Table 4). The activity tended to increase the liquor concentration-dependently. High correlation was demonstrated between the concentration of the liquor and this activity, with $R^2 = 0.9953$ (data not shown). The IC_{50} value against ACE activity was measured and was calculated to 51.7%.

Hyaluronidase Inhibitory Activity

Hyaluronidase inhibitory activity of the liquor prepared from the berries was determined. As a result, it showed high dependence on the concentration of the liquor; the correlation coefficient against the activity was $R^2 = 0.9957$ (data not shown). The liquor for 100% perfectly inhibited this activity (Table 4). The IC_{50} value against hyaluronidase activity was calculated to 48.6%.

Table 4. ACE and hyaluronidase inhibitory activities of the fruit liquor prepared from everbearing strawberry cultivar, ‘Summertiar’ berries

Liquor conc. (%)	ACE inhibition (%)	Hyaluronidase inhibition (%)
0.1	0.0	3.3±0.4
1.0	4.0±0.5	8.6±0.9
10	15.9±1.1	11.9±1.2
100	94.1±2.3	99.4±1.7

In Japan, annual consumption of alcoholic drink has reached the peak in 1996, and then after that is moderately decreasing. Among them, those of fruit liquor and liqueur are increasing, although those of Japanese sake, beer, beer like drink (happo-shu), and brandy are decreasing. It has been said that sake is the best of all medicines, but it shows a habit-forming, dependence-producing, and intoxicating properties. It is known that a large quantity of drinking for many years is also associated with an increased risk of diseases such as coronary heart disease, hepatopathy, stroke, and cancer [19-21]. Ndiaye et al. [22] reported that the enhancement of endothelial formation of nitric oxide by red wine polyphenols involved in the protective effect of chronic intake of red wine on coronary diseases. Sarr et al. [23] found out the prevention of angiotensin II-induced hypertension and endothelial dysfunction in rats by red wine polyphenols. Lefevre et al. [24] discovered the improvement ischemia-induced neovascularization in ApoE-deficient mice by moderate consumption of red wine.

In recent years, the patients with allergy are increasing year by year [25]. There are atopic dermatitis,

alimentary allergy (food allergy), pollinosis (hay fever), asthma bronchiale and so on in allergic symptoms. Allergic responses can be divided into four classes from type I to IV based on the mechanism of immunological involvement [25]. In particular, type I allergy is an immediate-type allergy. Hyaluronidase exists in some organs and in fluids of the body. This enzyme is released from mast cells with histamine as chemical mediators. Subsequently, it hydrolyzes hyaluronic acid in the extracellular matrix of connective tissue. Hyaluronidase is involved in not only the action that induces inflammation, but in the permeability of the vascular system [25]. Thus, hyaluronidase is responsible for the process causing allergic reactions and inflammatory. It is well known that hyaluronidase inhibitory activity and the suppression of histamine release have strong positive correlation [25]. Therefore, the effects on hyaluronidase inhibition are used to evaluate anti-allergic activity. Fruits contain a host of active phytochemicals. Among them, strawberries are abundant in natural antioxidants as ascorbic acid, phenolic acids, flavonoids, and anthocyanins. These anti-oxidants express a high level of anti-oxidative activity and radical scavenging activity, although these contents and functionalities considerably

affect by soil and whether conditions, planting site, and the degree of ripeness [26]. It is desired to positively eat for maintenance of a healthy biological system and for prevention of allergy and life-style related disease such as cancers, cardiovascular diseases, and neurological damage. The extensive research on the anti-oxidant and anti-allergic activities and phytochemical compositions of food materials or diets are progressing [27,28]. In the present study, we have developed fruit liquor made from everbearing strawberry cultivar, ‘Summertiarar’ berries. It will be capable of satisfying high nutrition and function, excellent taste, sweet-smelling flavor, and new palatability.

Conclusion

The fruit liquor prepared from everbearing strawberry cultivar, ‘Summertiarar’ berries showed not only an attractive color but also positive inference to fairly high anti-oxidative activity, free radical scavenging activity, anti-hypertensive activity, and anti-allergic activity for the major phytochemicals such as phenolic compounds, flavonoids, and anthocyanins. That is, the liquor can be used as protection or prevention for allergy and life-style related disease to over-production of free radicals.

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