

Fatty Acid-rich Trout Bone Soup Demonstrates Potential to Mask Bitterness of Food and Chinese Medicine

Ying Han¹, Zhizhou Zhang^{1,2,3,*}

¹School of Chemistry and Chemical Engineering, Harbin Institute of Technology, Harbin 150001, China

²School of Marine Science and Technology, Harbin Institute of Technology, Weihai 264209, China

³Marine Antifouling Engineering Technology Center of Shandong Province, Weihai, 264209, China

*Corresponding author: zhangzzbiox@hitwh.edu.cn

Received January 06, 2018; Revised February 24, 2019; Accepted March 16, 2019

Abstract Many Chinese medicine soups are very bitter and hard for children to drink. Some direct and simple bitter-masking approaches in daily food and Chinese medicine are always of great need. In this study, a rainbow trout bone soup-based efficient de-bittering method was discovered by serendipity when the authors tried to attenuate the bitterness of the bone soups made from cod and salmon. The bitterness of one of the bitterest daily food, bitter melon, was completely removed by the trout bone extract. The bitterness of the soups of *Coptis chinensis* and kuh-seng, two of the extreme bitter Chinese medicine, were also eliminated with high efficiency. Bone soups made of cod and salmon didn't have de-bittering function. Fatty acids composition analysis was performed with the bone soups made from cod, salmon and trout, and the results clearly demonstrated that the trout soup has much higher concentrations of myristic acid, palmitic acid, stearic acid, cis-9-hexadecenoic acid, and cis-9-octadecenoic acid. The combination of the five pure fatty acids did display the capacity to almost remove the bitterness of all above tested soup materials, either food or Chinese medicine. But other combinations (less than five components) cannot achieve the same level of de-bittering effect.

Keywords: fatty acids, bitterness, masking, trout, bone soup

Cite This Article: Ying Han, and Zhizhou Zhang, "Fatty Acid-rich Trout Bone Soup Demonstrates Potential to Mask Bitterness of Food and Chinese Medicine." *Journal of Food and Nutrition Research*, vol. 7, no. 3 (2019): 224-230. doi: 10.12691/jfnr-7-3-7.

1. Introduction

In traditional Chinese medicine, most drug materials are characterized with four-Qi and five-taste, in which four-Qi means chill, hot, warm or cool, and five-taste means sour, sweet, bitter, spicy, or salty [1]. Japanese scientists often classify basic tastes in food or drugs as sweet, salty, sour, bitter, and umami [2]. In particular, bitter taste is generally unfavorable and tends to be aversive to most people. Bitter-tasting foods are hard to be accepted in a long term except some slightly bitter ones such as beer, wine and coffee. For pharmaceutical compounds with medical benefits, bitterness taste is frequently encountered for patients, and it often reduce compliance with a treatment regimen especially for children. Therefore, bitterness masking technology is considered important in both food processing and pharmaceutical manufacturing.

In the BitterDB database [3,4], most of listed about 700 bitter substances are small molecules of various chemical categories. One type of the common bitterness in fish is often derived from cholic acid or bitter amino acids (histidine, leucine, isoleucine, methionine, etc.). Fish

tissue waste (skin, tail, bones, fishbone, fins, visceral, etc.) is often mixed with bile or bile acid and other bitter substances, resulting in a taste of obvious bitterness. In theory, it is possible to eliminate the cholic acid or bitter amino acids through specific biotransformation reactions. Many kinds of food will form a bitter taste after the hydrolysis, seriously affecting oral sensation. The bitterness of the hydrolates is partially due to the release of bitter peptides. At present, there are at least over one hundred known bitter peptides [5-14].

Decent progresses have been achieved in bitterness-masking technology development. Various protein hydrolysates with bitter tastes were found from 1950s [15]. Since then, researchers have proposed de-bittering methods such as activated-carbon adsorption, chloroform extraction, ethanol extraction, isoelectric point precipitation, hydrophobic interaction chromatography, and other bitter masking approaches [16-21]. Encapsulation or molecular binding plus other molecular levels of studies brought more specific approaches to mask bitterness. For example, coating and encapsulating are frequently used in the biomedical industry to mask the bitterness of drugs [22,23]. The formation of inclusion complexes between various target substances and cyclodextrin can be

employed to mask bitterness [24]. Phosphatidic acid and its lipoprotein derivative have been reported to deter the bitterness of quinine [25]. Bitterness can also be masked by introducing antagonists of bitter taste receptors (T2Rs) into food or drug coatings [26]. Besides, amino acid derivatives as low-molecular-weight bitterness-masking compounds are worthy considering in some cases [27]. Zinc chemicals were also found to mask the bitterness of quinine, tetralone, and denatonium benzoate [28]. For both food processing and drug manufacturing, bitterness-masking compounds or approaches must be harmless and biocompatible, so identifying safe bitterness-masking agents originating from foods is a desirable objective. More bitterness masking techniques derived from natural food or herbs with low cost are highly expected.

In the beginning of this study, the authors tried to attenuate the bitterness of one local soup product made from cod and salmon bones by using a bitter peptide digestion approach. The origin of the product's heavy bitterness was not very clear because cod and salmon bone soups themselves are only slightly bitter. The authors guessed that the product's bitterness might come from some released bitter peptides from cod and salmon proteins (and proteins of other origins in the product). After a series of experiments, it was found that bitter peptide digestion approach was only able to partially eliminate the product's heavy bitterness (data not shown). However, we found, by serendipity, that the trout bone soup can completely eliminate the product's bitterness. Then we tested whether the trout bone soup could mask bitterness from different typical bitter food and Chinese medicine. Satisfactory de-bittering effects were steadily observed, and the main fatty acids components were analyzed and compared among cod, salmon and trout bone soups. Key de-bittering or bitterness-masking fatty acids components in the trout bone soup were proposed and confirmed by a series of experiments. This study revealed the potential for the trout bone soup to be a low-cost de-bittering reagent that may be widely implicated in food processing and Chinese medicine production.

2. Materials and Methods

2.1. Bone Soups

All kinds of fresh fish bone were provided by The YueYi Biotech Ltd, Rizhao, Shandong, China. Each fish bone still has some fish meat around it, with about 15-20% W/W fish meat of the whole weight. Each bone was cut into pieces less than the size of 2cm×5cm×1cm. Cod (*Gadus macrocephalus*) fish bone was equally mixed with water (W/W), smashed with a laboratory stirrer (Insinkerator E200, JingDong, China) to get a cod bone suspension. The suspension was further equally mixed with water (V/V) and then heated to the boiling state for 20 min to get the cod bone soup. Trout (*Oncorhynchus mykiss*) and salmon (*Oncorhynchus masou*) bone soups were made in the same way.

2.2. Plants Bitter Soup

Coptis chinensis [29] and kuh-seng, two highly bitter types of Chinese medicine, were purchased in the

YanXiTang Pharmacy. Bitter melon (*Momordica charantia*) [30] was bought in Jiayiayue supermarket, Weihai, China. Each of the three above substances was cut into small pieces no larger than the size of 1cm×0.5cm×0.5cm. The initial concentration of *Coptis chinensis* soup, kuh-seng soup and bitter melon soup were 0.025g/ml, 0.025g/ml and 0.5g/ml, respectively, heated to the boiling state for 20 min to get the soup, and the three above soups were diluted 50, 10 and 10 times respectively in the sensory evaluation tests.

2.3. General Fat and Fatty Acids Analysis

Cod, trout and salmon bone soups were freshly prepared, each with three replicates, and were immediately subjected to lipid extraction according to the protocol of GBT 5009.6-2003. Well suspended and homogenated fish bone samples (50%V/V for cod, trout and salmon) 10ml each were mixed with 20g clean sea sand and dried upon the boiling water, then further dried at 105°C in an oven. The dried powder was further homogenated and put onto the folded filter-paper before loaded into Soxhlet's extractive tube. After extraction with ether for 10hr, the general fat biomass was dried completely to weight the amount of free fat biomass.

An aliquot of the total lipid extract was trans-methylated to produce fatty acid methyl esters according to the protocol of GB/T 17376-2008. Briefly, 250mg fat biomass in a 50ml flask was mixed with 7ml boron trifluoride (15% W/V in methanol). The flask was assembled with a condenser to boil the solution for 30min. Around 8ml isooctane was added into the boiling solution, then followed by 20ml saturated NaCl solution. Detach the flask and vortex vigorously for 15s. Keep adding saturated NaCl solution till the top of the flask. Wait 1hr for separation of solution layers. Take 1.5ml the upper isooctane supernatant and mix with some anhydrous sodium sulfate to get rid of residual water. Then the sample can be loaded for GC capillary analysis according to the protocol of GBT 17377-2008. The supernatant (1μL) was analyzed using GC-FID (Agilent 7890A) gas (Nitrogen) chromatography. The analytes were separated on an HP-88 fused silica capillary column (100 m × 0.25 mm, 0.20μm film thickness, Agilent Technologies, Santa Clara, CA, USA). The split ratio was 100:1 with a 1.25 mL/min flow rate. The injector and transfer line temperature were set at 250°C and 240°C, respectively. The oven temperature followed a program of 100°C for initial 13min, a ramp of 10°C/min to 180°C, holding at 6 min, another ramp of 1°C/min to 200°C and holding at 20 min; then the last ramp of 4°C/min to 230°C and holding at 10.5 min. The analytes were assigned by comparing retention times with authentic standards. The quantification was performed using calibration curves composed by plotting peak area ratios of the analyte to the internal standard against analyte concentrations. The general fat and fatty acids compositions were measured with the facilities in Merieux Nutrisciences, Sino Analytical (Qingdao) Ltd, China. Pure fatty acids were purchased from TCI (Shanghai) Development Co., Ltd., as (A) Myristic acid (Cat.Num M0476; CAS number 544-63-8), (B) Palmitic acid (Cat.Num P0002; CAS number 57-10-3), (C) Stearic acid (Cat.Num S0163; CAS number 57-11-4),

(D) cis-9-Hexadecenoic acid (Cat.Num H0072; CAS number 373-49-9) and (E) cis-9-Octadecenoic acid (Cat.Num O0011; CAS number 112-80-1).

2.4. Sensory Evaluation

The bitterness of each soup sample was estimated (averaged with three to four repetitions) by the quinine-sulfate (purchased from Sangon, Shanghai) equivalent test. The bitterness of a sample was compared with a series of quinine-sulfate dilutions using a sensory panel composed of ten trained students. The panelists were trained three times with standard quinine-sulfate solutions set at several concentrations [31] near the threshold levels. The panelists rinsed their mouths thoroughly with water and then keep 2ml soup in the mouth for 10 sec before evaluation. The degree of bitterness was rated as not (-), slightly (+), distinctly (++), moderately (+++), very (++++), and extremely bitter (+++++). It is very important to locate the concentration(s) of the level 'extremely bitter (+++++)' for the standard, because any higher concentration than this level will produce the same sensory result and bring significant errors. They rated the bitterness intensity on the six-level scale while the sample was swished in the mouth. Participants then expectorated the sample and rinsed with filtered water as needed. An inter-stimulus interval of at least 2 min between samples was confirmed. The panel repeated the testing of the standard quinine-sulfate solutions set and adjusted the solution concentrations until all agreed with the rated results of bitterness. Each adjusted degree represented a quinine-sulfate concentration of 1.6, 2.4, 3.2, 4.0, 4.8 and 5.6×10^{-5} mol L⁻¹, respectively [32]. Most kinds of the soups were then diluted in a way that their starting bitterness levels were not higher than (but as close as possible to) the level (++++), so the de-bittering effects can be compared more accurately. For each test, the highest and lowest scores from the ten ones accessed by ten panel members were deleted and the left eight scores were practically averaged as the final score.

3. Results

3.1 Cod and Salmon Bone Soups Had Little De-bittering Effects

The potential de-bittering effects of cod and salmon soups were checked out in the beginning. First, *Coptis chinensis*, kuh-seng, bitter melon, cod bone and salmon

bone were used to make respective soups as follow: 0.15g *Coptis chinensis* +330ml water, 0.75g kuh-seng +330ml water, 1.5g bitter melon +330ml water, 200g cod bone+350ml water, 200g salmon bone+350ml water, were well homogenated and mildly boiled for 20min to get *Coptis chinensis* soup 300ml (a), kuh-seng soup 300ml (b), bitter melon soup 300ml (c), cod bone soup 300ml (d), and salmon bone soup 300ml (e), respectively. Second, 100ml bone soup (d or e) plus 100 ml bitter substances (a, b or c) was further mildly boiled for 20 min and cooled down for 1 hour to room temperature before the sensory evaluation. The evaluation results were listed in Table 1, indicating that cod and salmon bone soups had no substantial de-bittering effects on the three typical bitter food or Chinese medicine.

Table 1. Cod and salmon bone soups had no de-bittering effects

	0hr	1hr	6hr	20hr
a	++++	++++	++++	++++
b	++++	++++	++++	++++
c	++++	++++	++++	++++
d+a	++++	+++	+++	+++
d+b	++++	+++	+++	+++
d+c	++++	+++	+++	+++
e+a	++++	+++	+++	+++
e+b	++++	+++	+++	++
e+c	++++	+++	++	++

Note: a, b, c, d and e represent 300ml *Coptis chinensis* soup, 300ml kuh-seng soup, 300ml bitter melon soup, 300ml cod bone soup, and 300ml salmon bone soup, respectively. The two soup combination (200ml) was made by 100ml bone soup (d or e) plus 100 ml bone soup (a, b or c).

Actually, cod and salmon bone soups are not only unable to debitter, but they themselves, either alone or mixed, had slight bitterness (data not shown) which can be eliminated completely by the trout bone soup.

3.2. De-bittering Effects of Trout Extract on Three Typical Bitter Soups

Freshly made 100ml *Coptis chinensis* soup was mixed with 100ml trout bone soup plus 200ml water. After boiled for 20 min and stayed for 1 hour at the room temperature, the bitter taste of the *Coptis chinensis* soup was evaluated. It was found that the bitter taste was completely eliminated only after 1 hour treatment by the trout bone soup, with the evaluation score from (++++), or (++++), to (-). The experiment repeated three times and got the similar results. Besides, the very similar results were obtained for kuh-seng and bitter mellon soups (Table 2).

Table 2. De-bittering effect of trout extract on three typical bitter soups

	bitter melon soup(5mg/ml)	kuh-seng soup(2.5mg/ml)	coptis chinensis soup(0.5mg/ml)
Initial bitterness	+++	++++	++++
	++++	++++	++++
	++++	++++	++++
Bitterness(1hr)	-	-	-
	-	-	-
	-	-	-
	-	-	-
Bitterness (24hr)	-	-	-
	-	-	-

3.3. Trout Bone Soup Composed of More Fat and Fatty Acids than Cod and Salmon Soups

Both unsaturated and saturated fatty acids were found to have bitterness-masking effects or functions of modulating human taste responses [33-39]. But only saturated fatty acids were reported in some details for masking bitterness. In this study, the authors also performed saturated fatty acids composition analysis for three types of bone soups. The trout bone soup was found rich in both the general fat and fatty acids as shown in Table 3 (also in Table 1s), consistent with the eye observations on these soups. Among forty different fatty acids (Table 3), 19 kinds were not detected, and 7 kinds (myristic acid, palmitic acid, stearic acid, cis-9-hexadecenoic acid, cis-9-octadecenoic acid, cis-11-Eicosenoic acid and all cis-11,14,17-Eicosatrienoic acid) were all detected in all three soups. Especially, the concentrations of the 5 kinds of fatty acids in the trout bone soup were all significantly higher than cod and

salmon soups, strongly suggesting that the combinations of the five kinds of fatty acids be responsible for the excellent bitterness-masking capacity.

3.4. De-bittering Effects of Pure Fatty Acids on Three Typical Bitter Soups

An 15ml sterile tube was used, to which was added 5 ml of ddH₂O, 2.5 ml of fresh *Coptis chinensis* soup, and 2.5 ml of a fatty acid mixture (the combination of five fatty acids used were mixed according to Table 1, the final molar concentration is the same as in the trout bone soup). After boiled for 20 min and stayed for 1hour at the room temperature, the bitter taste was evaluated. After 6 hours and 24 hours at room temperature, bitterness was evaluated again. According to the experimental control group, bitterness decreased from (++++) to (+ or -) as in Table 4. Similar results were observed for bitter melon soup (Supplementary Table 2s) and kuh-seng soup (Supplementary Table 3s).

Table 3. Fatty acid concentrations in three types of bone samples

Fatty acid	Average concentration (g kg ⁻¹)		
	cod	trout	salmon
1 Heneicosanoic acid	ND	ND	ND
2 Tricosanoic acid	ND	ND	ND
3 trans-9-Octadecenoic acid	ND	ND	ND
4 trans-trans-9,12-Octadecadienoic acid	ND	ND	ND
5 Butyric acid	ND	ND	ND
6 Caproic acid	ND	ND	ND
7 Caprylic acid	ND	ND	ND
8 Capric acid	ND	ND	ND
9 Undecanoic acid	ND	ND	ND
10 Lauric acid	ND	ND	ND
11 Tridecanoic acid	ND	ND	ND
12 Myristic acid	0.0028(α)	0.064(β)	0.017(α)
13 Pentadecanoic acid	ND	0.0046	ND
14 Palmitic acid	0.015(α)	0.254(β)	0.034(α)
15 Margaric acid	ND	0.0042	ND
16 Stearic acid	0.0026(α)	0.06(β)	0.0078(α)
17 Arachidic acid	ND	ND	ND
18 Behenic acid	ND	ND	ND
19 Lignoceric acid	ND	ND	ND
20 cis-9-Tetradecenoic acid	ND	ND	ND
21 cis-10-Pentadecenoic acid	ND	ND	ND
22 cis-9-Hexadecenoic acid	0.0022(α)	0.06(β)	0.0142(α)
23 cis-10-Heptadecenoic acid	ND	ND	ND
24 cis-11-Octadecenoic acid	ND	0.05	0.0056
25 cis-9-Octadecenoic acid	0.0146(α)	0.668(β)	0.034(α)
26 cis-11-Eicosenoic acid	0.0046(α)	0.007(α)	0.022(α)
27 cis-13-Docosenoic acid	ND	0.0084	0.0084
28 cis-15-Tetracosenoic acid	ND	0.0058	0.0022
29 all cis-9,12,15-Octadecatrienoic acid	ND	0.0074	ND
30 all cis-11,14-Eicosadienoic acid	ND	0.004	ND
31 all cis-11,14,17-Eicosatrienoic acid	0.0015(α)	0.042(α)	0.024(α)
32 all cis-8,11,14,17-Eicosatetraenoic acid	ND	ND	ND
33 all cis-5,8,11,14,17-Eicosapentaenoic acid	ND	ND	0.0036
34 all cis-7,10,13,16,19-Docosapentaenoic acid	ND	0.0068	ND
35 all cis-4,7,10,13,16,19-Docosahexaenoic acid	ND	0.0032	0.0022
36 cis,cis-9,12-Octadecadienoic acid	ND	0.062	0.0028
37 all cis-6,9,12-Octadecatrienoic acid	ND	0.0062	ND
38 all cis-8,11,14-Eicosatrienoic acid	ND	0.003	ND
39 all cis-5,8,11,14-Eicosateraenoic acid	ND	ND	ND
40 all cis-13,16-Docosadienoic acid	ND	0.0024	ND
41 Total fat	0.06(α)	1.54(β)	0.22(γ)

Note: Significant differences among the three groups in a row using Multiple comparisons (Tukey's test); Values with different letters (α , β , γ) in a row indicate that the average values are significantly different from each other ($P < 0.01$); ND: not detected; The original data for the three replicates were provided in Supplementary Table 1s.

Table 4. De-bittering effects of fatty acids on *Coptis chinensis* soup

	1hr	6hr	24hr		1hr	6hr	24hr		1hr	6hr	24hr		1hr	6hr	24hr
0	++++	++++	++++	AB	+-	+	+	ABC	++	++	+++	ABCD	++	+-	++
A	++	+++	+-	AC	+-	-	-	ABD	++	+-	++	ABCE	+-	+-	+-
B	++	++	+++	AD	+++	++	++	ABE	+-	++	++	ACDE	+++	+++	++
C	+++	+-	+-	AE	+-	++	+-	ACD	-	+-	+-	ABDE	+++	+++	++
D	+-	+-	+++	BC	+++	++	+++	ACE	+-	+++	+-	BCDE	+++	+-	-
E	+++	+-	+++	BD	+++	+-	+++	BCD	++	+++	++	ABCDE	-	-	-
				BE	+++	+-	++	BCE	++	+++	++				
				CD	+++	+-	++	BDE	+-	+-	+++				
				CE	+-	++	+-	CDE	+-	++	+-				
				DE	+++	+-	+++	ADE	+++	+++	++				

4. Discussion

The bitter taste of cod fish was previously studied using conventional methods [40] and the authors found that Flavourzyme® did not reduce the bitterness well while the use of butanol and cholestyramine resin separately or in combination reduced the bitter taste from fish protein hydrolysates to levels hardly discernible in 1% concentration. In this study, the bitter taste of cod fish bone soup was masked completely by trout bone soup, suggesting that the latter has some substance to interact with bitter taste receptors or scavenging bitter substances themselves. There are several types of naturally occurred substances that bind with bitter taste receptors, such as some lipoproteins, cyclodextrin and cyclofructan [41,42,43]. Among them, lipoproteins may be of a particular interest for this study because trout bone soup had a much higher lipid compositions as observed than other two kinds of bone soup, though the authors were still not sure whether lipoproteins [33] also play important roles for trout soup to mask bitter taste. Table 4 clearly indicated that the trout bone soup had higher concentrations of about 20 different fatty acids than cod and salmon soups. According to several reports, some fatty acids can be used to efficiently mask bitter taste [34,35,36,44]. Tomotake et al [34] and Fujita et al [35] found that fatty acid salts such as sodium stearate, palmitate, and laurate in relatively high concentrations of about 1% were able to reduce the bitter taste of a 100-ppm quinine solution significantly, while there was 2.54% palmitic acids in the trout soup (Table 4) and may contribute to mask the bitter taste. Ryousuke Homma et al [37] found that a 0.5mM mixture of palmitic acid, stearic acid and myristic acid significantly reduced the bitterness of quinine hydrochloride, while the concentration of the above mixture in the trout soup (99mM, 21mM and 28mM for the three types of fatty acids respectively) was about 40 times larger than that of the above mixture used for the sensory test, which shall be adequate to demonstrate the bitterness-masking effects. The literature strongly supports that fatty acids interwine with bitterness-sensing signaling pathway [38,39,45,46] and they did play an important role for trout soup to mask the bitter taste for both food and Chinese medicine.

It seems helpful to test whether concentrated cod or salmon bone soups can have similar de-bittering effect as trout bone soup in a dose-dependent manner. Because the concentrations of many fatty acids in the trout soup are 10-50 fold higher than the cod and salmon bone soups, it is not practical to concentrate cod and salmon bone soups to the similar fatty acid concentrations as in the trout bone

soup. Actually, 5 and 10 fold concentrated cod and salmon bone soups had no comparable de-bittering effects as the trout bone soup (data not shown).

Many Chinese medicine soups are very bitter and hard for children to drink. Some direct and simple bitter-masking approaches in daily food and Chinese medicine are always of great need. In this study, a rainbow trout bone soup-based efficient de-bittering method was accidentally discovered when the authors tried to attenuate the bitterness of the bone soups made from cod and salmon. Moreover, the total bitter taste of one typical food and two typical Chinese medicines was surprisingly removed simply by addition of the trout bone extract [47]. The strong de-bittering power of the trout bone soup was further confirmed by five pure fatty acids (myristic acid, palmitic acid, stearic acid, cis-9-hexadecenoic acid, and cis-9-octadecenoic acid) that had much higher concentrations in the trout soup than cod and salmon bone soups, though other combinations (less than five components) cannot achieve the same level of de-bittering effect. Potential molecular mechanisms under the five fatty acids combination are to be investigated in the future.

Acknowledgements

This study was supported by the following funds: NSFC (No.31071170); the Key research and development plan of Shandong Province (2016GSF115022); 2018 Weihai Scientific Innovation Project and the Natural Science Foundation of Shandong Province (ZR2018MC002).

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Supplemental Information

Table 1s. Original data of some fatty acid concentrations in three replicates of bone soup samples

		cod			trout			salmon					
		1	2	3	1	2	3	1	2	3			
Myristic acid	A	0.0028	0.0030	0.0023	α	0.064	0.061	0.070	β	0.017	0.019	0.016	α
Palmitic acid	B	0.015	0.019	0.0139	α	0.254	0.258	0.249	β	0.034	0.031	0.039	α
Stearic acid	C	0.0026	0.0031	0.0022	α	0.06	0.065	0.049	β	0.0078	0.0082	0.0073	α
cis-9-Hexadecenoic acid	D	0.0022	0.0025	0.0020	α	0.06	0.063	0.056	β	0.0142	0.0152	0.0166	α
cis-9-Octadecenoic acid	E	0.0146	0.0160	0.0138	α	0.668	0.675	0.661	β	0.034	0.038	0.029	α
cis-11-Eicosenoic acid		0.0046	0.0050	0.0041	α	0.007	0.0074	0.0066	α	0.022	0.021	0.022	α
all cis-11,14, 17 -Eicosatrienoic acid		0.0015	0.0018	0.0012	α	0.042	0.041	0.046	α	0.024	0.027	0.019	α
Total fat		0.06	0.066	0.051	α	1.54	1.58	1.47	β	0.22	0.24	0.21	γ

Note: Significant differences among the three groups in a row using Multiple comparisons (Tukey test); Values with different letters (α, β, γ) in a row indicate that the average values are significantly different from each other ($P < 0.01$);

Table 2s. De-bittering effects of fatty acids on bitter melon soup

	1hr	6hr	24hr		1hr	6hr	24hr		1hr	6hr	24hr		1hr	6hr	24hr
0	++++	++++	++++	AB	+-	+-	+++	ABC	+	++	+-	ABCD	+-	+-	+-
A	+-	+-	+++	AC	++	++	+-	ABD	++	++	+-	ABCE	+-	+-	++
B	++	+-	+-	AD	+-	+++	++	ABE	+++	+-	++	ACDE	+-	++	+-
C	++	++	++	AE	++	++	++	ACD	++	++	+-	ABDE	++	+-	+-
D	+-	+-	++	BC	+++	+-	+-	ACE	+-	++	++	BCDE	++	+-	++
E	++	++	++	BD	+-	+-	+++	BCD	+++	+-	+-	ABCDE	-	+	+
				BE	+++	+++	+++	BCE	++	+++	++				
				CD	++	+-	++	BDE	+-	+++	++				
				CE	+-	+-	+-	CDE	+-	+-	+-				
				DE	+++	+++	+++	ADE	+++	+-	++				

Table 3s. De-bittering effects of fatty acids on kuh-seng soup

	1hr	6hr	24hr		1hr	6hr	24hr		1hr	6hr	24hr		1hr	6hr	24hr
0	++++	++++	+++	AB	+-	+-	+-	ABC	+	++	+++	ABCD	+-	+-	++
A	+-	++	+-	AC	+-	+-	+++	ABD	++	+-	+++	ABCE	++	++	++
B	+-	++	++	AD	++	++	+-	ABE	+++	++	+++	ACDE	+-	+-	+++
C	+-	+-	+++	AE	+-	++	+-	ACD	++	+-	+++	ABDE	++	+-	++
D	+-	++	+-	BC	+++	+-	++	ACE	+-	++	+++	BCDE	++	++	+++
E	++	+-	+-	BD	++	++	++	BCD	+++	++	+++	ABCDE	-	+	+-
				BE	+-	+-	+-	BCE	++	+-	+++				
				CD	+-	++	+-	BDE	+-	+-	+-				
				CE	+-	++	+-	CDE	+-	+++	++				
				DE	++	+-	+++	ADE	+++	++	+++				

