# HOSTED BY

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Review doi: 10.1016/S2222-1808(15)60894-5

©2015 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

A review on pharmacological properties of Bidens biternata: A potential nutraceutical

Kulsoom Zahara<sup>\*</sup>, Yamin Bibi, Shaista Tabassum, Mudrikah, Tasneem Bashir, Shakeela Haider, Anum Araa, Maryam Ajmal

Department of Botany, PirMehr Ali Shah, Arid Agriculture University, Rawalpindi 46300, Pakistan

## ARTICLE INFO

Article history: Received 30 Apr 2015 Received in revised form 7 May, 2nd revised form 18 May 2015 Accepted 13 Jun 2015 Available online 9 Jul 2015

*Keywords: Bidens biternata* Phytochemicals Nutritional values Bioactivities

## ABSTRACT

*Bidens biternata* (Lour.) Merr. and Sheriff. (*B. biternata*) belonging to family Asteraceae, is a common easy to grow, widespread, pestiferous crop weed and a wasteland plant species. It is a wild edible plant rich in macronutrients and micronutrients. *B. biternata* is extensively used in traditional medicine against inflammation, infections, diabetes, malaria, leprosy, ulcers and diarrhea and digestive disorders. Present review highlights the up-to-date information on the botanical properties, phytochemistry, bioactivities, traditional and medicinal uses of *B. biternata* in over 26 diseases, scientific studies investigating the potential medicinal uses of *B. biternata* and its constituent phytochemicals are presented and discussed. The present review provides preliminary information and gives direction for further research into this plant.

#### **1. Introduction**

Plants have been used as a source of therapeutic compounds since time immemorial<sup>[1]</sup>. They play a vital role in conventional health care system as well as in international pharmaceutical market<sup>[2]</sup>. The medicinal properties of these plants are due to some bioactive compounds such as alkaloids, phenolic compounds, flavonoids and tannins which produce a definite physiological action in human body<sup>[3]</sup>.

Bidens biternata (B. biternata), belonging to family Asteraceae, is an important plant species with several ethnomedicinal and nutritional values. Traditionally, *B. biternata* is used widely in both Chinese medicine and western herbalism to cure a variety of diseases *i.e.* nausea, leprosy, fever, cough and asthma[4]. In last few decades, many species of genus *Bidens i.e. Bidens pilosa* (*B. pilosa*) and *Bidens bipinnata* are extensively studied for their medicinal properties by advanced scientific techniques and a variety of bioactive compounds *i.e.* sesquiterpenes, germacrene-D,  $\beta$ -caryophyllene,  $\beta$ -carotene *etc* have been isolated[5], but no significant work is reported on pharmacological properties of *B. biternata*. The present review highlights the overall outline

E-mail: kulsoomzahara@gmail.com

of the distribution, morphology, phytochemistry, nutritional, ethnomedicinal and medicinal properties of *B. biternata* and its future prospects for the further scientific investigation for the development of effective therapeutic compounds.

## 2. Botany

B. biternata is an easy-to-grow herb that is widely distributed all over the world. Plant has variety of vernacular names (Table 1) and is commonly known as Spanish needles because of its sticky achenes that entrench themselves into hair and clothing. It is a widespread weed occurring in moist and shady places of gardens, in village, along the roadside, cultivated areas and along the bank of small channels<sup>[4]</sup>. Stem of *B. biternata* is quadrangular, grooved and hairy. Leaves are opposite, trifoliate, imparipinnate and acuminated with dentate margins. Hairy bracts are present at base. Outer bracts are green in colour with dark, thick mid nerves and green or whitish scarious margins. Ray florets are ligulate, white or yellow in colour and starshaped whereas disc florets are yellow in colour. Achenes are 8-13 mm  $\times$  1 mm in length (Figure 1)[4]. B. biternata produce large number of seeds with 100% germination capacity which is resulting in rapid spread of this species over a large area[6]. B. biternata is quite similar to B. pilosa and Bidens bipinnata. B. pilosa can be differentiated from B. biternata in possessing 3-foliate leaves and white (or no) ray florets. Similarly, Bryophyllum pinnata

<sup>\*</sup>Corresponding author: Kulsoom Zahara, Department of Botany, PirMehr Ali Shah, Arid Agriculture University, Rawalpindi 46300, Pakistan.

differs in the 2- pinnatisects[7].
Table 1

Vernacular names of B. biternata.

Names	Regions
Phutjom, Kerrai, Kathori, Kuro	India
Agedi, Ketul	Indonasia
Konchem	Thiland
Jinzhan, Yinpan	China
Dipmal, Phutium	Pakistan



**Figure 1.** *B. biternata.* a: Plant body; b: Floral part.

## 3. Origin and geographical distribution

*B. biternata* is a troublesome invasive weed native to Egypt, Eritrea, Ethiopia, Somalia, Sudan, Kenya, Tanzania, Uganda, Zaire, Ghana, Nigeria, Angola, Malawi, Mozambique, Zambia, Zimbabwe, Arabian Peninsula, China, Japan, Korea, Taiwan, Indonesia, Philippines, New Caledonia and Solomon Islands (Figure 2). It is naturalized in Botswana, Lesotho, Namibia, South Africa, Swaziland, Reunion, Afghanistan, India, Pakistan and Sri Lanka[8].



Figure 2. Distribution of B. biternata (Encyclopedia of Life, 2014).

### 4. Ethnobotanical importance

The genus *Bidens* has been used in traditional medicine as antiinflammatory, anti-malarial, anti-ulcer, anti-allergic, anti-cancer, anti-diabetic and antibacterial agents<sup>[9]</sup>.

## 4.1. Ethnomedicinal uses

All parts of *B. biternata*, the whole plant, the roots, fresh or dried, and/or the aerial parts (flowers, seeds, leaves and stems) are used as ingredients in folk medicines. Plant has been used as stimulant, anti-inflammatory, febrifuge, diuretic anthelmintic and has wound

healing properties<sup>[10]</sup>. As summarized in Table 2, *B. biternata*, either as a whole plant or different parts, has been reported to be useful in the treatment of many diseases such as toothache, leprosy, fever, cough, asthma, liver infection, diabetes, toothache, cutaneous infections and many others. In China, the whole plant is used for detoxification, respiratory tract infections, blood stasis effect, acute appendicitis, sore throat, acute jaundice, hepatitis, gastroenteritis, malaria, rheumatoid joint pain, topical cure boils, traumatic swellings, snakebite and pain<sup>[11]</sup>.

In Chinese-English Manual of Common-Used Herbs, under guizhencao, *B. biternata* along with *Bidens bipinnata* and *B. pilosa* are listed as source materials in order to clear away the superficial heat for common cold of wind-heat type and prevention of influenza. It also clears away heat and toxic materials for sore throat, appendicitis, snake bite, and centipede bite, diarrhea, dysentery and stomach ache. *Bidens bipmnata*, *B. pilosa* and *B. biternata* are called herba *Bidens* and their decoction together with radix dichroae is anti-inflammatory<sup>[12]</sup>.

## Table 2

Traditional methods of application of B. biternata.

Ailments	Mode of applications	Reference
Cutaneous infections	Poultice of leaf is applied	[10]
Liver infections	Decoction of leaves is used	[13]
Headache	Bruised leaves are applied on forehead	[10]
Cold	Decoction of whole plant is given	[14]
Eye and ear complaints	Juice of fresh leaves is used as eye and ear drops	[15]
Toothache	Roots are chewed	[16]
Cough	Infusion is given	[17]
snake bite	Fresh roots are made into a paste and given to drink	[18]
Pimples	Leaves paste is applied	[19]
Wounds	Leaves are rubbed as a haemostatic	[10]
Diabetes	Decoction of leaves and root is given	[10]
Chronic dysentery		
Eczema	Decoction of whole plant is used	[20]

## 4.2. Other uses

Leaves of the plant are used for combustion<sup>[16]</sup>. *B. biternata* is a wild edible plant brimming with nutrients. Its young shoots are eaten raw or steamed. In times of scarcity of staple food, tribes use this plant as a vegetable<sup>[21]</sup>.

#### 5. Phytochemicals

Phytochemical constituents of a plant are associated with its pharmacological activity<sup>[22]</sup>. The total phenolic content could be used as an indicator of antioxidant properties (Piluzza and Bullitta, 2011). Stems of *B. biternata* have highest concentration of phenols *i.e.* 0.16 mg/g than the root (0.15 mg/g), mature leaf (0.042 mg/g) and young leaf (0.04 mg/g)<sup>[21]</sup>. Another study conducted by Pradeesh *et al.* proved that *B. biternata* have adequate amount of phenol content in stem compared to leaves<sup>[23]</sup>.

High phytic acid content inhibits the absorption of nutrients by the body so it leads to nutrients deficiency[24]. A very low amount of phytic acid is found in *B. biternata i.e.* 0.35[21]. Tannic acid is responsible for decreases in growth rate, feed intake, feed efficiency and protein digestibility[25]. A very low concentration of tannic acid was found only in mature leaf and young leaf of *B. biternata i.e.* 0.02

## 596

and 0.01 (Table 3)[21]. Various alkaloids, glycosides, steroids and tannins were present actively in all parts of plant, but the presence of anthraquinones, phlobatannins, iridoids, *etc.* was not yet detected [21].

#### Table 3

Phenols, phytic acid and tannic acid concentration in different parts of *B. biternata*.

Phytochemicals	Mature leaf	Young leaf	Stem	Root
Phytic acid	0.035	0.034	0.037	0.036
Total phenol	0.042	0.040	0.160	0.150
Tannic acid	0.010	0.020	-	-

#### 6. Compound isolation

Species from genus *Bidens* are rich in quercetin and phenolic compounds (Lastra Valdes, 2001). *Bidens* plants contain chalcone glycosides; okanin is one of the most abundant chalcone (1,3-diphenyl-2-propen-1-one) compounds found in the genus *Bidens* (Figure 3)[26]. In last few decade, compound isolation of various species of genus *Bidens* is done *i.e.* about two hundred compounds have been isolated from *B. pilosa*[27]. But to the best of our knowledge, no study regarding compound isolation of *B. biternata* has been done.



Figure 3. Chemical structures of okanin.

Recently, a new potential allelochemical A,m.f.  $C_{33}H_{40}O_{20}$ , [M<sup>+</sup>] 756, mp.228-231°C has been isolated from ethanolic extract of the stems of *B. biternata* and the structure of the compound was characterized as 5, 7, 8, 4' tetra hydroxy 3, 5' di-methoxy flavone-7-O- $\alpha$ -Lrahmnopranosyl-4'-O- $\beta$ -D-arabinopyranosyl(1 $\rightarrow$ 4)-O- $\beta$ -D-xylopyranoside (Figure 4). Some other unknown chemical compounds are also identified from this plant[28].



Figure 4. Chemical compound isolated from B. biternata[28].

#### 7. Bioactivities

Genus *Bidens* is widely studied for its biological activity and phytochemical<sup>[29]</sup>. Medicinal properties of members of genus *Bidens* are due to polyacetylenic glycosides, aurons, auron glycosides, *p*-coumaric acid derivatives, flavonoids and flavonoid glycosides, sesquiterpenes, phenylpropanoid glucosides and diterpenes<sup>[22,30]</sup>.

## 7.1. Anti-malarial activity

The extensive use of synthetic drugs against pathogens has resulted in drug-resistant mutants. Examples of drug resistance pathogens can be found in the species of the *Plasmodium*, the causative agent of malaria. Genus *Bidens* has significant antimalarial activity which is may be due to the presence of acetylene compounds. All species of genus *Bidens* having aliphatic acetylenes 6-14 each were also very active. However, different extracts of *B. biternata* containing only 3 acetylenes showed only 38% growth inhibition of *Plasmodium falciparum in vitro*. Therefore, it considered to be inactive or have a borderline activity *in vitro*[31].

## 7.2. Antioxidant activity

Free radicals can harm the cellular components by a series of chemical reactions leading to progression of neurodegenerative diseases, cardiovascular disease, cancer and ageing[32,33]. Antioxidant based drugs have appeared during the last 3 decades for prevention and treatment of complex diseases like atherosclerosis, Alzheimer's disease, diabetes, stroke and cancer[34]. Literatures suggest that essential oil from stems, leaves and flowers of genus *Bidens* have significant antioxidant activities[35].

Free radical scavenging activity of crude extract, fractions, and compounds of *B. biternata* using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power assays showed that the butanolic and chloroform extract of *B. biternata* exhibited highest ferric reducing antioxidant power value in the range of 8.5  $\mu$ mol/L/g of extract and in case of DPPH assay the hexane extract of *B. biternata* was the most active extract which showed a significant result with lowest IC<sub>50</sub> = (55 ± 3)  $\mu$ g/mL. However, butanol extract and chloroform extract exhibited very close results of % inhibition with significantly different IC<sub>50</sub> values[35]. Another study conducted by Nair *et al.* revealed that this wild leafy plant possess high free-radical scavenging properties[23]. *B. biternata* extracts effectively reduced the generation of nitric oxide from sodium nitro peroxide and effectively inhibited hydroxyl radicals.

Alzheimer's disease resulting in loss of memory is a neurologic disorder. Controlling the activity of acetylcholinesterase (AChE) through acetylcholine esterase inhibitors is one of the ways to treat this disease. Nine extracts of *B. biternata* were tested for their AChE inhibitory potential by Ellman's colorimetric method. All the extracts of *B. biternata* significantly inhibited AChE. Therefore, this

plant could be a better candidate for AChE inhibitors[35].

## 8. Nutritional values

Young leaf contains high proportion of moisture (0.03%), sugar content (0.08 mg/g), crude fiber (0.55%), total protein (22.1 mg/g), total carbohydrate (16.06 mg/g), vitamins A and E while stem is rich in free fatty acid and lipids[21].

## 8.1. Micronutrients

*B. biternata* is used as an appetizer in various cultures. It contains sufficient quantities of micronutrients like iron, magnesium, manganese, aluminium, zinc and copper. The proportion of manganese, iron, copper and zinc are highest in root as compared with the other plant parts such as mature leaf, young leaf and stem[21].

#### 8.1.1. Total protein

Plant proteins constitute large part of human diet. *B. biternata* is reported to have high concentration of total proteins, especially in their leaves *i.e.* 22.1 mg/g[21]. This is very close to total protein contents in wheat (22–35 mg/g), 30–36 mg/g protein in rice, and 28–42 mg/g in maize[24].

## 8.1.2. Amino acid

Young leaf and stem of *B. biternata* contain high amino acid contents where as among the different amino acids, methionine was found in higher amounts in young leaf (Figure 5). It contains more amounts of proteins, total reducing sugar, carbohydrates, amino acids and crude fiber compared with many leafy vegetables *i.e. Cassia tora* and *Cissus quadrangularis* (Table 4).



Figure 5. Amino acids concentrations in different parts of B. biternata[27].

#### Table 4

A comparison of macronutrient composition between *B. biternata* and other leafy vegetables (mg/g).

Components	Cassia tora	Cissus quadrangularis	B. biternata
Carbohydrates	11.90	12.12	16.06
Protein	5.57	3.97	22.10

It also exhibited very low quantities of total phenols, phytic acid, tannic acid *etc*. (Table 5) in comparison with other reported leafy vegetables *i.e. Sesamum indicum* and *Sesamum aegyptica*. Phytic acid, phenols and tannic acid are known as antinutritional factors<sup>[36]</sup>. These anti-nutritional factors if consumed in large quantity have harmful impact on human beings<sup>[24]</sup>.

#### Table 5

A comparison of phytochemical composition between *B. biternata* and other vegetables (mg/g).

Components	Sesamum indicum	Sesamum aegyptica	B. biternata
Phytic acid	10.9	2.4	0.35
Tannic acid	4.8	4.1	0.02
Total phenol	2.4	2.1	0.16

### 9. Conclusion

In this era of price rise and inflation, this leafy vegetable is not only inexpensive, but also brimming with nutrients like iron, magnesium, manganese, zinc, aluminium and copper and if domesticated and consumed on a regular basis, it can be used to reduce malnutrition (Figure 6). In terms of bioactivities, this plant is rich in antioxidant and has potential to be used in treating Alzheimer's disease but it shows borderline antimalarial activity *in vitro*. However, in order to authenticate the ethnomedicinal uses, no significant work is done on compound isolation and pharmacological properties of this plant. In conclusion, further *in vitro* clinical and pathological studies must be conducted to investigate the unexploited potential of this plant.



Figure 6. Pharmacological and nutritional profile of *B. biternata*.

## **Conflict of interest statement**

We declare that we have no conflict of interest.

### References

- Petrovska BB. Historical review of medicinal plants' usage. *Pharmacogn Rev* 2012; 6(11): 1-5.
- [2] Okigbo RN, Mmeka EC. An appraisal of phytomedicine in Africa. *KMITL Sci Technol J* 2006; 6(2): 83-93.
- Bugno A, Nicoletti MA, Almodóvar AAB, Pereira TC, Auricchio MT. Antimicrobial efficacy of *Curcuma zedoaria* extract as assessed by linear

regression compared with commercial mouthrinses. *Braz J Microbiol* 2007; **38**: 440-5.

- [4] Bhatt JR, Singh JS, Singh SP, Tripathi RS, Kohli RK. Invasive alien plants: an ecological appraisal for the Indian subcontinent. UK: CABI; 2012.
- [5] Yuan LP, Chen FH, Ling L, Dou PF, Bo H, Zhong MM, et al. Protective effects of total flavonoids of *Bidens pilosa* L. (TFB) on animal liver injury and liver fibrosis. *J Ethnopharmacol* 2008; **116**: 539-46.
- [6] Sharma A, Bargali K, Pande N. The allelopathic potential of bryophyte extract on seed germination and seedling growth of *Bidens biternata*. *Nat Sci* 2011; 7(6): 30-8.
- [7] Hyde MA, Wursten BT, Ballings P, Palgrave CM. Flora of Zimbabwe.
  [Online] Available from: http://www.zimbabweflora.co.zw/speciesdata/ species.php?species\_id=126760 [Accessed on 12th April, 2015]
- [8] United States Department of Agriculture. Taxon: *Bidens biternata* (Lour.) Merr. & Sherff. Beltsville: United States Department of Agriculture; 2012. [Online] Available from: http://www.ars-grin.gov/cgibin/npgs/html/taxon.pl?428099 [Accessed on 12th April, 2015]
- [9] Newton SM, Lau C, Wright CW. A review of antimyocobacterial natural products. *Phytother Res* 2000; 14: 303-22.
- [10] Panda H. Medicinal plants cultivation and their uses. Delhi: Asia Pacific Business Press Inc.; 2002.
- [11] Shi Z, Chen YL, Chen YS, Lin YR, Liu SW, Ge XJ, et al. Asteraceae (Compositae). In: Wu ZY, Raven PH, Hong DY, editors. *Flora of China Volume 20-21 (Asteraceae)*. Beijing and St. Louis: Science Press and Missouri Botanical Garden; 2007.
- [12] Borten P. Chinese herbs: a digital materia medica compilation. [Online] Available from: http://chineseherbinfo.com/chineseherbinfo.html [Accessed on 12th April, 2015]
- [13] Nanda Y, Singson N, Rao AN. Ethnomedicinal plants of Thadou tribe of Manipur (India) -1. *Pleione* 2013; 7(1): 138-45.
- [14] Shah S, Ram J, Pala AN, Tripathi P, Kumar M. Medicinal plant wealth of oak dominated forests in Nainital catchment area of Uttarakhand. *Acad J Med Plants* 2014; 2(1): 6-13.
- [15] Ghosh G, Ghosh DC, Melkania U, Majumdar U. Traditional medicinal plants used by the Adi, Idu and Khamba tribes of Dehang-Debang Biosphere Reserve in Arunachal Pradesh. *Int J Agric Environ Biotechnol* 2014; 7(1): 165-71.
- [16] Priyadi H, Takao G, Rahmawati I, Supriyanto B, Nursal WI, Rahman I. Five hundred plant species in Gunung Halimun Salak National Park, West Java: a checklist including Sundanese names, distribution and use. Bogor: CIFOR; 2010.
- [17] Bhat JA, Kumar M, Bussmann RW. Ecological status and traditional knowledge of medicinal plants in Kedarnath Wildlife Sanctuary of Garhwal Himalaya, India. *J Ethnobiol Ethnomed* 2013; 9: 1.
- [18] Kadel C, Jain AK. Folklore claims on snakebite among some tribal communities of Central India. *Indian J Tradit Know* 2008; 7(2): 296-9.
- [19] Kala CP. Current status of medicinal plants used by traditional Vaidyas in Uttaranchal state of India. *Ethnobot Res Appl* 2005; **3**: 267-78.
- [20] Sasidharan N, Bhasha SC, Renuka C. Botanical studies in the medicinal plant conservation areas in Kerala. KFRI Research Report 99. Thrissur:

Kerala Forest Research Institute Peechi; 1994.

- [21] Sukumaran P, Nair AG, Chinmayee DM, Mini I, Sukumaran ST. Phytochemical investigation of *Bidens biternata* (Lour.) Merr. and Sheriff.-a nutrient-rich leafy vegetable from Western Ghats of India. *Appl Biochem Biotechnol* 2012; **167**: 1795-801.
- [22] Bairwa K, Kumar R, Sharma RJ, Roy RK. An updated review on *Bidens pilosa* L. Der Pharma Chemica 2010; 2(3): 325-37.
- [23] Swapna TS, Nair AG, Mini I, Pradeesh S. Free-radical scavenging activity of leaves of *Bidens Biternata* (Lour.) Merr. & Sherif. *Int J Pharm Res Dev* 2014; 6(8): 127-35.
- [24] Bos C, Juillet B, Fouillet H, Turlan L, Dare S, Luengo C, et al. Postprandial metabolic utilization of wheat protein in humans. *Am J Clin Nutr* 2005; 81: 87-94.
- [25] Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: a review. *Crit Rev Food Sci Nutr* 1998; **38**(6): 421-64.
- [26] Kill JS, Son Y, Cheong YK, Kim NH, Jeong HJ, Kwon JW, et al. Okanin, a chalcone found in the genus *Bidens*, and 3-penten-2-one inhibit inducible nitric oxide synthase expression via heme oxygenase-1 induction in RAW264.7 macrophages activated with lipopolysaccharide. *J Clin Biochem Nutr* 2012; **50**(1): 53-8.
- [27] Lastra Valdes HA. Bidens pilosa Linné. Rev Cuba Planta Med 2001; 1: 28-33.
- [28] Surywanshi V, Yadava RN. Isolation and characterization of new potential allelochemical from *Bidens biternata* (Lour.) Merrill & Sherff. *J Chem Pharm Res* 2015; 7(4): 175-9.
- [29] Brandao MG, Krettli AU, Soares LS, Nery CG, Marinuzzi HC. Antimalarial activity of extracts and fractions from *Bidens pilosa* and other *Bidens* species (Asteraceae) correlated with the presence of acetylene and flavonoid compounds. *J Ethnopharmacol* 1997; 57(2): 131-8.
- [30] Zulueta MCA, Tada M, Ragasa CY. A diterpene from *Bidens pilosa*. *Phytochemistry* 1995; **38**: 1449-50.
- [31] Alonzo DS, Hildebrand JW. Bidens L. In: de Padua LS, Bunyapraphatsara N, Lemmens RHM, editors. Plant resources of South-East Asia. Leiden: Backhuys Publishers; 1999, p. 150-5.
- [32] Chiang YM, Chuang DY, Wang SY, Kuo YH, Tsai PW, Shyur LF. Metabolite profiling and chemopreventive bioactivity of plant extracts from *Bidens pilosa*. J Ethnopharmacol 2004; 95(2-3): 409-19.
- [33] Muchuweti M, Mupure C, Ndhlala AR, Murenje T, Benhura MAN. Screening of antioxidant and radical scavenging activity of *Vigna* unguiculata, Bidens pilosa and Cleome gynandra. Am J Food Technol 2007; 2(3): 161-8.
- [34] Devasagayam TP, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. Free radicals and antioxidants in human health: current status and future prospects. *J Assoc Physicians India* 2004; **52**: 794-804.
- [35] Yang HL, Chen SC, Chang NW, Chang JM, Lee ML, Tsai PC. Protection from oxidative damage using *Bidens pilosa* extracts in normal human erythrocytes. *Food Chem Toxicol* 2006; 44: 1513-21.
- [36] Schlemmer U, Frølich W, Prieto RM, Grases F. Phytate in foods and significance for humans: food sources, intake, processing, bioavailability, protective role and analysis. *Mol Nutr Food Res* 2009; **53**: S330-75.