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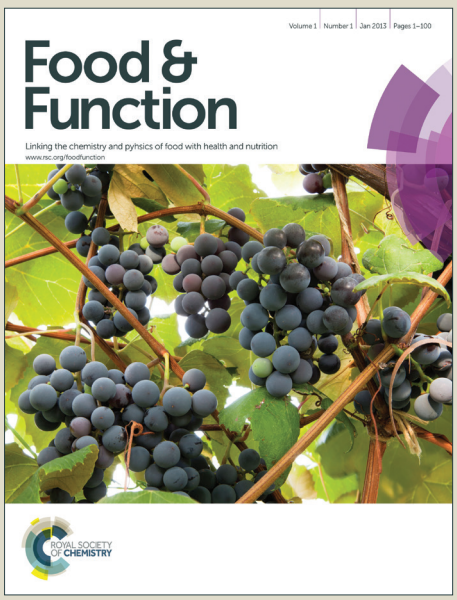

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Inhibition of DNA polymerase λ and associated inflammatory activities of extracts from steamed germinated soybeans

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Running title: Anti-inflammatory actions of processed soybean extracts

(Footnotes)

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Abstract

During the screening of selective DNA polymerase (pol) inhibitors from more than 50 plant food materials, we found that the extract from steamed germinated soybeans (*Glycine max* L.) inhibited human pol λ activity. Among the three processed soybean samples tested (boiled soybeans, steamed soybeans, and steamed germinated soybeans), both the hot water extract and organic solvent extract from the steamed germinated soybeans had the strongest pol λ inhibition. We previously isolated two glucosyl compounds, a cerebroside (glucosyl ceramide, AS-1-4, compound **1**) and a steroidal glycoside (eleutheroside A, compound **2**) from dried soybean, and these compounds were prevalent in the extracts of the steamed germinated soybeans as pol inhibitors. The hot water and organic solvent extracts of the steamed germinated soybeans and compounds **1** and **2** selectively inhibited the activity of eukaryotic pol λ *in vitro* but did not influence the activities of other eukaryotic pols, including those from the A-family (pol γ), B-family (pols α, δ, and ε), and Y-family (pols η, ι, and κ), and also showed no effect on the activity of pol β, which is of the same family (X) as pol λ. The tendency for *in vitro* pol λ inhibition by these extracts and compounds showed a positive correlation with the *in vivo* suppression of TPA (12-*O*-tetradecanoylphorbol-13-acetate)-induced inflammation in mouse ear. These results suggest that steamed germinated soybeans, especially the glucosyl compound components, may be useful for their anti-inflammatory properties.

Introduction

Pol (DNA-dependent DNA polymerase, E.C. 2.7.7.7) catalyzes deoxyribonucleotide addition to the 3′-hydroxyl terminus of primed double-stranded DNA (dsDNA) molecules.[1] The human genome encodes at least 14 pols, which function in cellular DNA synthesis.[2,3] Eukaryotic cells contain three replicative pols (α, δ, and ε), one mitochondrial pol (γ), and at least 10 non-replicative pols (β, ζ, η, θ, ι, κ, λ, μ, ν, and REV1).[4,5] Pols have a highly conserved structure, with their overall catalytic subunits showing little variation among species; conserved enzyme structures are usually preserved over time as they perform important cellular functions that confer evolutionary advantages. Based on sequence homology, eukaryotic pols can be divided into four main families, A, B, X, and Y.[4] Family A includes mitochondrial pol γ as well as pols θ and ν; family B includes the three replicative pols α, δ, and ε and also pol ζ; family X is comprised of pols β, λ, and μ; and family Y includes pols η, ι, and κ in addition to REV1.[5] We have been studying selective inhibitors of each eukaryotic pol derived from natural products, including food materials and components, and over the past 18 years, we have discovered more than 100 inhibitors of mammalian pols.[6,7]

During our pol inhibitor studies, we have found that pol λ-selective inhibitors, such as curcumin derivatives,[8-10] display 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced anti-inflammatory activity.[8,9,11] Although tumor promoters are classified as compounds that promote tumor formation,[12] they also cause inflammation and are commonly used as artificial

inducers of inflammation to screen for anti-inflammatory agents.[13] Tumor promoter-induced inflammation can be distinguished from acute inflammation, which is exudative and is accompanied by fibroblast proliferation and granulation. The tumor promoter TPA is frequently used to search for new types of anti-inflammatory compounds. TPA not only causes inflammation but also influences mammalian cell growth,[14] suggesting that the molecular basis of the inflammation stems from pol reactions related to cell proliferation. However, this relationship needs to be investigated more closely.

In screening for pol inhibitors from more than 50 plant food materials, we found that the extract from steamed germinated soybeans inhibited pol activity. In this report, we focused on processed soybeans (boiled in water, steamed, and steamed germinated soybeans) and investigated whether pol activity was inhibited by the hot water and organic solvent extracts from these three types of processed soybeans. We previously isolated two glucosyl compounds as potential selective eukaryotic pol λ inhibitors from dried soybean,[15] and the processed soybean extracts and/or their components, such as these glucosyl compounds, may hold promise as chemotherapeutic agents based on their specific inhibitory activities for pol λ.

Materials and methods

Materials

The three processed soybean samples (water-boiled, steamed, and steamed germinated soybeans), were obtained from Oguraya Yanagimoto Co., Ltd. (Kobe, Japan). The processed soybeans were all of the same species, *Glycine max* L. cv. Toyomasari and are available for purchase at Japanese supermarkets. The soybeans were freeze-dried to use for extraction (Fig. 1).

A chemically synthesized DNA template, poly(dA), was purchased from Sigma-Aldrich Inc., and a customized oligo$(dT)_{18}$ DNA primer was produced by Sigma-Aldrich Japan K.K. (Hokkaido, Japan). Radioactive nucleotide [^{3}H]-labeled 2′-deoxythymidine-5′-triphosphate (dTTP; 43 Ci/mmol) was obtained from Moravek Biochemicals Inc. (Brea, CA, USA). All other reagents were analytical grade from Nacalai Tesque Inc. (Kyoto, Japan).

Enzymes

Pol α was purified from calf thymus by immunoaffinity column chromatography as described by Tamai *et al.*.[16] Recombinant rat pol β was purified from *Escherichia coli* JMpβ5 as described by Date *et al.*.[17] The human pol γ catalytic gene was cloned into pFastBac. Histidine-tagged enzyme was expressed using the BACTO-BAC HT Baculovirus Expression

System according to the manufacturer's instructions (Life Technologies, Frederick, MD, USA) and purified using ProBound resin (Invitrogen Japan, Tokyo Japan).[18] Human pols δ and ε were purified by nuclear fractionation of human peripheral blood cancer cells (Molt-4) using the second subunit of pol δ- and ε-conjugated affinity column chromatography, respectively.[19] A truncated form of human pol η (residues 1–511) tagged with His_6 at its C-terminus was expressed in *E. coli* cells and purified as described by Kusumoto *et al.*.[20] A recombinant mouse pol ι tagged with His_6 at its C-terminus was expressed and purified by Ni–NTA column chromatography.[21] A truncated form of pol κ (residues 1–560) with 6× His-tags attached at the C-terminus was overexpressed in *E. coli* and purified as described by Ohashi *et al.*.[22] Recombinant human His-pol λ was overexpressed and purified according to a method described by Shimazaki *et al.*.[23] Pol α from a higher plant, cauliflower inflorescence, was purified according to the methods outlined by Sakaguchi *et al.*.[24] Recombinant rice (*Oryza sativa* L. cv. Nipponbare) pol λ tagged with His_6 at the C-terminus was expressed in *E. coli* and purified as described by Uchiyama *et al.*.[25] *Taq* pol, T4 pol, T7 RNA polymerase, and T4 polynucleotide kinase were purchased from Takara Bio Inc. (Kyoto, Japan). The Klenow fragment of pol I from *E. coli* and human immunodeficiency virus type-1 (HIV-1) reverse transcriptase were purchased from Worthington Biochemical Corp. (Freehold, NJ, USA). Bovine pancreas deoxyribonuclease I was obtained from Stratagene Cloning Systems (La Jolla, CA, USA).

Measurement of pol activity

The reaction mixtures for calf pol α, rat pol β, plant pol α, and prokaryotic pols have been described previously;[26,27] reaction mixtures for pol γ as well as pols δ and ε were previously described by Umeda *et al.*[18] and Ogawa *et al.*,[28] respectively. The reaction mixtures for pols η, ι, and κ are the same as for pol α, and the reaction mixture for pol λ is the same as for pol β. For the pol reactions, poly(dA)/oligo(dT)$_{18}$ (A/T, 2/1) and dTTP were used as the DNA template-primer substrate and nucleotide (dNTP; 2′-deoxynucleoside-5′-triphosphate) substrate, respectively.

The test compounds were dissolved in distilled dimethyl sulfoxide (DMSO) to various concentrations and sonicated for 30 sec. Subsequently, 4 µL aliquots were mixed with 16 µL of each enzyme (0.05 units) in 50 mM Tris-HCl at pH 7.5, containing 1 mM dithiothreitol, 50% glycerol (by vol), and 0.1 mM ethylenediaminetetraacetic acid (EDTA) and held at 0°C for 10 min. These inhibitor-enzyme mixtures in 8 µL volumes were added to 16 µL of enzyme standard reaction mixture and incubated at 37°C for 60 min, except for *Taq* pol, which was incubated at 74°C for 60 min. The activity without an inhibitor was considered 100%, and the relative activity was determined for each inhibitor concentration. One unit of pol activity was defined as the amount of each enzyme that catalyzed the incorporation of 1 nmol dTTP into synthetic DNA template-primers in 60 min at 37°C and under standard

reaction conditions.[26,27]

Other enzyme assays

The activities of calf primase of pol α, HIV-1 reverse transcriptase, T7 RNA polymerase, mouse IMP dehydrogenase (type II), T4 polynucleotide kinase, and bovine deoxyribonuclease I were measured in standard assays according to the manufacturer's specifications, as described by Tamiya-Koizumi *et al.*,[29] Mizushina *et al.*,[27] Nakayama and Saneyoshi,[30] Mizushina *et al.*,[31] Soltis *et al.*,[32] and Lu and Sakaguchi,[33] respectively.

Anti-inflammatory assay by TPA-induced inflammation in mice

Female 8-week-old ICR mice were obtained from Japan SLC, Inc. (Hamamatsu, Japan) and maintained on a standard diet (MF; Oriental Yeast Co., Ltd., Osaka, Japan) and provided water ad libitum. The mice that had been bred in-house with free access to food and water were used for all experiments. All of the mice were maintained under a 12-h light/dark cycle and housed at a room temperature of 25°C. This animal study was approved by the Institutional Animal Care and Use Committee of Kobe Gakuin University, and was performed according to the guidelines outlined in the Care and Use of Laboratory Animals of Kobe Gakuin University. The animals were anesthetized with pentobarbital before undergoing cervical dislocation.

The mouse inflammatory test was performed according to Gschwendt's method.[34] Briefly, an acetone solution of compounds (250 or 500 μg in 20 μL) or 20 μL of acetone as a vehicle control was applied to the inner part of the mouse ear. Thirty minutes after the test compound was applied, a TPA solution (0.5 μg/20 μL of acetone) was applied to the same part of the ear. To the other ear of the same mouse, the TPA solution was applied as a control. After 7 h, a disk (6 mm diameter) was obtained from the ear and weighed. The inhibitory effect (IE) is presented as a ratio of the increase in weight of the ear disks:

$$IE = [(\text{TPA only}) - (\text{tested compound plus TPA})]/[(\text{TPA only}) - (\text{vehicle})] \times 100$$

Results

Preparation of hot water and organic solvent extracts from the processed soybeans

Initially, the soybean extract was prepared from the three processed soybean samples (*Glycine max* L. cv. Toyomasari) (water-boiled, steamed, and steamed germinated soybeans) as outlined in Fig. 2. These processed soybeans were freeze-dried (Fig. 1) and powdered using a mill mixer. Each powder (250 mg) was added to 1.5 mL of (A) hot water or (B) organic solvent (i.e., chloroform:methanol = 1:1) and extracted by stirring and sonication. The samples were centrifuged. A portion (0.5 mL) of the supernatant was removed, and the solvent was evaporated *in vacuo* to obtain the soybean extract.

Amounts of extracts from the processed soybeans

The amounts of the prepared (A) hot water extract and (B) organic solvent extract from each of the processed soybean samples were measured. The hot water extracts from both the steamed soybeans (2) and the steamed germinated soybeans (3) were approximately 2-fold higher than that from the soybeans boiled in water (1) (Fig. 3). These results suggested that the steaming process that was used to prepare samples (2) and (3), could prevent the dissolution of water-soluble compounds from soybeans, while the boiling process, which was used to prepare sample (1), must lose water-soluble compounds to broth. Both the steamed soybeans (2) and the steamed germinated soybeans (3) were more yellow than the soybeans

boiled in water (1) (Fig. 1); therefore, the hot water extracts from (2) and (3) might contain yellow hydrophilic materials.

However, the organic solvent extracts from the three processed soybean samples produced nearly identical amounts (Fig. 3). These results suggested that the hydrophilic compounds in the soybean extract were not affected by the boiling and steaming processes.

Previously, we isolated two glucosyl compounds, a cerebroside (glucosyl ceramide, AS-1-4, compound **1**) and a steroidal glycoside (eleutheroside A, compound **2**) from dried soybeans (Fig. 4), and we found that these compounds inhibited pol activity and suppressed TPA-induced inflammation in mouse ear.[15] The amounts of these compounds in the six prepared soybean extracts were measured by HPLC and TLC, and it was found that both the (A) hot water and (B) organic solvent extracts from the steamed germinated soybeans contained compounds **1** and **2**, but the other processed soybean extracts did not (data not shown). Thus, we focused on the prepared extracts from the steamed germinated soybeans and the purified glucosyl compounds **1** and **2** from dried soybean in the latter part of this study.

Effect of the extracts and glucosyl compounds from the processed soybeans on the activities of mammalian pols α and λ

The inhibitory activity of each soybean extract against mammalian pols was investigated

using calf pol α and human pol λ. In mammalian pols, pols α and λ represent DNA replicative pols of the B family and DNA repair/recombination pols of the X family, respectively.[4,5] The six extracts and compounds **1** and **2** from the processed soybeans at the concentrations of 10 and 100 μg/mL did not influence the activity of calf pol α (Fig. 5A). In contrast, some soybean extracts inhibited human pol λ activity, and the extract activity could be ranked in the following order: (3) steamed germinated soybeans > (2) steamed soybeans > (1) boiled soybeans (Fig. 5B). The two glucosyl compounds also inhibited the activity of pol λ, and the inhibitory effect of compound **2** was slightly stronger than that of compound **1**. In the soybean extracts, the (A) hot water extracts were stronger pol λ inhibitors than the (B) organic solvent extracts, suggesting that water soluble compounds might strongly inhibit pol λ activity.

When activated DNA (bovine deoxyribonuclease I-treated DNA) was used as the DNA template-primer substrate instead of synthesized DNA [poly(dA)/oligo(dT)$_{18}$ (A/T = 2/1)] and dNTP was used as the nucleotide substrate instead of dTTP, the inhibitory effects of these compounds did not change (data not shown).

Effects of the extracts and glucosyl compounds from steamed germinated soybeans on the activities of various pols and other DNA metabolic enzymes

We focus on the extracts and two glucosyl compounds from the steamed germinated soybeans in this section. As described briefly in the introduction, we succeeded in obtaining 9

mammalian pol species (α, β, γ, δ, ε, η, ι, κ, and λ); however, pols ζ, θ, μ and ν, and REV1 are not yet available. Currently, eukaryotes are thought to express at least 14 species of pols,[2,3] and we are still in an era when most pols are very difficult to obtain in their purified form in a laboratory. Table **1** shows the inhibitory effect (50% inhibitory concentration: IC_{50} value) of the two steamed germinated soybean extracts and their glucosyl compounds **1** and **2** against various pol species including the 9 mammalian pols that could be obtained. The two extracts from the (A) hot water and (B) organic solvents inhibited the activity of human pol λ, with IC_{50} values of 25.2 and 41.8 μg/mL, respectively; therefore, the hydrophilic compounds of (A) the hot water extract had a stronger inhibitory effect than the hydrophobic compounds of (B) the organic solvent extract. Compounds **1** and **2** also inhibited human pol λ activity, with IC_{50} values of 6.5 and 5.0 μg/mL, respectively; therefore, compound **2** was a stronger inhibitor than compound **1** by approximately 1.3-fold. These extracts and compounds had no influence at all on the activities of replicative pols, such as those from family B (calf pol α, human pol δ, and human pol ε), human mitochondrial pol γ (family A), or repair-related pols such as those from family X (rat pol β) and family Y (human pol η, mouse pol ι, and human pol κ). In particular, although pols β and λ both belong to the X-family, it seemed interesting that these extracts and glucosyl compounds showed no effect on the activity of pol β, which is thought to have a similar homology and three-dimensional structure to pol λ. The extracts and compounds **1** and **2** also inhibited the activity of plant (i.e., rice) pol λ to the same extent as

they inhibited human pol λ. These compounds had no inhibitory effect on plant (i.e., cauliflower) pol α or prokaryotic pols, such as the Klenow fragment of *E. coli* pol I, *Taq* pol, and T4 pol. When activated DNA (i.e., DNA with gaps digested by bovine deoxyribonuclease I) and dNTP were used as the DNA template-primer substrate and nucleotide substrate pair instead of synthesized DNA [poly(dA)/oligo$(dT)_{18}$ (A/T = 2/1)] and dTTP, respectively, the inhibitory effects of these compounds were unchanged (data not shown).

The extracts and glucosyl compounds from the steamed germinated soybeans did not inhibit the activities of other DNA-metabolic enzymes, such as calf primase of pol α, HIV-1 reverse transcriptase, T7 RNA polymerase, mouse IMP dehydrogenase (type II), T4 polynucleotide kinase, and bovine deoxyribonuclease I (Table 1). These results suggest that the extracts and compounds can selectively inhibit the activity of eukaryotic pol λ. The fact that these glucosyl compounds, which are components of the steamed germinated soybeans, are inhibitors of a pol species, pol λ, is of great interest.

We next performed specific assays to determine whether compounds **1** and **2**-induced inhibition resulted from the ability of these compounds to bind to DNA or to the enzyme. The interaction of compound **1** or **2** with dsDNA was investigated based on the thermal transition of dsDNA by measuring the melting temperature (T_m) of dsDNA with an excess amount of compound **1** or **2** (100 μg/mL each) using a spectrophotometer equipped with a thermoelectric cell holder. A thermal transition of T_m was not observed within the concentration range of the

compounds used in the assay, whereas a typical intercalating compound used as a positive control (ethidium bromide, 15 μg/mL) produced a clear thermal transition (data not shown). We next investigated whether an excessive amount of nucleic acid [poly(rC)] or protein [bovine serum albumin (BSA)] prevented the inhibitory effect of compounds **1** and **2** to determine whether the inhibitory effect resulted from non-specific adhesion of these molecules to pol λ or from selective binding to specific sites. Poly(rC) and BSA had little or no influence on the pol λ inhibitory effect of compounds **1** and **2** (data not shown), suggesting that these compounds selectively bind to the pol λ molecule. These observations indicated that these compounds do not act as DNA intercalating agents as a template-primer substrate, but that these compounds can directly bind to the enzyme and inhibit its activity.

These results suggest that compounds **1** and **2**, containing the extract from the steamed germinated soybeans, may be potent and specific inhibitors of eukaryotic pol λ.

Anti-inflammatory effect of the extracts and glucosyl compounds from the processed soybeans on TPA-induced inflammation *in vivo*

In a previous pol inhibitor study, we found that there was a relationship between pol λ inhibitors and TPA-induced anti-inflammatory activity.[6,7,9,35] Thus, using the *in vivo* mouse ear inflammatory test, we examined the anti-inflammatory activity of the six soybean extracts and compounds **1** and **2**. The application of TPA (0.5 μg) to the mouse ear induced edema,

resulting in a 241% increase in the weight of the ear disk 7 h after application. The pretreatment with the extracts and glucosyl compounds suppressed inflammation in a dose-dependent manner, and the effect of (A) the hot water extract was stronger than that of (B) the organic solvent extract (Fig. 6). The anti-inflammatory activity of the processed soybean extracts could be ranked as follows: (3) steamed germinated soybean extract > (2) steamed soybean extract > (1) extract from soybeans boiled in water. Compound **2** showed stronger anti-inflammatory activity than compound **1** by approximately 1.3-fold. Therefore, the *in vivo* anti-inflammatory effect of the extracts and compounds from soybeans displayed the same order as their *in vitro* inhibitory effect on pol λ (Fig. 5B and Table 1). These results suggest that the inhibition of pol λ activity has a positive correlation with anti-inflammatory activity.

Discussion

Eukaryotic cells reportedly contain 14 pol species, which belong to four families: family A (pols γ, θ, and ν), family B (pols α, δ, ε, and ζ), family X (pols β, λ, and μ) and family Y (pols η, ι, and κ, and REV1).[36,37] Among the X family of pols, pol λ and pol β seems to function similarly.[38] The exonuclease-deficient pol λ (64 kDa) contains all the important residues required for DNA binding, nucleotide binding and selection, and catalysis of DNA polymerization, which are conserved in pol β (39 kDa), the smallest known mammalian pol. Hence, the 3D-structure and the primary sequence of the catalytic core in the C-terminal part of pol λ (residues 244−575) are highly homologous to pol β.[39,40] The difference of inhibitory activity between pol β and pol λ by the purified glucosyl compounds **1** and **2** from soybean is unknown, and the effects of these compounds on the molecular mechanism of mammalian pol λ inhibition will be addressed in further studies.

Pol β is involved in the short-patch base excision repair (BER) pathway[41-44] and plays an essential role in neural development.[45] Pol λ is capable of synthesizing DNA de novo and template-dependent. Furthermore, it displays 5'-deoxyribose-5-phosphate (dRP)-lyase activity.[46] Pol λ is implicated in V(D)J recombination,[47] translesion synthesis (TLS),[48] and BER.[49] Moreover, studies with eukaryotic cells and reactive oxygen species (ROS) indicate that pol λ functions as a backup for pol β in BER[50] and protects cells from oxidative damage.[51] There is also evidence that pol λ is required for cell cycle progression and is

functionally connected to the S phase DNA damage response machinery in cancer cells.[51]

As well as causing inflammation, TPA influences cell proliferation and has physiological effects on cells because it has tumor promoter activity.[14] Therefore, anti-inflammatory agents are expected to suppress DNA replication/repair/recombination in nuclei in relation to the action of TPA. Because pol λ is a repair/recombination-related pol,[38] our finding—that the molecular target of glucosyl compounds **1** and **2** in steamed germinated soybean extract is pol λ —is in good agreement with an expected mechanism where anti-inflammatory agents suppress DNA repair/recombination. The detailed mechanism by which these compounds prevent mammalian pol λ inhibition and, hence, inhibit inflammation is unclear; therefore, to clarify the exact mechanism of the anti-inflammatory effect of these glucosyl compounds, further studies will be conducted.

Cerebroside (glucosyl ceramide; compound **1**) and steroidal glucoside (compound **2**) are common components of plant membranes.[52] Whether these glucosyl compounds are active metabolic compounds is unknown. Some hypotheses consider these conjugates to be final products of ceramide and sterol metabolism, whereas others suggest that the processes of deglucosylation of glucosyl compounds incorporated into membranes can play a significant role in the membrane properties or in the modulation of membranous enzyme activity. Several biological effects of glucosyl compounds are known, including anti-inflammatory effects.[53] The anti-inflammatory action of compounds **1** and **2** may be through their selective inhibition

of pol λ activity. If the analogues of these glucosyl compounds can be synthesized, it is much worth learning the structure-activity relationship and exploring more potent compounds. We are trying to chemically synthesize compounds **1** and **2** and their analogues and related compounds for further studies.

Conclusion

In this study, we found that both human pol λ inhibitory activity and anti-inflammatory activity of the steamed germinated soybean extract was higher than that from boiled soybean and steamed soybean extracts. These results are important for food (i.e., soybean) science and processed food technology fields. Additionally, glucosyl compounds **1** and **2** contained within the steamed germinated soybean extract demonstrated both human pol λ inhibitory activity and anti-inflammatory activity. Some glucosyl compounds are found in sprout-derived sterols of plants[54] and play an important role as sprout-growing biochemical factors that are newly generated during the process of germination. Therefore, purified glucosyl compounds **1** and **2** and/or purified steamed germinated soybean extract, which contains large amounts of these compounds, could be used as an anti-inflammatory functional food and/or cosmetic based on the inhibitory activity of eukaryotic pol λ. Because glucosyl compounds **1** and **2** are present in steamed germinated soybeans, these processed soybeans may provide effective nutrients for human anti-inflammatory health promotion.

Conflict of interest

The authors declare that there are no conflicts of interest.

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(Figure legends)

Fig. 1 The three processed soybean samples (*Glycine max* L. cv. Toyomasari). (A) boiled (water) soybeans, (B) steamed soybeans, and (C) steamed germinated soybeans. These are freeze-dried samples.

Fig. 2 Processed soybean extract preparation method.

Fig. 3 Amount of hot water and organic solvent extracts from the three processed soybeans. Data are shown as the mean ± SD (n = 3).

Fig. 4 Structures of the identified human pol λ inhibitors from the steamed germinated soybean extract. (A) Compound **1**: cerebroside (glucosyl ceramide, AS-1-4) and (B) compound **2**: steroidal glycoside (eleutheroside A). The purification and structure determination of these compounds were reported previously [15].

Fig. 5 Inhibitory effects of hot water extract and organic solvent extract from the three processed soybeans and their glucosyl components (compounds **1** and **2**) on the activity of mammalian pols α and λ. (A) Calf pol α and (B) human pol λ. Each test compound (10 μM, gray bars; 100 μM, black bars) was incubated with 0.05 units of calf pol α (B-family pol, a

DNA replicative pol) and 0.05 units of human pol λ (X-family pol, a DNA repair/recombination pol). Pol activity in the absence of the compound was taken as 100%, and the relative activity is shown. Data are shown as the mean ± SD (n = 3).

Fig. 6 Anti-inflammatory activity of hot water extract and organic solvent extract from the three processed soybeans and their glucosyl components (compounds **1** and **2**) against TPA-induced edema in a mouse ear model. Each test compound (250 μg, gray bars; 500 μg, black bars) was applied individually to one ear of a mouse. After 30 min, TPA (0.5 μg) was applied to both ears. Edema was evaluated after 7 h. The inhibitory effect is expressed as the percentage of edema. Data are shown as the mean ± SD (n = 6).

Table 1 IC_{50} of the two extracts from steamed germinated soybeans and their glucosyl compounds on the activities of mammalian, plant, and prokaryote pols, and other DNA metabolic enzymes

Enzyme	IC_{50} values (μM)			
	Steamed germinated soybeans		Compound **1**	Compound **2**
	(A) Hot water extract	(B) Organic solvent extract		
– Mammalian pols –				
[A-Family of pol]				
Human pol γ	>100	>100	>100	>100
[B-Family of pols]				
Calf pol α	>100	>100	>100	>100
Human pol δ	>100	>100	>100	>100
Human pol ε	>100	>100	>100	>100
[X-Family of pols]				
Rat pol β	>100	>100	>100	>100
Human pol λ	25.2 ± 1.5	41.8 ± 2.5	6.5 ± 0.4	5.0 ± 0.3
[Y-Family of pols]				
Human pol η	>100	>100	>100	>100
Mouse pol ι	>100	>100	>100	>100
Human pol κ	>100	>100	>100	>100
– Plant pols –				
Cauliflower pol α	>100	>100	>100	>100
Rice pol λ	29.1 ± 1.8	48.6 ± 2.9	8.6 ± 0.5	6.5 ± 0.4
– Prokaryotic pols –				
E. coli pol I	>100	>100	>100	>100
Taq pol	>100	>100	>100	>100
T4 pol	>100	>100	>100	>100
– Other DNA metabolic enzymes –				
Calf primase of pol α	>100	>100	>100	>100
HIV-1 reverse transcriptase	>100	>100	>100	>100
T7 RNA polymerase	>100	>100	>100	>100

Mouse IMP dehydrogenase (type II)	>100	>100	>100	>100
T4 polynucleotide kinase	>100	>100	>100	>100
Bovine deoxyribonuclease I	>100	>100	>100	>100

These extracts and compounds were incubated with each enzyme (0.05 units). Enzyme activity in the absence of inhibitor was considered 100%; data is expressed as the mean ± SD (n = 3).

Fig. 1 Mizushina *et al.*

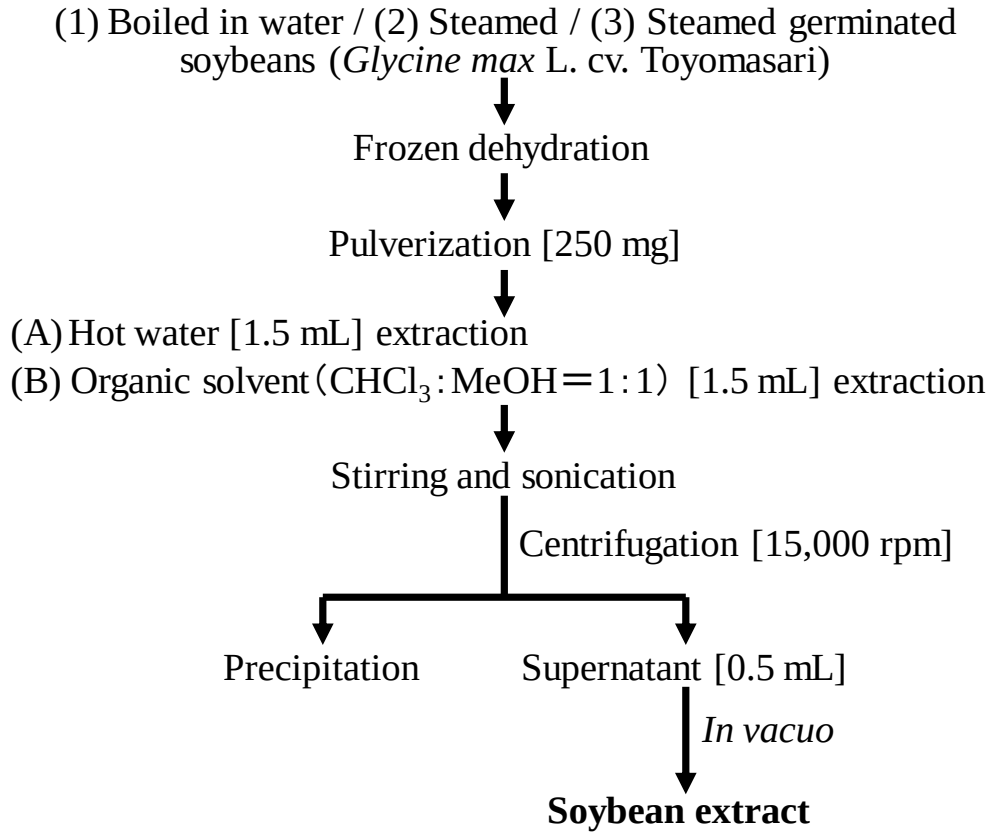


Fig. 2 Mizushina *et al.*

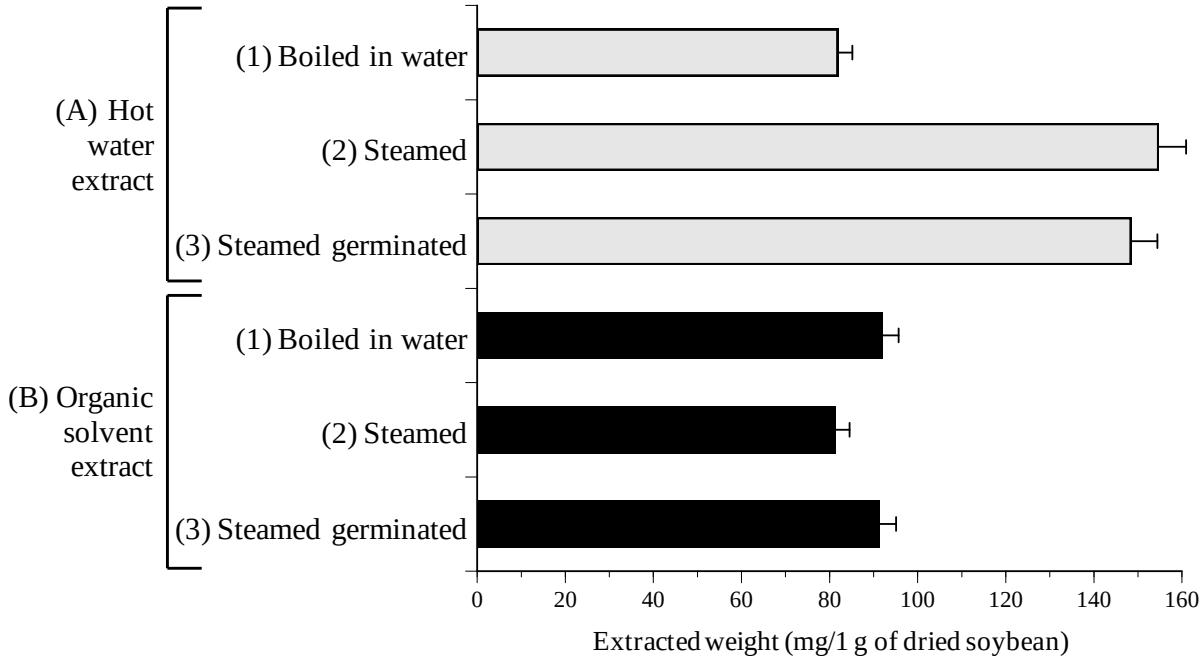
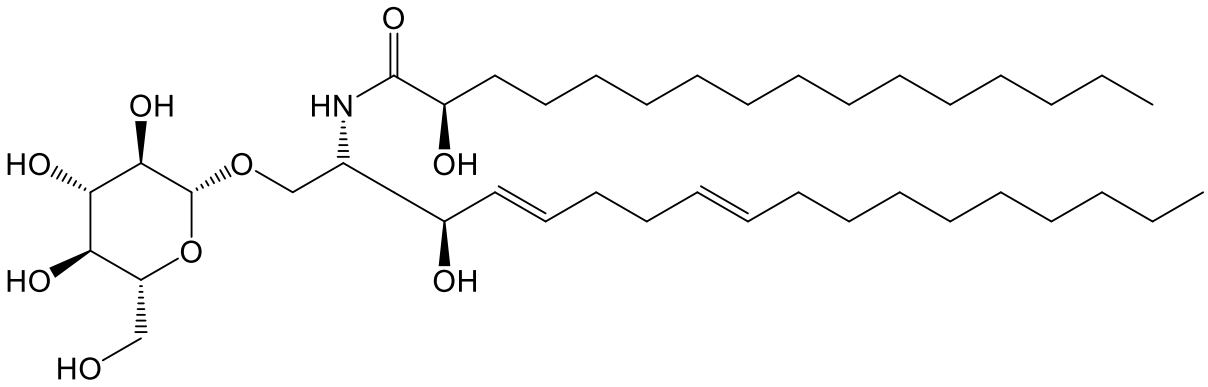


Fig. 3 Mizushina *et al.*

A



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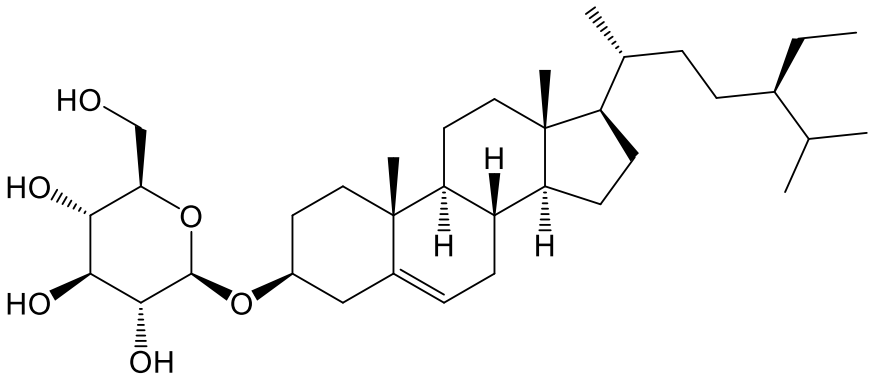
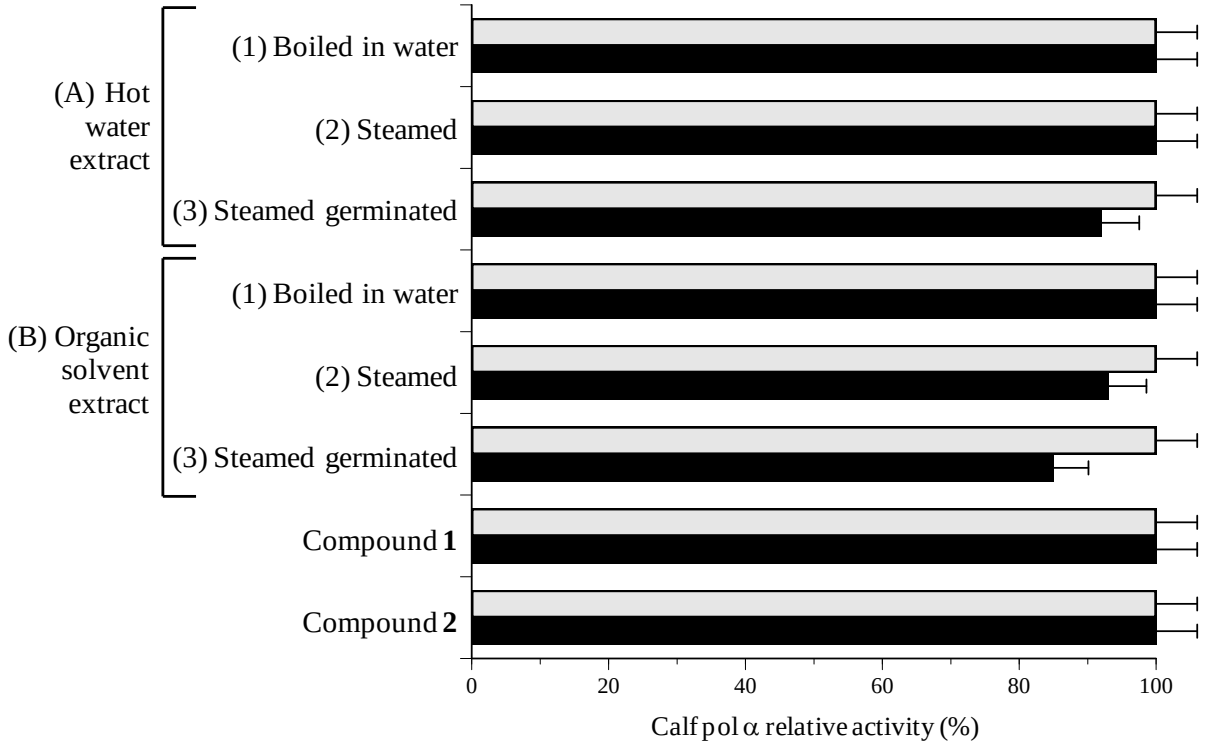


Fig. 4 Mizushina *et al.*

A



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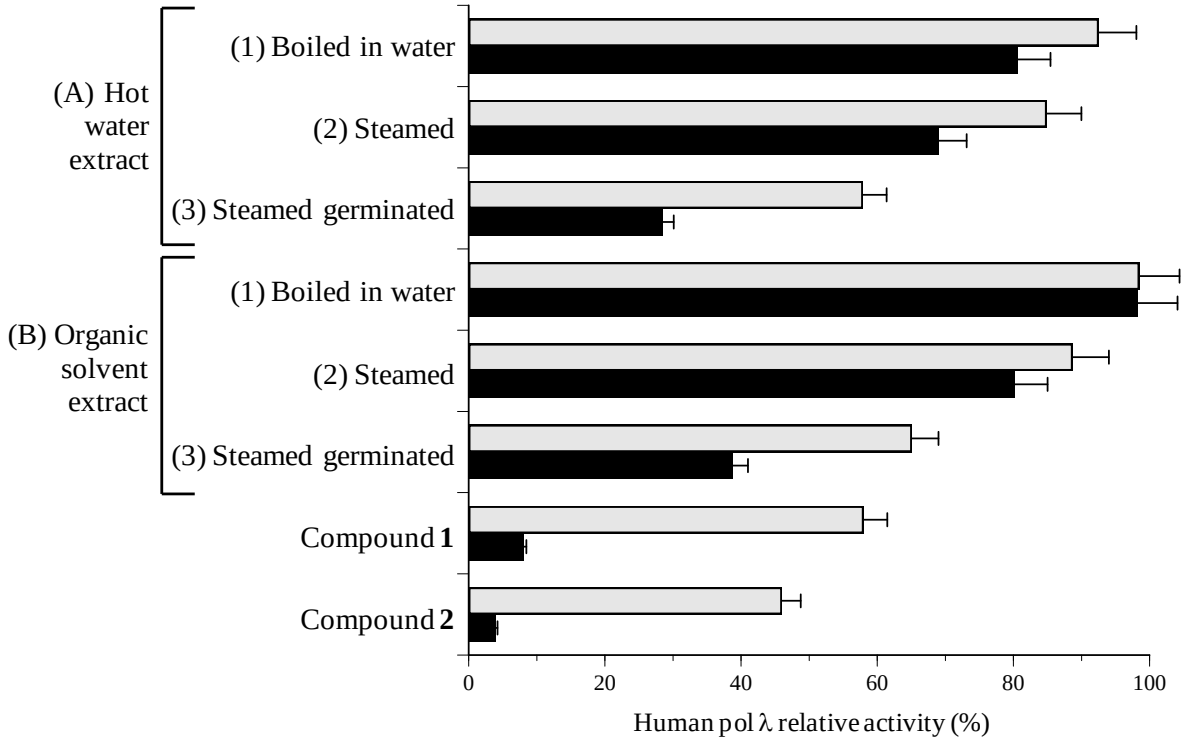

Fig. 5 Mizushina *et al.*

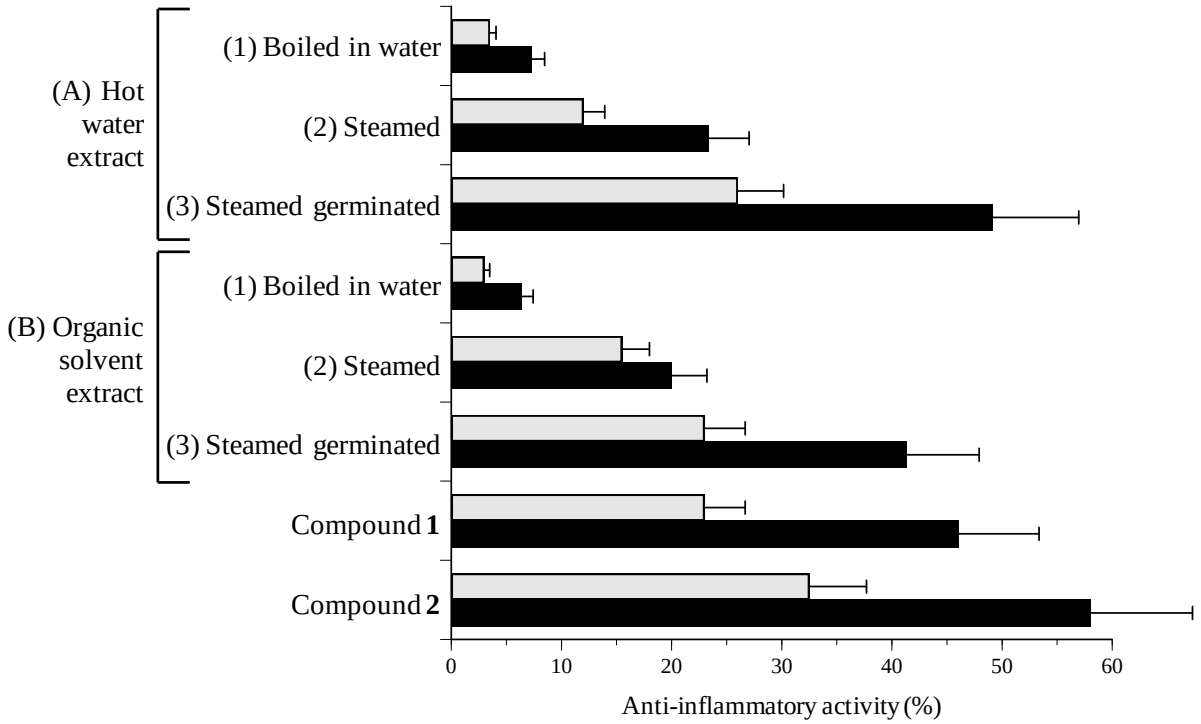

Fig. 6 Mizushina *et al.*