Application of *Sargassum fusiforme* (Harv.) Setch. Extract in Cigarettes

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Abstract [**Objectives**] This study was conducted to investigate the application of *Sargassum fusiforme* (Harv.) Setch. in cigarettes. [**Methods**] Tobacco-specific nitrosamines in the smoke of cigarettes added with ethanol extract of *S. fusiforme* were determined, and the compounds related to the aroma of *S. fusiforme* were identified by flavor-smelling experiment. [**Results**] With the addition of the ethanol extract of *S. fusiforme*, the decrease in the total amount of four tobacco-specific nitrosamines in mainstream cigarette smoke reached 16.42%. The results of the flavor-smelling experiment showed that the aroma of *S. fusiforme* might be related to (R)-5,6,7,7A-tetrahydro-4,4,7A-trimethyl-2 (4H) -benzofuranone, glycerol, ethyl palmitate, methyl palmitate, ethyl linoleate, methyl (Z, Z, Z) -9,12,15-octadecatrienoate, ethyl (Z, Z, Z) -9,12,15-octadecatrienoate, phytol, and tetradecanoic acid. [**Conclusions**] The ethanol extract of *S. fusiforme* has the potential function of reducing the content of tobacco-specific nitrosamines in smoke and improving the taste of cigarettes.

Key words *Sargassum fusiforme* (Harv.) Setch.; Tobacco-specific nitrosamines (TSNAs); Flavor smelling

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Sargassum fusiforme (Harv.) Setch. is a plant in *Sargassum* of Sargassaceae in Phaeophyta, commonly known as Haidamai, Lujiaojian and Haicaiya, *etc.* *S. fusiforme* mainly grows in the warm temperate waters of the western North Pacific Ocean, and is widely distributed in the shallow sea areas of Liaoning, Shandong, Zhejiang, Fujian and other places in China[1-2].

S. fusiforme is a kind of edible and medicinal plant. In recent years, its nutritional value and medical functions have been gradually recognized by people, but most of the studies mainly focus on the research of polysaccharides[3-6], polyphenols[7-10] and inorganic elements[11-12], especially in the aspects of anti-tumor, anti-oxidation, hypoglycemic and anti-aging activities and immune activity regulation of polysaccharides[3]. However, there have been no reports on the effect of ethanol extract from *S. fusiforme* on tobacco-specific nitrosamines. Moreover, *S. fusiforme* has a special aroma, and there have been no reports on which compounds are its main aroma components.

In this study, tobacco-specific nitrosamines in the smoke of cigarettes added with extract from *S. fusiforme* were investigated, aiming to provide reference for their application in cigarettes, and compounds related to the aroma of *S. fusiforme* were identified through flavor-smelling experiment (post-column sensory evaluation, GC/MS determination).

Materials and Methods

Materials and instruments

Reference cigarettes; blended type cigarettes of a certain domestic brand; virginia type cigarettes of a certain domestic brand; Cambridge filter, provided by Zhengzhou Tobacco Research Institute of China Tobacco Corporation; *S. fusiforme*, native to Zhejiang Province.

Anhydrous ethanol (AR), Laiyang Shuangshuang Chemical Co., Ltd; alkaline alumina (200 - 300 mesh), produced by Sinopharm Chemical Reagent Co., Ltd.; anhydrous sodium sulfate (analytically pure), Tianjin Kaitong Chemical Reagent Co., Ltd.

CIJECTOR essence injection machine, BURGHART company, Germany; JJZ-20 linear automatic smoking machine, Wuxi Tongchen Technology Development Company; KC automation automatic cigarette picker, Mettler Toledo; MO-2 temperature-regulating electric heating sleeve, Xinxing Electric Appliance Factory, Huanghua City, Hebei Province; GC-TEA, Thermo Finnigan; HP 6890N/5975GC/MS instrument, Agilent Technologies Inc., USA; ODP olfactory detection channel, GERSTEL; RE52-99 rotary evaporator, Shanghai Yarong Biochemical Instrument Factory; CP224S electronic balance (sensitive quality: 0.000 1 g), Beijing Sartorius Scientific Instruments (Beijing) Co., Ltd.

Experimental methods

Sample preparation The original seaweed of *S. fusiforme* was rinsed with tap water three times to remove impurities. The cleaned *S. fusiforme* was air-dried first and then oven-dried at 45 ℃, and crushed with a grinder. The obtained powder was sieved with a 45-mesh sieve[13].

Next, 10 g of *S. fusiforme* powder was weighed and added with 150 ml of anhydrous ethanol. The sample was extracted with reflux at 95 ℃ for 5 h, and the extract was concentrated under reduced pressure at 40 ℃ to 10 ml.

Analysis of tobacco-specific nitrosamines in the smoke of cigarettes The above concentrated solution was prepared into three concentrations of 0.2, 0.5 and 1 g/ml. Qualified cigarettes after balancing were selected and injected with an essence injection machine. Each of the above three concentrations of extract solution was injected to cigarettes according to 20 μl per cigarette. The blank was experimental cigarettes injected with the same

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amount of anhydrous ethanol.

The injected cigarettes were equilibrated in a chamber with constant temperature and humidity for 48 h, and the experimental cigarettes were smoked by a linear smoking machine. The smoking quantity of 10 cigarettes was taken as one sample, and 20 cigarettes were smoked as two parallel samples for each concentration. The filters were processed and analyzed by GC-TEA.

Flavor-smelling experiment The above concentrated liquid was filtered through a 0.45 μm organic phase filter membrane for GC/MS analysis.

GC/MS operating parameters The GC/MS analysis conditions[13] used in this experiment are shown in Table 1.

Table 1 GC/MS parameters in flavor-smelling experiment

Parameters	Analysis conditions
Chromatographic column	HP-INNOWAX (30 m × 0.25 mmi.d. × 0.25 μmd.f.) capillary column
Heating program	60 ℃ (0 min) $\xrightarrow{4\ ℃/min}$ 240 ℃ (40 min)
Carrier gas	He, flow rate 1 ml/min
Injector temperature	280 ℃
Sampling mode	Constant current mode
Sample size	2 μl
Split ratio	5 : 1
Transmission line temperature	250 ℃
ODP transmission line temperature	150 ℃
Solvent delay	6.0 min
Ionization mode	EI
Ionization energy	70 ev
Ion source temperature	230 ℃
Quadrupole temperature	150 ℃
Mass scan range	30 – 550 amu

Post-column sensory evaluation The flavor-smelling device was locally modified from GC/MS. The modified device made the capillary tube that originally led to a mass spectrometer allow a portion of the sample to enter the mass spectrometer and a portion to enter a flavor-smelling end (extending outside the chromatographic furnace) through a three-way valve, thereby realizing simultaneous flavor smelling while chromatographic peaks were flowing out. A total ion chromatogram of the sample was first obtained, and then, the flavors were smelled under the same conditions. The retention time, odor characteristics and intensity of the chromatographic effluent were recorded on the total ion chromatogram.

Results and Analysis

Analysis of tobacco-specific nitrosamines in mainstream cigarette smoke

The analysis results of nitrosamines in the smoke of cigarettes added with *S. fusiforme* extract are shown in Table 2.

From Table 2, it can be seen that the addition amounts were all 20 μl. With the concentration of *S. fusiforme* extract increasing, the contents of the four tobacco-specific nitrosamines in the mainstream cigarette smoke showed a decreasing trend. The total amounts of the four tobacco specific nitrosamines decreased by 5.76%, 11.93%, and 16.42%, respectively. Furthermore, it could be determined that the anhydrous ethanol heat reflux extract of *S. fusiforme* had the effect of reducing tobacco-specific nitrosamines in mainstream smoke.

Table 2 Results of tobacco-specific nitrosamines (TSNAs) ng/cig.

Nitrosamine	1#	2#	3#	0#
NNN	258.75	237.59	229.34	268.92
NAT	216.55	206.38	193.75	234.24
NAB	17.15	15.75	13.22	19.94
NNK	23.50	22.45	21.27	24.39
TSNAs	515.95	482.17	457.58	547.49

1#, 2# and 3# experimental cigarettes were added with 0.2, 0.5 and 1 g/ml of *S. fusiforme* extract, respectively. 0# referred to blank cigarettes added with the same amount of anhydrous ethanol.

Flavor-smelling experiment

It was preliminarily judged by searching chromatographic peaks corresponding to the retention time of characteristic aroma that the aroma of *S. fusiforme* might be related to (R)-5,6,7,7A-tetrahydro-4,4,7A-trimethyl-2 (4H) - benzofuranone, glycerol, ethyl palmitate, methyl palmitate, ethyl linoleate, methyl (Z, Z, Z) -9,12,15-octadecatrienoate, ethyl (Z, Z, Z) -9,12,15-octadecatrienoate, phytol, and tetradecanoic acid.

Conclusions and Discussion

The analysis results of nitrosamines in the smoke of cigarettes added with *S. fusiforme* extract showed that with the concentration of *S. fusiforme* extract increasing, the contents of four tobacco-specific nitrosamines in mainstream cigarette smoke decreased, and the decrease in the total amount of the four nitrosamines reached 16.42%. The flavor-smelling experiment showed that the substances related to the aroma of *S. fusiforme* might be several moderately-volatile compounds. The above experimental results suggest that the ethanol extract of *S. fusiforme* has the potential effect of reducing the content of tobacco-specific nitrosamines in cigarette smoke and improving the taste of cigarettes.

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construction atmosphere that environmental protection is everyone's responsibility.

Conclusions

Effluent outfalls are a bridge connecting land and water, and they are important point sources for land-based pollutants entering natural water bodies. We should do a good job in land and water planning, and complete investigation, tracing, classification and regulation, and only when the supervision and management of effluent outfalls are in place can we promote the continuous improvement of the ecological environment quality of water bodies in basins.

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