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ORIGINAL PAPER

# Potent Inhibitory Effect of Chinese Dietary Spices on Fatty Acid Synthase

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**Abstract** Dietary spices have been adopted in cooking since ancient times to enhance flavor and also as food preservatives and disease remedies. In China, the use of spices and other aromatic plants as food flavoring is an integral part of dietary behavior, but relatively little is known about their functions. Fatty acid synthase (FAS) has been recognized as a remedy target, and its inhibitors might be applied in disease treatment. The present work was designed to assess the inhibitory activities on FAS of spices extracts in Chinese menu. The *in vitro* inhibitory activities on FAS of 22 extracts of spices were assessed by spectrophotometrically monitoring oxidation of NADPH at 340 nm. Results showed that 20 spices extracts (90.9 %) exhibited inhibitory activities on FAS, with half inhibition concentration ($IC_{50}$) values ranging from 1.72 to 810.7 μg/ml. Among them, seven spices showed strong inhibitory effect with $IC_{50}$ values lower than 10 μg/ml. These findings suggest that a large proportion of the dietary spices studied possess promising inhibitory activities on FAS, and subsequently might be applied in the treatment of obesity and obesity-related human diseases.

**Keywords** Spices · Fatty acid synthase · Inhibitor · Obesity

## Abbreviations

| | |
|---|---|
| Ac-CoA | Acetyl-CoA |
| EGCG | (-)-epigallocatechin gallate |
| EtOH | Ethanol |
| FAS | Fatty acid synthase |
| $IC_{50}$ | Half inhibition concentration |
| Mal-CoA | Malonyl-CoA |
| NADPH | β-Nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt. |

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## Introduction

Obesity, which broadly refers to excess body fat, has become a serious public health problem, threatening both the developed and the developing countries [1, 2]. It results from a long-term disbalance between energy intake and expenditure, that is, over consumption of nutrient-poor foods containing high levels of sugar and saturated fats in combination with insufficient physical activity [2]. Obesity is now characterized by a serious of chronic metabolic disorders, such as insulin resistance, type 2 diabetes, fatty liver disease, hypertension, atherosclerosis, hypercholesterolemia, and inflammation [3]. However, public health acts and current agents did little on the increasing epidemic of non-genetic obesity caused by so many environmental elements that could not be solved by single simple method [4]. With a global prevalence of obesity, both nutrition and exercise play key roles in its prevention and treatment. Food products influence specific physiological functions and show real potential for body weight management.

Animal fatty acid synthase (EC 2.3.85, FAS) is an essential enzyme participating in energy metabolism *in vivo* [5]. It catalyzes *de novo* synthesis of long chain fatty acids with acetyl-CoA (Ac-CoA) as a primer, malonyl-CoA (Mal-CoA) as a two-carbon donor, and NADPH as a reducing equivalent [6]. In the past several years, FAS had been reported many bio-activities related to many chronic diseases and play an important role in the regulation of body weight and the development of obesity [7]. As FAS inhibitors can reduce appetite and the amount of food intake, thus the body weight, FAS is regarded as a potential target for therapeutic applications in preventing human obesity.

However, although there have been a number of reports on natural and synthetic FAS inhibitors, only a few possess health care potential, such as cerulenin, C75 (a synthetic compound) and (-)epigallocatechin gallate (EGCG) have been reported [8–10]. In addition, each of them has been found disadvantages to be a suitable drug component, thus new FAS inhibitors with promising health care functions remain to be discovered.

Spices are common food additives, which have been used as flavoring, seasoning, and coloring agents and sometimes as preservative, throughout the world for thousands of years, especially in China, India, and many other Asian countries. Besides bringing color and taste to the menu, some spices have long been considered to possess special function and used in the indigenous systems of medicine. Moreover, a host of beneficial physiological effects have been brought to the fore by extensive animal studies [11]. Among these are their beneficial effects on lipid metabolism, efficacy as antidiabetics, antimicrobial, digestive stimulant action, anticarcinogenic potential, antioxidant property, and anti-inflammatory [12–15]. Molecular and cellular mechanisms underlying the protective effects of such spices are, however, not very clear.

Despite considerable advances over the years in elucidating the underlying mechanisms, the present knowledge of the beneficial effects of spices is far from sufficient. In the present study, we screened the inhibitory effect on FAS by 22 crude extracts of common Chinese dietary spices, and found that 20 of them showed strong inhibitive potential on FAS.

## Materials and Methods

### Reagents

Ac-CoA, Mal-CoA, NADPH, DMSO were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other reagents were of analytical grade.

### Preparation of FAS and Its Substrates

The preparation of FAS from chicken liver was performed as described previously [16]. The amino acid sequence of chicken FAS has 63 % identity with the sequence of human enzyme [16]. The purified FAS was homogenized by polyacrylamide gel electrophoresis in the presence and absence of sodium dodecyl sulfate, respectively. The concentrations of FAS and its substrates were determined by UV–Vis spectrophotometer (Amersham Pharmacia Ultrospec 4300, England, UK) using the following experimental parameters: FAS, $4.83\times10^5\ M^{-1}\ cm^{-1}$ at 279 nm; Ac-CoA, $1.54\times10^4\ M^{-1}\ cm^{-1}$ at 259 nm, pH 7.0; Mal-CoA, $1.46\times10^4\ M^{-1}\ cm^{-1}$ at 260 nm, pH 6.0; acetoacetyl-CoA, $1.59\times10^4\ M^{-1}\ cm^{-1}$ at 259 nm, pH 7.0; NADPH, $6.02\times10^3\ M^{-1}\ cm^{-1}$at 340 nm, and $1.59\times10^4\ M^{-1}\ cm^{-1}$ at 259 nm, pH 9.0.

### Preparation of Crude Sample of Spices

The following 22 species of Chinese spices were selected for research. They were (Common name and Scientific name in order for each spice) star anise, *Illicium verum* (1), lilac, *Syzygium aromaticum* (2), katsumadai seed, *Alpinia katsumadai* Hayata (3), nutmeg, *Myristica fragrans* Houtt (4), cassia bark, *Cinnamomum cassia* (5), Fructus Tsaoko, *Amomum tsaoko* Crevost (6), sweet fruit, *Myristica fragrans* Houtt (7), Chinese wolfberry, *Lycium chinense* Miller (8), Chinese red pepper, *Zanthoxylum bungeanum* (9), Sichuan pepper, *Zanthoxylum bungeanum* (Sichuan province) (10), cumin, *Cuminum cyminum* (11), fennel, *Foeniculum vulgare* Mill (12), mustard, *Wasabia japonica* (13), myrcia, *Laurus nobilis* (14), hot pepper, *Capsicum annuum* L (15), angelica root, *Angelica dahurica* (16), sand ginger, *Kaempferia galanga* (17), long pepper, *Piper longum* (18), fructus amomi, *Amomum villosum* (19), amomun kravanh, *Amomum kravanh* (20), black pepper, *Piper nigrum* (21), ginger, *Zingiber officinale* Roscoe (22) (Fig. 1).

The spices were purchased from Chaoshifa Supermarket in Yuquanlu, Beijing, China. All of these spices were identified by Professor Chuanchu Chen. The voucher specimens were deposited in the enzymatic laboratory, University of Chinese Academy of Sciences. These chosen spices have been used for thousands of years in China and some of them are recorded in the pharmacopoeia of the People's Republic of China and are reported to have no apparent toxicity [17].

These spices were air dried, crushed, and extracted with 50 % ethanol (15 ml solvent per gram for root, stem, fruit and seed; or 20 ml solvent per gram for leaf). The mixtures were stirred at 25 °C for 2 h (for leaf) or 4 h (for others). After centrifugation, the 22 supernatants were air dried to yield crude extracts. These extracts were dissolved in DMSO and were investigated for their ability to inhibit FAS *in vitro*.

### Assays of FAS Activity

The FAS activity was measured at 37 °C by spectrophotometer at 340 nm of NADPH absorption. The overall reaction system contained 100 mM $KH_2PO_4$-$K_2HPO_4$ buffer, 1 mM EDTA, 1 mM dithiothreitol, 3 μM Ac-CoA, 10 μM Mal-CoA, 35 μM NADPH, and 10 μg FAS in a total volume of 2 ml as previously described [18].


**Fig. 1** Chinese dietary spices tested in this study

### Assays of FAS Inhibition

Reversible inhibition was determined by adding the inhibitor to the reaction system before the reaction was initiated. The activities of FAS in the presence and absence of spice extracts were designated as $A_i$ and $A_0$ respectively. The value of $A_i/A_0 \times 100$ % was defined as the relative activity (RA) of FAS. In the experiments the largest volume of DMSO added to the reaction was far less than 0.5 % (*v/v*); thus the DMSO existing in the reaction system had no influence on the results of the activity assay.

### Half-Inhibition Concentration ($IC_{50}$) Assay

The reversible inhibition of FAS was determined under different concentrations of spice extracts. Curve reflecting decreasing FAS activity with increasing herbal concentrations were obtained. The concentrations of spice extract (μg/ml) and residual FAS activity were represented on the X- and Y coordinates, respectively. The $IC_{50}$ values were obtained from these graphs at the point of 50 % remaining (residual) activity. This parameter indicates the inhibitory potency of the inhibitor - the smaller the $IC_{50}$ value, the stronger the activity of the inhibitor. Each experiment repeated three times.

## Results and Discussion

The inhibitory effects of spices on the overall reaction of FAS were measured *in vitro*, and showed that an overwhelming majority of the spices exhibited inhibition on FAS. As shown in Table 1, 20 among 22 spices extracts (90.9 %) exhibited inhibitory activities on FAS, with $IC_{50}$ values ranging from 1.72 to 810.7 μg/ml.

Moreover, the inhibitory effects of seven spices–cassia bark, *Cinnamomum cassia* (5), lilac, *Syzygium aromaticum* (2), star anise, *Illicium verum* (1), Fructus Tsaoko, *Amomum tsaoko* Crevost (6), katsumadai seed, *Alpinia katsumadai* Hayata (3), sweet fruit, *Myristica fragrans* Houtt (7), nutmeg, *Myristica fragrans* Houtt (4),–were very strong. The $IC_{50}$ values of them ranged from 1.72 to 9.40 μg/ml. Compared with classical well-known FAS inhibitors such as cerulenin

**Table 1** The inhibitory activity of the 50 % ethanol extract of spices against FAS

| No. | Spices | Latin name | Family | Genus | Part | $IC_{50}$ (μg/ml) |
|---|---|---|---|---|---|---|
| 1 | star anise | *Illicium verum* | Winteraceae | Illicium | Fruit | 4.24 |
| 2 | lilac | *Syzygium aromaticum* | Myrtaceae | Syringa | Male bud | 3.83 |
| 3 | katsumadai seed | *Alpinia katsumadai* Hayata | Zingiberaceae | Alpinia | Seed | 6.22 |
| 4 | nutmeg | *Myristica fragrans* Houtt | Myristicaceae | Myristica | Seed | 9.40 |
| 5 | cassia bark | *Cinnamomum cassia* | Lauraceae | Cinnamomm | Bark | 1.72 |
| 6 | Fructus Tsaoko | *Amomum tsaoko* Crevost | Zingiberaceae | Amomum | Fruit | 4.59 |
| 7 | sweet fruit | *Myristica fragrans* Houtt | Myristicaceae | Myristica | Hall | 8.59 |
| 8 | Chinese wolfberry | *Lycium chinense* Miller | Solanaceae | Lycium | Fruit | >1000[a] |
| 9 | Chinese red pepper | *Zanthoxylum bungeanum* | Rutcee | Zanthoxylm | Seed | 16.18 |
| 10 | Sichuan pepper | *Zanthoxylum bungeanum* (Sichuang) | Rutcee | Zanthoxylm | Seed | 23.17 |
| 11 | cumin | *Cuminum cyminum* | Umbelliferae | Cuminum | Fruit | 166.70 |
| 12 | fennel | *Foeniculum vulgare* Mill | Umbelliferae | Foeniculum | Fruit | 52.66 |
| 13 | mustard | *Wasabia japonica* | Crucifers | Sinapis | Seed | 74.70 |
| 14 | myrcia | *Taurus nobilis* | Lauraceae | Taurus | Leaf | 39.28 |
| 15 | hot pepper | *Capsicum annuum* L | Solanaceae | Capsicum | Fruit | 810.75 |
| 16 | angelica root | *Angelica dahurica* | Umbelliferae | Angelica | Root | 663.05 |
| 17 | sand ginger | *Kaempferia galanga* | Zingiberaceae | Kaempferia | Rhizoe | >1000[a] |
| 18 | long pepper | *Piper longum* | Pipcraceac | Piper | Fruit | 27.66 |
| 19 | fructus amomi | *Amomum villosum* | Zingiberaceae | Amomum | Fruit | 17.47 |
| 20 | amomun kravanh | *Amomum kravanh* | Zingiberaceae | Amomum | Fruit | 81.29 |
| 21 | black pepper | *Piper nigrum* | Pipcraceac | Piper | Fruit | 20.26 |
| 22 | ginger | *Zingiber officinale* Roscoe | Zingiberaceae | Zingiber | Rhizoe | 323.04 |
| 23 | (-)epigallocatechin gallate (EGCG) | | | | | 24.24[b] |

$IC_{50}$ values were determined by regression analyses and expressed as means for three distinct experiments

[a] The level of $IC_{50}$ value over 1000 μg/ml standed for weak activity against FAS

[b] Positive control

($IC_{50}$=20 μg/ml) and EGCG ($IC_{50}$=24 μg/ml) [8–10], these spices showed much stronger inhibition.

Spices are proven to have benefits on human health and some of them are adopted in traditional medicine as crucial part of therapeutic formulations. Ethnopharmacological studies revealed spices a wide range of biological activities, including anti-oxidantion, anti-inflammation, anti-tumor and immunomodulation [19–22]. It is worth mentioning that all the spices of this investigation are well-known and have been widely used over hundreds of years in China. The long history of their important roles in traditional prescription lighted their applications in later medical development definitely [23–27]. In addition, the active concentrations of these spices used in this work were very low, so oral intake of these spices in the conventional dosage may reach the effective concentrations.

From 1970 to 2005, the overall *per capita* consumption of spices in the United States doubled, increasing from about 1.6 to 3.3 lb per year. As expected, the consumption of some spices, such as garlic, increased at an amount of more than six-fold [28]. As functional foods, spices are important components of human nutrition all over the world. Based on our previous studies, grape, pomegranate, green tea and many other functional foods were found to have inhibitory effects on FAS [10, 29]. Epidemiological, preclinical, and clinical studies continue to provide fundamental insights into the dynamic relationships between functional foods and health. Today, claims about the ability of foods, including spices, to lower disease risk or to enhance the quality of life continue to capture our eyes [30–33].

Spices are reported to contain natural chemicals like vitamins, phytonutrients, mineral elements, alkaloids, flavonoids, terpenoids and sesquisterpenes [11]. With a low calorie content and relative low cost, spices are reliable sources of potential bioactive compounds in daily diet. As the potential functional value of spices has not been fully investigated, we try to provide new idea to take advantage of spices in the present study. Further investigations on the functions of these spices will offer new strategies for obesity treatment, as well as for effective obesity prevention. Basing on this point, seek and find high active FAS inhibitors from spices has important significance.

It was noteworthy that the effective components of these spices are not totally pure, which are different from cerulenin and EGCG, thus we proposed that there must be some more active FAS inhibitors presented in these spices. In sum, spices have greater potential to be favorable FAS inhibitors and are worthy to be explored deeper.

## Conclusion

The positive effect of a functional food may include the maintenance of health or wellbeing, or a reduction in the risk of suffering a given illness. In the present study, we found that 20 Chinese dietary spices showed strong inhibitory activity on FAS. Elucidation of their mechanism of anti-obesity activity would give their wider prospect and more effective application, and the discovery of these spices would help specify their potential benefits on human health.

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**Conflict of Interest** The authors declare that they have no conflict of interest.

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