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Determination of anti-inflammatory activities of standardised preparations of plant- and mushroom-based foods

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Abstract

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Results: Ten food samples including lime zest, English breakfast tea, honey-brown mushroom, button mushroom, oyster mushroom, cinnamon and cloves inhibited NO production in N11 microglia, with IC50 values below 0.5 mg/ml. The most active samples were onion, oregano and red sweet potato, exhibiting IC50 values below 0.1 mg/ml. When these ten food preparations were retested in RAW 264.7 macrophages, they all inhibited NO production similar to the results obtained in N11 microglia. In addition, English breakfast tea leaves, oyster mushroom, onion, cinnamon and button mushroom preparations suppressed TNF-α production, exhibiting IC50 values below 0.5 mg/ml in RAW 264.7 macrophages.

Conclusion: In summary, anti-inflammatory activity in these food samples survived 'cooking'. Provided that individual bioavailability allows active compounds to reach therapeutic levels in target tissues, these foods may be useful in limiting inflammation in a variety of age-related inflammatory diseases. Furthermore, these foods could be a source for the discovery of novel anti-inflammatory drugs.

Keywords

foods, inflammatory, cinnamon, oregano, oyster mushroom

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Determination of anti-inflammatory activities of standardised preparations of plant and mushroom based foods

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ABSTRACT

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Conclusion In summary, anti-inflammatory activity in these food samples survived 'cooking'. Provided that individual bioavailability allows active compounds to reach therapeutic levels in target tissues, these foods may be useful in limiting inflammation in a variety of age-related inflammatory diseases. Furthermore, these foods could be a source for the discovery of novel anti-inflammatory drugs.

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1. INTRODUCTION

The increase in both the absolute number as well as relative proportion of the elderly is arguably one of the most important developments facing human society in the next decades [1]. Age is the leading risk factor for many devastating diseases such as acute and chronic neurodegenerative diseases, degenerative musculoskeletal diseases, cardiovascular diseases, diabetes, asthma, rheumatoid arthritis and inflammatory bowel disease. Increasing evidence suggests that systemic low grade inflammation is a contributing factor in these age-related diseases [2-6]. To date, pharmacotherapy of inflammatory conditions is based on the use of non-steroidal anti-inflammatory drugs (NSAIDs). However, NSAIDs can cause serious gastrointestinal toxicity such as gastric bleeding and the formation of stomach ulcers [7, 8]. Even more ominously, some NSAIDs, particularly COX-2 inhibitors, have been linked to increased blood pressure, greatly increased risk of congestive heart failure, occurrence of thrombosis and myocardial infarction [9-11]. Together, these findings provide the motivation for the development of anti-inflammatory treatments with fewer adverse effects.

Herbal medicines derived from plants rich in the secondary metabolite salicylic acid such as the bark of the willow tree (*Salix alba*) have been used for the treatment of diseases with a prominent inflammatory component for thousands of years. Many other medicinal plants are known to have anti-inflammatory activity but neither the underlying mechanisms nor their potential for the development of new drugs have been fully explored. Several mechanisms are proposed to explain their anti-inflammatory action, including inhibition of cyclooxygenases and lipoxygenases or modulation of pro-inflammatory gene expression such as inducible nitric oxide synthase, and several pivotal cytokines including TNF-α [12, 13].

However, very few studies have attempted a systematic comparison of a large number of commercially available plant and mushroom foods in search of those with the highest degree of anti-inflammatory activities. To close this knowledge gap, we have screened 115 dietary plants and mushrooms for *in vitro* anti-inflammatory activities using a generic food-compatible sample processing method involving heating in water, or in the presence of glucose.

2. MATERIALS AND METHODS

2.1. Materials

Plant and mushroom samples in the screening library were obtained from retail suppliers in Melbourne, Australia. DMSO, 95% ethanol, bovine serum albumin, lipopolysaccharide (LPS) (*E.coli* serotype 0127:B8), EDTA, N-(1-1-napthyl) ethylenediaminedihydrochloride, penicillin G sodium benzyl, resazurin sodium, streptomycin, sulfanilamide, tetra methyl benzidine (TMB), trypan blue were purchased from Sigma-Aldrich, (Castle Hill, NWS, Australia). Antibiotics, Dulbecco's modified Eagle's medium (DMEM), foetal bovine serum (FBS) and glutamine were purchased from Invitrogen (Mulgrave, Vic, Australia). Murine IFN-γ and TNF-α ELISA kits were purchased from Peprotech (Rocky Hill, NJ, USA).

2.2 Methods

2.2.1 Preparation of the food and plant library samples

Plant and mushroom library samples were prepared by methods usually employed with food preparation, involving heating (i.e. 'cooking'), mechanical dispersion and treatments intended to solubilise both hydrophobic and hydrophilic solutes. Samples were prepared by blending in a food processor with water (1:2 ratio w/v) before heating in a microwave for 10 min. under control conditions or including 1% glucose, to enhance Maillard reaction products. After cooling to room temperature, ascorbic acid (0.1% of initial solids) and ethanol (1% of initial solids) were added for microbial stabilisation. Samples were ultrasonicated using a 400 W probe at 100% power for 2 min. (Hielscher 400UPS) before freeze drying.

2.2.2 Maintenance of N11 microglia and RAW 264.7 macrophages

Cells were cultured in 175 cm^2 flasks in DMEM containing 5% FBS, supplemented with glutamine (2 mM), penicillin (200 U/ml), streptomycin (200 μg/ml) and fungizone (2.6 μg/ml). The cell lines were maintained in 5% CO_2 at 37°C.

2.2.3 Pro-inflammatory activation of cells

Cells were seeded at a density of 75,000 /well into each well of a 96-well plate. After 24h, a combination of 25 µg/ml LPS and 10 U/ml IFN-γ diluted in DMEM was used for activation. The food samples were dissolved in DMEM and insoluble solids were removed by centrifugation at 16,900 x g for 2 min. Samples were added to the cells an hour prior to addition of the inflammatory activation mix at a maximal concentration of 2.5 mg/ml, from which 6 doses were made by serial 1:2 dilutions. Cells were incubated for 24 h at 37^{o}C with the inflammatory activation mix before NO and TNF-α levels were measured.

2.2.4 Measurement of nitrite levels by the Griess assay

NO release was measured through the quantification of nitrite by the Griess assay. The Griess reagent was made up of equal volumes of 1% sulfanilamide and 0.1% napthyethylene-diamine in 5% HCl. From each well, 80 µl of cell culture medium was transferred to a fresh 96-well plate and mixed with 80 µl of Griess reagent and after 30 minutes at room temperature absorbance was measured at 540 nm in a POLARstar Omega microplate reader (BMG Labtech, Mornington, Australia) and expressed as a percentage of that in control cells.

2.2.5 Measurement of TNF-α levels by enzyme linked immunosorbent assay (ELISA)

Determination of TNF-α was performed by ELISA, according to the manufacturer's instructions (Peprotech) with minor modifications. Briefly, the capture antibody was diluted to 0.5 µg/ml in phosphate buffered saline (PBS; 1.9 mM NaH_2PO4, 8.1mM Na_2HPO4, 154 mM NaCl pH 7.4) and 100 µl of diluted capture antibody was added to each well of a 96-well plate, sealed with parafilm and incubated overnight at room temperature. The plate was washed 4 times with washing buffer (0.05% Tween-20 in PBS); 250 µl of blocking buffer (1% BSA in PBS) was added to each well, the plate was covered and incubated for 1 hour at room temperature. The plate was again washed 4 times in washing buffer. TNF-α standard was serially diluted from 0 to 10,000 pg/ml in diluent (0.05% Tween-20, 0.1% BSA in PBS). 50µl of cell supernatant or TNF-α standard in duplicate was added to each well, the plate was covered and incubated for 2.5 hours at room temperature. Each well was washed 4 times with washing buffer. Detection antibody was diluted to 0.125 µg/ml in diluent (supplied by the manufacturer) and 100 µl of diluted detection antibody was added to each well. The plate was covered and incubated for 2.5 hours before being washed 4 times with washing buffer. Avidin Peroxidase conjugate was diluted to a ratio of 1:4000 in diluent and 100µl of diluted Avidin Peroxidase conjugate solution was added to each well, the plate was covered and incubated for 30 minutes before being washed 4 times with washing buffer.

100µl of 3,3',5,5'-tetramethybenzidine (TMB) Liquid Substrate Solution (0.5 mg TMB dissolved in 1 ml DMSO, supplemented with 9ml phosphate-citrate buffer and 1µl 30% H_2O_2) was added to each well. Absorbance was measured at 655 nm in a POLARstar Omega microplate reader (BMG Labtech, Mornington, Australia), taking readings every 5 min. After 25 min, the reaction was stopped using 0.5M sulphuric acid, and then the absorbance was measured at a wavelength of 455nm.

2.2.6 Determination of cell viability

The Alamar Blue assay is a colorimetric assay involving the cellular reduction of resazurin to resorufin. Resazurin was dissolved in PBS to give a concentration of 0.001% (w/v), sterile filtered (0.22 µm), protected from light with aluminium foil and stored at 4°C for up to six months. To determine cell viability, incubation media was aspirated from wells and replaced with 100 µl of resazurin solution and incubated at 37°C for 1 h. After incubation for 1 h, fluorescence of formed resorufin was measured with excitation at 530 nm and emission at 590 nm in a POLARstar Omega microplate reader (BMG Labtech, Mornington, Australia) and expressed as a percentage of that in control cells, after background fluorescence was subtracted.

2.2.7 Data calculation and statistics

Data calculations were performed using MS-Excel 2010 software. IC_{50} values were obtained by using the sigmoidal dose-response function in GraphPad Prism. IC_{50} values from three experiments were averaged (all performed in triplicate). The results were expressed as mean ± standard deviation. Differences between the same sample heated in water or glucose were analysed by t-tests using GraphPad Prism.

3. RESULTS

3.1 Primary screen of 115 food and mushroom samples using nitric oxide as pro-inflammatory readout

115 commercially available dietary plants and mushrooms were selected (Table 1). Samples were prepared using a generic food-compatible sample processing method involving heating in water or in the presence of 1% glucose (to allow modification by the Maillard reaction for form Maillard reaction products, MRPs).

These 115 samples (including 115 identical samples heated with glucose) were then tested for their ability to down-regulate LPS + IFN-γ induced NO production in N11 microglia (Table 1). Ten foods demonstrated significant anti-inflammatory activity with IC_{50} values below 0.5 mg/ml (Table 2). Seven of these food preparations, including lime zest, English breakfast tea, honey brown mushroom, button mushroom, oyster mushroom, cinnamon and cloves demonstrated potent anti-inflammatory activity with IC_{50} values between 0.1 and 0.5 mg/ml (Table 2). However, the most active food samples were onion, followed by oregano and red sweet potato exhibiting IC_{50} values below 0.1 mg/ml (Table 3). Furthermore, the MRP modified samples showed IC_{50} values in the same order of magnitude as the unmodified samples and, despite significant differences in some samples between the unmodified and MRP modified samples, no general increase in anti-inflammatory activity by the inclusion of glucose in the heating mixture could be detected. All of the active preparations were non-toxic at the IC_{50} values (cell viability > 75 %), suggesting that the reduction in NO production was not simply caused by a decrease in cell number or cell viability.

Table 1: List of food and mushroom samples tested for their anti-inflammatory activity

Category	Foods	*Botanical name*	Plant part used	Plant family or class of organism
HERBS	Continental parsley	*Petroselinium crispum*	dried aerial parts	Apiaceae/Umbelliferae
	Tarragon	*Artemisia dracunculus*	dried leaf	Asteraceae/Compositae
	Oregano	*Origanum vulgare*	dried leaf	Lamiaceae/Labiatae
	Rosemary	*Rosmarinus officinalis*	dried leaf	Lamiaceae/Labiatae
	Sage	*Salvia officinalis*	dried leaf	Lamiaceae/Labiatae
	Thyme	*Thymus vulgaris*	Dried leaf	Lamiaceae/Labiatae
SPICES	Fennel	*Foeniculum vulgare*	seeds	Apiaceae/Umbelliferae
CONDIMENTS	Coriander	*Coriandrum sativum*	seeds	Apiaceae/Umbelliferae
	Yellow mustard	*Brassica hirta*	seeds	Cruciferae

	Bay leaves	*Laurus nobilis*	leaves	Lauraceae
	Cinnamon	*Cinnamomum zeylanicum*	bark	Lauraceae
	Garlic	*Allium sativum*	bulb	Lilliaceae
	Brown linseed	*Linum Usitatissimum*	seeds	Linaceae
	Cloves	*Syzygium aromaticum*	flower buds	Myrtaceae
	Cardamom	*Elattaria cardamomum*	fruit	Zingiberaceae
	Ginger	*Zingiber officinale*	rhizome	Zingiberaceae
	Turmeric	*Curcuma longa*	rhizome	Zingiberaceae
VEGETABLES	Spinach	*Spinacia oleracea*	aerial parts	Amaranthaceae
	Silver beet	*Beta vulgaris*	tuber	Amaranthaceae
	Rainbow silver beet	*Beta vulgaris*	tuber	Amaranthaceae
	Beet root	*Beta vulgaris*	tuber	Amaranthaceae
	Carrot	*Daucus carota*	tuber	Apiaceae/Umbelliferae
	Coriander	*Coriandrum sativum*	leaves	Apiaceae/Umbelliferae
	Black carrot	*Daucus carota*	tuber	Apiaceae/Umbelliferae
	Asparagus	*Asparagus officinalis*		Asparagaceae
	Radiccio	*Cichorium intybus*		Asteraceae/Compositae
	Red coral lettuce	*Lactuca sativa*	aerial parts	Asteraceae/Compositae
	Bok Choi	*Brassica chinensis var.parachinensis*	fresh herb	Cruciferae
	Brussel srouts	*Brassica oleracea gemmifera?*	fresh herb	Cruciferae
	Chinese Broccoli	*Brassical oleracea*	fresh herb	Cruciferae
	Chinese cabbage	*Brassica rapa*	fresh herb	Cruciferae
	Choi sum	*Brassica chinenssis*	fresh herb	Cruciferae
	Red cabbage	*Brassica oleracea var.capitata rubra*	aerial parts	Cruciferae
	Savoy cabbage	*Brassica oleracea var savoy*		Cruciferae
	Water cress	*Nasturtium officinale*		Cruciferae
	Red Sweet Potato	*Ipomoea batatus*	tuber	Convolvulaceae
	Choko flesh	*Sechium edule*	fruit flesh	Cucurbitaceae
	Pumpkin	*Cucurbita maxima*	fruit	Cucurbitaceae
	Squash	*Cucurbita moschata*	fruit	Cucurbitaceae
	Choko skin	*Sechium edule*	skin	Cucurbitaceae
	Basil green	*Ocimum basilicum*	fresh herb	Lamiaceae/Labiatae

	Basil purple	*Ocimum basilicum*	fresh herb	Lamiaceae/Labiatae
	Basil Thai	*Ocimum basilicum var. thrysiflora*	fresh herb	Lamiaceae/Labiatae
	Green peas	*Pisum sativum*	seeds	Leguminosae
	Green beans			
	Chives	*Allium schoenoprasum*	bulb	Lilliaceae
	Leek	*Allium porrum*	herb	Lilliaceae
	Onion	*Allium cepa*	buld	Lilliaceae
	Shallot	*Allium cepa var. aggregatum*	bulb	Lilliaceae
	Spring onion	*Allium fistulosum*	bulb	Lilliaceae
	Red onion	*Allium cepa*	bulb	Lilliaceae
	Corn	*Zea mays*	seeds	Poaceae
	Lemon grass	*Cimbopogon citratus*	aerial parts	Poaceae
	Rhubarb	*Rheum palmatum*		Polygonaceae
	White potato	*Solanum tuberosum*	tuber	Solanaceae
MARINE ALGEA	Spirulina	*Arthrospira platensis*	Whole organism	Cyanobacteria
FRUITS	Kiwi Gold	*Actinidia chinensis*		Actinidiaceae
	Kiwi green	*Actinidia chinensis*		Actinidiaceae
	Cucumber	*Cucumis sativus*		Cucurbitaceae
	Zucchini white	*Cucurbita peop*		Cucurbitaceae
	Blueberries	*Vaccinium corymbosum*		Ericaceae
	Avocado	*Persea americana*		Lauraceae
	Coconut	*Cocos nucifera*		Palmae
	Quince	*Pyrus cydonia*		Rosaceae
	Raspberries	*Rubus idaeus*		Rosaceae
	Strawberries	*Fragaria X ananassa*		Rosaceae
	Apples Red delicious	*Malus domestica*		Rosaceae
	Ruby grapefruit	*Citrus paradisi*		Rutaceae
	Lime	*Citrus aurantifolia*		Rutaceae
	Mandarine (imperial)	*Citrus reticulata*		Rutaceae
	Lemon flesh	*Citrus limon*		Rutaceae
	Lime zest	*Citrus aurantifolia*		Rutaceae
	Oranges Navel	*Citrus sinensis*		Rutaceae

	Oranges Valencia	*Citrus sinensis*		Rutaceae
	Orange zest	*Citrus sinensis*		Rutaceae
	Lemon zest	*Citrus limon*		Rutaceae
	Citrus yellow flesh	*Citrus limon*		Rutaceae
	Citrus red skin	"		"
	Citrus red flesh	"		"
	Citrus yellow skin	"		"
	Citrus green	"		"
	Tomato	*Lycoperisicon esculentum*		Solanaceae
	Grapes M48-42	*Vitis vinifera*		Vitaceae
	Grapes Chambourcin	"		"
	Grapes Cabernet Sauvignon	"		"
	Grapes M12-41	"		"
	Grapes Sanmuscat	"		"
	Grapes Concord	"		"
	Grapes CR101-13	"		"
	Grapes CR101-10	"		"
MUSHROOMS	Maitake	*Grifola frondosa*	fruit body	Polyporaceae
	Jew's ear	*Auricularia auricula-judae*	fruit body	Auriculariaceae
	Oyster	*Pleurotus ostreatus*	fruit body	Pleurotaceae
	Honey brown	*Agaricus bisporus*	fruit body	Agaricaceae
	Button	*Agaricus bisporus*	fruit body	Agaricaceae
	Enoki	*Flammulina veluptipes*	fruit body	Physalacriaceae
	Shiitake	*Lentinus edodes*	fruit body	Tricholomataceae
BEVERAGES	Coffe beans dark columbia	*Coffea arabica*		Rubiaceae
	Coffee beans mocha	*Coffea arabica*		Rubiaceae
	Cocoa beans	*Theobroma cocoa*		Malvaceae
	Tea leaves English breakfast	*Camellia sinensis*		Theaceae
	Sencha tea leaves Japanese	*Camellia sinensis*		Theaceae

Table 2: Food samples with anti-inflammatory activity (IC_{50}: 0.1 – 0.5 mg/ml)

Food Sample	Heated in	IC_{50} for inhibition of NO production (mg/ml)	Cell viability at IC_{50} for NO (%)
Oyster mushroom	water	0.47 ± 0.28	96 ± 6
Oyster mushroom	glucose	0.27 ± 0.10	98 ± 8
Lime zest	water	0.36 ± 0.06	108 ± 4
Lime zest	glucose	0.13 ± 0.04	93 ± 10
Honey brown mushroom	water	0.16 ± 0.13	106 ± 12
Honey brown mushroom	glucose	0.11 ± 0.04	104 ± 14
Button mushroom	water	0.14 ± 0.01	105 ± 15
Button mushroom	glucose	0.35 ± 0.01	124 ± 2
English Breakfast Tea leaves	water	0.24 ± 0.13	94 ± 3
English Breakfast tea leaves	glucose	0.30 ± 0.21	92 ± 2
Cinnamon	water	0.21 ± 0.10	88 ± 12
Cinnamon	glucose	0.29 ± 0.14	89 ± 14
Cloves	water	0.28 ± 0.06	83 ± 5
Cloves	glucose	0.15 ± 0.01	95 ± 9

Table 3: Food samples with anti-inflammatory activity (IC_{50}< 0.1mg/ml)

Food sample	Heated in	IC_{50} for inhibition of NO production (mg/ml)	Cell viability at IC_{50} for NO (%)
Onion	water	0.087 ± 0.030	99 ± 5
Onion	glucose	0.075 ± 0.010	101 ± 1
Oregano	water	0.066 ± 0.020	96 ± 2
Oregano	glucose	0.083 ± 0.030	96 ± 3
Red sweet potato	water	0.067 ± 0.020	95 ± 7
Red sweet potato	glucose	0.054 ± 0.020	90 ± 5

Table 4: Food samples re-tested in RAW 264.7 macrophages

Food Sample	IC_{50} for NO production (mg/ml)	IC_{50} for TNF-α production (mg/ml)	LC_{50} (mg/ml)
Onion	0.13 ± 0.02	0.30 ± 0.01	> 2.5
Oregano	0.13 ± 0.01	0.73 ± 0.06	> 2.5
Oyster mushroom	0.07 ± 0.01	0.23 ± 0.02	> 2.5
Red sweet potato	1.54 ± 0.10	0.51 ± 0.03	> 2.5
Lime zest (glucose)	0.41 ± 0.05	1.02 ± 0.11	> 2.5
Honey brown mushroom	0.81 ± 0.03	1.0 3± 0.14	> 2.5
Button mushroom	0.73 ± 0.01	0.39 ± 0.04	> 2.5
Cinnamon	0.10 ± 0.01	0.45 ± 0.01	> 2.5
Cloves	0.12 ± 0.01	2.12 ± 0.02	> 2.5
English breakfast tea leaves	0.40 ± 0.01	0.30 ± 0.02	> 2.5
Anti-inflammatory drug			
Prednisone	0.25 ± 0.09 (0.69 ± 0.26 mM)	0.7 ± 0.07 (1.95 ± 0.21 mM)	1.36 ± 0.21 (3.81 ± 0.59 mM)
Ibuprofen	0.79 ± 0.08 (3.83 ± 0.43 mM)	0.42 ± 0.16 (1.19 ± 0.16mM)	0.34 ± 0.09 (1.63 ± 0.44 mM)

3.2 Secondary screen using a second cell line (RAW 264.7 macrophages) and nitric oxide plus TNF-α as pro-inflammatory readouts

The 10 most active samples (defined as having an IC50 value of < 0.5 mg/ml or 0.1 mg/ml, respectively) with demonstrated anti-inflammatory properties from the primary screen were retested in a second cell line with an

additional pro-inflammatory readout, TNF-α. Since the previous experiment did not show any striking difference between the original and glucose-modified preparations, no glucose-modified samples were used in the second screen, except for lime zest, where the availability of the unmodified sample was limited.

These 10 active samples (English breakfast tea leaves, onion, oregano, oyster mushroom, red sweet potato, lime zest, honey brown mushroom, button mushroom, cinnamon and cloves) were tested by measuring their ability to down-regulate LPS + IFN-γ induced release of NO and, as a second marker of inflammation, TNF-α (Table 4).

Of the 10 products, oyster mushroom and cinnamon, demonstrated the most significant anti-inflammatory activities (with IC_{50} values below 0.1 mg/ml), followed by cloves, oregano, onion, English breakfast tea leaves and lime zest (with IC_{50} values below 0.5 mg/ml) in terms of suppression of LPS and IFN-γ induced NO production. In addition, English breakfast tea leaves, oyster mushroom, onion, cinnamon and button mushroom preparations also suppressed TNF-α release (with IC_{50} values below 0.5 mg/ml). Oyster mushroom was the most active anti-inflammatory sample for both NO inhibition and TNF-α inhibition with IC_{50} values 0.067 mg/ml and 0.23 mg/ml respectively (Table 4).

In addition, two anti-inflammatory drugs, including prednisone and the non-steroidal anti-inflammatory drug (NSAID) ibuprofen were tested in the same assay systems. Ibuprofen was toxic to cells at concentrations of 1.63 ± 0.44 mM, and NO and TNF-α production were down-regulated at the same concentrations (Table 4). Prednisone inhibited LPS + IFN-γ induced NO and TNF-α production with IC_{50} values of 0.25 ± 0.09 mg/ml (0.69 ± 0.26 mM) and 0.7 ± 0.07 mg/ml (1.95 ± 0.21 mM), respectively (Table 4).

4. DISCUSSION

The activation of macrophages and microglia leads to secretion of inflammatory molecules such as the pro-inflammatory cytokine TNF-α and the free radical NO, which play an important role in inflammation and nitroxidative stress in many age-related diseases, including Alzheimer's disease (AD) [14]. In the assay systems used here, the bacterial surface molecule lipopolysaccharide and the cytokine IFN-γ were used to activate microglia, and NO and TNF-α release were used as readouts [15].

As control substances, the synthetic corticosteroid prednisone and the non-steroidal anti-inflammatory drug (NSAID) ibuprofen were also tested in the same assay systems. As expected, ibuprofen as a selective COX-1 and 2 inhibitor did not specifically inhibit LPS and IFN-γ induced NO and TNF-γ production. Interestingly, prednisone was quite a weak inhibitor of LPS + IFN-γ induced NO and TNF-α production, since in many other inflammatory paradigms corticosteroids are effective at low micromolar, and in some instances at nanomolar concentrations [13]. However, it appears that macrophages treated with a combination of LPS and IFN-γ (compared to LPS alone) are insensitive to anti-inflammatory corticosteroids such as dexamethasone [14, 15], which is consistent with our observations.

Among the food samples, one hundred and fifteen distinct plants and mushroom preparations were screened for anti-inflammatory properties by employing *in vitro* cell-based bio-assays. The extracts of several plants including onion, oregano, red sweet potato, lime zest, cinnamon and cloves exhibited the strongest capacity to suppress NO and TNF-α. Interestingly, oyster mushroom, honey brown mushroom and button mushroom showed also significant anti-inflammatory potential.

Interestingly, a differential effect of MRP modification on the activity of the food preparations was observed, depending on the sample. Since high molecular MRPs (also termed advanced glycation endproducts, AGEs) are known to increase inflammation [16, 17], and low molecular MRPs (MW < 1 kDa) have been shown to possess anti-inflammatory potential [18], it might be the balance between these species which shift the IC_{50} value up or down depending on the composition of the sample.

Some of our data are in accordance with published studies on anti-inflammatory properties of the foods identified as potent in this study. For example, onions have been shown to exhibit anti-inflammatory properties, e.g. downregulation of adipokine expression in the visceral adipose tissue of rats or attenuation of vascular inflammation and oxidative stress in fructose-fed rats [19, 20]. Among the polyphenols in onions, quercetin was suggested to be the responsible anti-inflammatory ingredient, as evidenced by the downregulation of *COX2* transcription in human lymphocytes [21]. Furthermore, the anti-inflammatory activity of the onion has been studied also in relation to the presence of thiosulfinates and cepaenes [22, 23].

Anti-inflammatory properties of cinnamon has been demonstrated for *Cinnamomum osmophloem* kaneh [24, 25], but less is known about the 'true' cinnamon of India, *Cinnamomum zeylanicum*. Some authors reported significant inhibitory effects of inflammatory signalling by the extracts of *C. cassia* [26]. Sodium benzoate appears to be one of the active ingredients in cinnamon, since it inhibits LPS-induced expression of inducible

NO synthase (iNOS), pro-inflammatory cytokines (TNF-α and IL-1β) and surface markers for inflammatory activation such as CD11b, CD11c, and CD68 in mouse microglia [27].

Clove (*Syzygium aromaticum)* extracts have been identified as having potent free radical (including superoxide anion) scavenging properties, and metal chelating activities, which may be due to the presence of flavonoids. Cloves contain considerable concentrations of eugenol, beta-caryophyllene, quercetin and kaempferol as well as rhamnetin and kaempferol and their glycosides [28]. Our study is in line with studies showing that eugenol suppresses NF-κB activation, thereby down-regulating cyclooxygenase-2 expression in lipopolysaccharide-stimulated macrophages [29-31].

Red sweet potato (*Ipomoea batatas*), a species rich in β-carotene and anthocyanins [32], has been demonstrated to have anti-inflammatory properties for the first time by our study. Lime (*Citrus aurantifolia*) rich in flavonol glycosides, especially of kaempferol-type are known for their anti-oxidant properties [33], however our study is also the first to report on its anti-inflammatory activities.

The anti-inflammatory properties of the various mushroom species were quite surprising, since there are only a handful studies showing such activity. For example, oyster mushroom concentrate (OMC) was shown to suppress LPS-induced secretion of TNF-α, IL-6 and IL-12p40 in RAW264.7 macrophages and also suppressed PGE2 and NO by down-regulation of COX-2 and iNOS expression, respectively. OMC also inhibited LPS-dependent DNA binding activity of AP-1 and NF-κB in RAW264.7 cells [34]. Our results correlate with these observations as oyster mushroom extracts inhibited LPS+IFN-γ induced production of NO. In mushrooms, water-soluble polysaccharides, especially the β-glucans, are most likely to be the substances responsible for the anti-inflammatory properties. For example, β-glucans isolated *Pleurotus ostreatus* were able to potentiate the anti-inflammatory effects of methotrexate in rat models of experimental arthritis or colitis [35, 36]. A further potential anti-inflammatory compound in mushrooms could be ergothioneine (ET), a sulfur containing amino acid that functions as an antioxidant and is present in mushrooms at a concentration of up to 2.0mg/g [37]. In Acute Respiratory Distress Syndrome (ARDS), ergothioneine given intravenously 1h before or 18 h after cytokine (IL-1 and IFN-γ) insufflation, decreased lung injury and lung inflammation in cytokine insufflated rats [38].

In summary, it is suggested that foods containing anti-inflammatory properties might be useful in the prevention of age-related inflammatory conditions, provided that dose and individual bioavailability lead to therapeutic concentrations in affected tissues. Bioavailability of plant substances including polyphenols is highly variable,

and will depend on the nature of the compound [39, 40]. Therefore, validation of the active samples in animal models should eventually follow. Furthermore, we speculate that these natural sources could lead to the discovery of potent novel anti-inflammatory compounds.

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CONFLICT OF INTEREST STATEMENT

Louse Bennett and Richard Head are employed by CSIRO, the organization, that sponsored the research.

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