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Saponins: Properties, Applications and Processing

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Saponins are a diverse group of compounds widely distributed in the plant kingdom, which are characterized by their structure containing a triterpene or steroid aglycone and one or more sugar chains. Consumer demand for natural products coupled with their physicochemical (surfactant) properties and mounting evidence on their biological activity (such as anticancer and anticholesterol activity) has led to the emergence of saponins as commercially significant compounds with expanding applications in food, cosmetics, and pharmaceutical sectors. The realization of their full commercial potential requires development of new processes/processing strategies to address the processing challenges posed by their complex nature. This review provides an update on the sources, properties, and applications of saponins with special focus on their extraction and purification. Also reviewed is the recent literature on the effect of processing on saponin structure/properties and the extraction and purification of sapogenins.

Keywords Triterpenes, sapogenins, ginsenosides, health products, surfactants, extraction

INTRODUCTION

Saponins, glycosides widely distributed in the plant kingdom, include a diverse group of compounds characterized by their structure containing a steroidal or triterpenoid aglycone and one or more sugar chains. Their structural diversity is reflected in their physicochemical and biological properties, which are exploited in a number of traditional (as soaps, fish poison, and molluscicides) and industrial applications (Price et al., 1987; Oakenfull, 1981; Fenwick et al., 1991; Hostettmann and Marston, 1995; Oakenfull and Sidhu, 1989). While plant extracts containing saponins have been widely used in food and other industrial applications mainly as surface active and foaming agents (San Martin and Briones, 1999); saponins in foods have traditionally been considered as "antinutritional factors" (Thompson, 1993) and in some cases have limited their use due to their bitter taste (Ridout et al., 1991). Therefore, most of the earlier research on processing of saponins targeted their removal to facilitate human consumption (Khokhar and Chauhan, 1986; Ridout et al., 1991). However, food and non-food sources of saponins have come into renewed focus in recent years due to increasing evidence of their health benefits such as cholesterol lowering and anticancer properties (Gurfinkel and Rao, 2003; Kim et al., 2003b). Recent research has established saponins as the active components in many herbal medicines (Liu and Henkel, 2002; Alice et al., 1991) and highlighted their contributions to the health benefits of foods such as soybeans (Kerwin, 2004; Oakenfull, 2001) and garlic (Matsuura, 2001).

The commercial potential of saponins has resulted in the development of new processes/processing strategies and reevaluation of existing technologies (Muir et al., 2002) for their extraction/concentration (Rickert et al., 2004b). The objective of this review is to provide a timely update on the sources, properties and applications of saponins with special focus on their extraction and purification.

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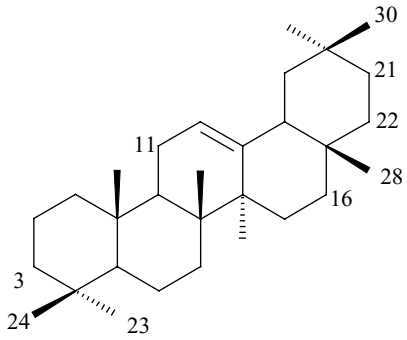
SOURCES

The presence of saponins has been reported in more than 100 families of plants, and in a few marine sources such as star fish and sea cucumber (Hostettmann and Marston, 1995). The steroidal saponins are mainly found in monocotyledons (such as *Agavaceae, Dioscoreaceae* and *Liliaceae*), and triterpene saponins are predominantly present in dicotyledons (*Leguminosae, Araliaceae, Caryophyllaceae*) (Sparg et al., 2004). While the main dietary sources of saponins are legumes (soybeans,

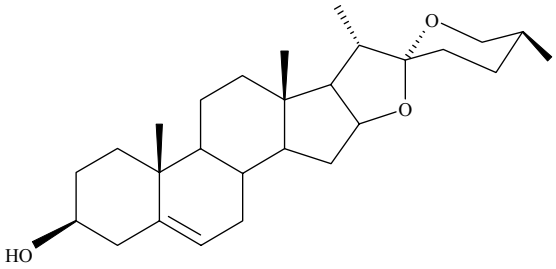
(A) AGLYCONES

Triterpene aglycones



Aglycone	-OH	=O	-COOH
Glycyrrhetinic acid	3β	11	30
Gypsogenin	3β	23	28
Oleanolic acid	3β		28
Quillaic acid	3β, 16α	23	28
Soyasapogenol A	3β, 21β, 22β, 24		
Soyasapogenol B	3β, 22β, 24		
Soyasapogenol E	3β, 24	22	

Steroid aglycone
Diosgenin



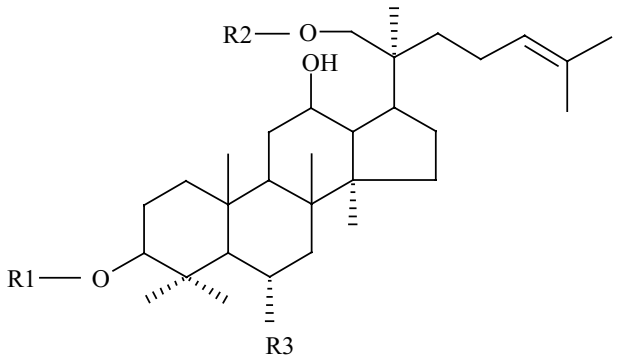
(B) SOYA SAPONINS

Soya saponin	Soya sapogenol	Structure[a]
Group A		
Aa	A	glc(1→2)gal(1→2)glcUA(1→3)**A**(22←1)ara(3←1)xyl(2,3,4-tri-*O*-Acetyl)
Ab	A	glc(1→2)gal(1→2)glcUA(1→3) **A**(22←1)ara(3←1) glc(2,3,4,6-tetra-*O*-Acetyl)
Ac	A	rha(1→2)gal(1→2)glcUA(1→3) **A**(22←1)ara(3←1) glc(2,3,4,6-tetra-*O*-Acetyl)
Ad	A	glc(1→2)ara(1→2)glcUA(1→3) **A**(22←1)ara(3←1) glc(2,3,4,6-tetra-*O*-Acetyl)
Ae	A	gal(1→2)glcUA(1→3) **A**(22←1)ara(3←1)xyl(2,3,4-tri-*O*-Acetyl)
Af	A	gal(1→2)glcUA(1→3) **A**(22←1)ara(3←1) glc(2,3,4,6-tetra-*O*-Acetyl)
Ag	A	ara(1→2)glcUA(1→3) **A**(22←1)ara(3←1)xyl(2,3,4-tri-*O*-Acetyl)
Ah	A	ara(1→2)glcUA(1→3) **A**(22←1)ara(3←1) glc(2,3,4,6-tetra-*O*-Acetyl)
Group B		
Ba	B	glc(1→2)gal(1→2)glcUA(1→3)**B**
Bb	B	rha(1→2)gal(1→2)glcUA(1→3)**B**
Bc	B	rha(1→2)ara(1→2)glcUA(1→3)**B**
Bb'	B	gal(1→2)glcUA(1→3)**B**
Bc'	B	ara(1→2)glcUA(1→3)**B**
Group E		
Bd	E	glc(1→2)gal(1→2)glcUA(1→3)**E**
Be	E	rha(1→2)gal(1→2)glcUA(1→3)**E**
DDMP		
αg	B_{DDMP}[b]	glc(1→2)gal(1→2)glcUA(1→3)$\mathbf{B_{DDMP}}$
βg	B_{DDMP}[b]	rha(1→2)gal(1→2)glcUA(1→3)$\mathbf{B_{DDMP}}$
βa	B_{DDMP}[b]	rha(1→2)ara(1→2)glcUA(1→3)$\mathbf{B_{DDMP}}$
γg	B_{DDMP}[b]	gal(1→2)glcUA(1→3)$\mathbf{B_{DDMP}}$
γa	B_{DDMP}[b]	ara(1→2)glcUA(1→3)$\mathbf{B_{DDMP}}$

[a] glc:D-glucose, ara:L-arabinose, gal:D-galactose, glcUA:D-glucuronic acid, xyl:D-xylose, rha: L-rhamnose
[b] B_{DDMP}: DDMP (2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one) attached through an acetal linkage to the C-22 hydroxyl of soyasapogenol B

Figure 1 Structure of (A) aglycones (Hostettman and Marston, 1995), (B) soyasaponins (Berhow et al., 2002; Gu et al., 2002), (C) ginsenosides (Li et al., 1996), (D) glycyrrhizic acid (Ong and Len, 2003), and (E) quillaja saponins (Reprinted from Nord and Kenne, 2000, Copyright (2002) with permission from Elsevier). (Continued)

(C) GINSENOSIDES



Ginsenosides	R1	R2	R3
Rb_1	-glc[2→1]glc	-glc[6→1]glc	-H
Rb_2	-glc[2→1]glc	-glc[6→1]ara(p)	-H
Rc	-glc[2→1]glc	-glc[6→1]ara(f)	-H
Rd	-glc[2→1]glc	-glc	-H
Re	-H	-glc	-O-glc[2→1]rha
Rf	-H	-H	-O-glc[2→1]glc
Rg_1	-H	-glc	-O-glc

glc: D-glucose, ara(p): L-arabinopyranose, ara(f): L-arabinofuranose, rha: L-rhamnose

(D) GLYCYRRHIZIC ACID (GLYCYRRHIZIN)

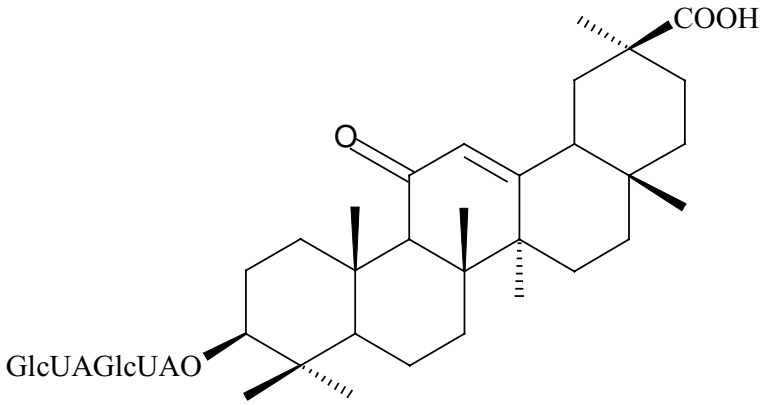


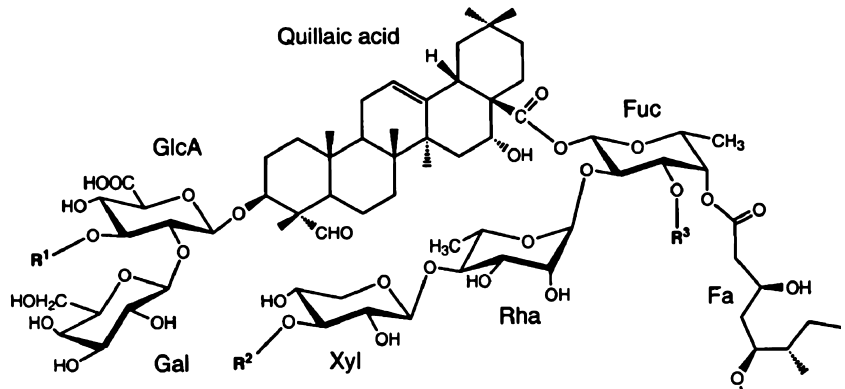
Figure 1 (Continued)

chickpeas, mungbeans, peanuts, broad beans, kidney beans, lentils), they are also present in oats, allium species (leek, garlic), asparagus, tea, spinach, sugarbeet, and yam (Price et al., 1987). Soap bark tree (*Quillaja saponaria*), fenugreek *(Trigonella foenum-graceum)*, alfalfa (*Medicago sativa*), horse chestnut (*Aesculus hippocastanum*), licorice (Glycyrrhiza species such as *Glycyrrhiza glabra*), soapwort (*Saponaria officinalis)*, Mojave yucca (*Yucca schidigera*), gypsophila genus (such as *Gypsophila paniculata*), sarsaparilla (*Smilax regelii* and other closely related species of Smilax genus) and ginseng (Panax genus) are the main non-food sources of saponins used in health and industrial applications (Hostettmann and Marston, 1995; Balandrin, 1996).

A single plant species may contain a complex mixture of saponins. For example, the characterized soybean saponins include three groups of compounds: soyasaponins A, B and E categorized according to the soyasapogenol in their structure (Figure 1B). Similarly ginseng contains a mixture of saponins (ginsenosides), the main components of which are Rb_1, Rb_2, Rc, Rd, Re, Rf, and Rg_1 (Figure 1C). Commonly used plant sources and their main saponins are presented in Table 1.

The saponin content of plant materials is affected by the plant species, genetic origin, the part of the plant being examined, the environmental and agronomic factors associated with growth of the plant, and post-harvest treatments such as storage and processing (Fenwick et al., 1991) (Table 2).

(E) QUILLAJA SAPONINS



	R^1	R^2	R^3
S1	α-L-Rha*p*	H	H
S2	β-D-Xyl*p*	H	H
S3	α-L-Rha*p*	β-D-Xyl*p*	H
S4	β-D-Xyl*p*	β-D-Xyl*p*	H
S5	α-L-Rha*p*	β-D-Api*f*	H
S6	β-D-Xyl*p*	β-D-Api*f*	H
S7	α-L-Rha*p*	H	Acetyl
S8	β-D-Xyl*p*	H	Acetyl
S9	α-L-Rha*p*	β-D-Xyl*p*	Acetyl
S10	β-D-Xyl*p*	β-D-Xyl*p*	Acetyl
S11	α-L-Rha*p*	β-D-Api*f*	Acetyl
S12	β-D-Xyl*p*	β-D-Api*f*	Acetyl

Figure 1 (Continued)

STRUCTURE AND PROPERTIES

Structure

Saponins are glycosides containing one or more sugar chains on a triterpene or steroid aglycone backbone also called a sapogenin (Figure 1). They are categorized according to the number of sugar chains in their structure as mono, di-, or tridesmosidic. Monodesmosidic saponins have a single sugar chain, normally attached at C-3. Bidesmosidic saponins have two sugar chains, often with one attached through an ether linkage at C-3 and one attached through an ester linkage at C-28 (triterpene saponins) or an ether linkage at C-26 (furastanol saponins). The most common monosaccharides include: D-glucose (Glc), D-galactose (Gal), D-glucuronic acid (GlcA), D-galacturonic acid (GalA), L-rhamnose (Rha), L-arabinose (Ara), D-xylose (Xyl), and D-fucose (Fuc). The nature of the aglycone and the functional groups on the aglycone backbone and number and nature of the sugars can vary greatly resulting in a very diverse group of compounds (Figure 1; Price et al., 1987; Hostettmann and Marston, 1995).

Properties

Physicochemical Properties

The structural complexity of saponins results in a number of physical, chemical, and biological properties, only a few of which are common to all members of this diverse group. Properties of a few selected aglycones and saponins are summarized in Table 3.

Due to the presence of a lipid-soluble aglycone and water-soluble sugar chain(s) in their structure (amphiphilic nature), saponins are surface active compounds with detergent, wetting, emulsifying, and foaming properties (Wang et al., 2005; Sarnthein-Graf and La Mesa, 2004; Mitra and Dungan, 1997; Ibanoglu and Ibanoglu, 2000). In aqueous solutions surfactants form micelles above a critical concentration called critical micelle concentration (cmc). Saponins, including soybean saponins, saponins from *Saponaria officinalis,* and *Quillaja saponaria*, form micelles in aqueous solutions, the size and structure of which are dependent on type of saponin (Oakenfull, 1986). The micelle forming properties (cmc and the aggregation number (number of monomers in a micelle)) of quillaja

Table 1 Selected plant sources and their constituent saponins

Source	Aglycone	Saponin	Reference
Soybean	Soyasapogenol A	Acetyl soyasaponins A_1 (Ab), A_2 (Af), A_3, A_4 (Aa), A_5 (Ae), A_6, A_c, A_d	Yoshiki et al., 1998
	Soyasapogenol B	Soyasaponin DDMP[a] conjugated I (Bb) βg II (Bc) βa III (Bb′) γg IV (Bc′) γa V (Ba) αg	Yoshiki et al., 1998
	Soyasapogenol E	Soyasaponin Be, Bd	Yoshiki et al., 1998
Chickpea	Soyasapogenol B	DDMP[a] conjugated saponins	Kerem et al., 2005; Price et al., 1988
Quillaja	Quillaic acid	QS 1-22, S1-12	Kensil and Marciani, 1991; Nord and Kenne, 2000
Horse chestnut	Protoescigenin, barringtogenol C	Aescin (escin): β-aescin, cryptoaescine, α-aescine	World Health Organization, 2001
Alfalfa	Medicagenic acid	I-XV	Oleszek, 1995
	Hederagenin	XVI-XIX	Oleszek, 1995
	Soyasapogenol B, E	XX- XXVI	Oleszek, 1995
	Zanhic acid	XXV-XXVI	Oleszek, 1995
Licorice	Glycyrrhetic acid	Glycyrrhizic acid[b]	World Health Organization, 1999a
Ginseng	20(s)-protopanaxadiol	Ra_{1-3}, Rb_{1-3}, Rc, Rc_2, Rd, Rd_2, Rh_2	World Health Organization, 1999b
	20(s)-protopanaxatriol	Re_2, Re_3, Rf, Rg_1, Rg_2, Rh_1	World Health Organization, 1999b
Quinoa	Phytolaccagenic acid Oleanolic acid Hederagenin	Quinoa saponins	Mizui et al., 1990
Oat	Nuategenin	Avenacoside A, B	Önning et al., 1994
Yam (Dioscoera species)	Diosgenin	Dioscin	Hostettmann and Marston, 1995
Fenugreek	Diosgenin, yamogenin, tigogenin, neotigogenin, yuccagenin, lilagenin, gitogenin, neogitogenin, smilagenin, sarsasapoenin	Trigofoenoside A-G, Trigonelloside B (C)	Sauvaire et al., 1995

[a] 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one.
[b] Synonyms: glycyrrhizin, glycyrrhizinic acid.

saponins were affected by temperature, salt concentration, and pH of the aqueous phase (Mitra and Dungan, 1997). At 25°C, the values of cmc of quillaja saponins were in the range of 0.5 and 0.8 g/L. It increased with temperature and pH but decreased with increasing salt concentration. The incorporation of cholesterol into the saponin micelles increased their cmc, size, viscosity, and the aggregation number (Mitra and Dungan, 2000) resulting in the solubility enhancement of cholesterol as much as a factor of 10^3 at room temperature (Mitra and Dungan, 2001).

Quillaja saponins also had a solubilizing effect on phenantherene, and fluoranthene, which increases linearly with saponin concentration at values higher than cmc (Soeder et al., 1996). A similar linear relationship has been observed between the concentration of the saponin extract from *Sapindus mukurossi* and aqueous solubility of hexachlorobenzene and naphthalene up to a surfactant concentration of 10% (Kommalapati et al., 1997; Roy et al., 1997).

Solubility enhancement has also been observed for Yellow OB (Nakayama et al., 1986), and progesterone (Nakayama et al., 1986) in the presence of bidesmoside saponins from *Sapindus mukurossi*, and for α-tocopherol, and oleanolic acid in the presence of glucoside and glucuronide esters of glycyrrhizic acid (Sasaki et al., 1988). Purified saponins and saponin mixtures resulted in both enhancements and reductions in water solubility of test compounds quercetin (Schöpke and Bartlakowski, 1997), digitoxin (Walthelm et al., 2001), rutin (Walthelm et al., 2001), and aesculin (Walthelm et al., 2001), the extent of which was determined by concentration of saponin and the model compound. Solubility enhancement of quercetin obtained by pure

Table 2 Saponin content of some selected plant materials

Source	Saponin content (%)	Reference
Soybean	0.22–0.47	Fenwick et al., 1991
Chickpea	0.23	Fenwick et al., 1991
Green pea	0.18–4.2	Price et al., 1987
Quillaja bark	9–10	San Martin and Briones, 1999
Yucca	10	Oleszek et al., 2001
Fenugreek	4–6	Sauvaire et al., 2000
Alfalfa	0.14–1.71	Fenwick et al., 1991
Licorice root	22.2–32.3	Fenwick et al., 1991
American ginseng *(P. quinquefolium L).*		
Young leaves	1.42–2.64	Li et al., 1996
Mature leaves	4.14–5.58	Li et al., 1996
Roots (4 year old)	2.44–3.88	Li et al., 1996
Oat	0.1–0.13	Price et al., 1987
Horse chest nut	3–6	Price et al., 1987
Sugar beet leaves	5.8	Price et al., 1987
Quinoa	0.14–2.3	Fenwick et al., 1991

Table 3 Physical properties of some selected aglycones and saponins (Adapted from Budavari et al., 1996; Biran and Baykut, 1975)

Compound	Formula	Solubility	Source	MW	MP
Aglycone					
Oleanolic acid	$C_{30}H_{48}O_3$	Insoluble in water, sol in 65 parts ether, 106 parts 95% alcohol, 35 parts boiling 95% alcohol, 118 parts chloroform, 180 parts acetone, 235 parts methanol.	Quinoa	457	310
Quillaic acid	$C_{30}H_{46}O_5$	Soluble in alcohol, ether, acetone, ethyl acetate, glacial acetic acid	Quillaja	487	292–293
Diosgenin	$C_{27}H_{42}O_3$	Soluble in the usual organic solvents, in acetic acid	Dioscorea, fenugreek, yam	415	204–207
Glycyrrhetic acid	$C_{30}H_{46}O_4$		Licorice	471	298–300
Saponin					
Glycyrrhizic acid (Glycyrrhizin)	$C_{42}H_{62}O_{16}$	Freely soluble in hot water, alcohol, practically insoluble in ether	Licorice	823	
Escin			Horse chestnut		
α-escin		Very soluble in water and methanol, only slightly soluble in acetone, insoluble in ether and hydrocarbons			225–227
β-escin		Readily soluble in methanol, slightly soluble in acetone, practically insoluble (very little solubility) in water, insoluble in ether and hydrocarbons			222–223
Gypsophia saponin	$C_{35}H_{61}O_{24}$	Soluble in water (0.5147 g/100 mL at 25°C)	Gypsophia	863	221–227

saponins at concentrations > cmc values can be attributed to micellar solubilization, whereas solubilization effect of some saponin mixtures at concentrations < cmc points to an alternative mechanism (Schöpke and Bartlakowski, 1997).

Purified saponins or saponin mixtures may also have a solubilizing effect on other saponins. Solubility enhancement of monodesmosides (such as monodesmosides of *Sapindus mukurossi* (Nakayama et al., 1986; Kimata et al., 1983), *Bupleuri radix* (saikosaponins) (Kimata et al., 1985; Morita et al., 1986; Watanabe et al., 1988) and soyasaponins Bb, Bb′ and B-G (Shimoyamada et al., 1993)), which have very low water solubility, in the presence of bidesmoside saponins is well documented. The extent of the enhancement is dependent on the structure of the monodesmoside saponin, and the composition/concentration of the saponin bidesmosides. Solubility of *Sapindus mukurossi* monodesmosides was enhanced in the presence of mukurossi bidesmoside saponins containing hederagenin (Y1, Y2, X) (Nakayama et al., 1986; Kimata et al., 1983). However, mukurossi bidesmosides did not affect the solubility of saikosaponins (Kimata et al., 1985), which was enhanced by oleanolic acid bidesmosides with a glucuronide moiety such as ginsenosides (chikusetsusaponin-V (ginsenoside Ro) and IV) (Kimata et al., 1985; Watanabe et al., 1988), *Hemsleya macrosperma* (cucurbitaceae) bidesmosides (Ma2 and Ma3) (Morita et al., 1986), and cyclic bidesmoside tubeimoside I isolated from tubers of *Bolbostemma paniculatum* Franquet (Kasai et al., 1986b).

The solubility of saikosaponin-a in water at 37°C (0.14 mg /mL) increased with concentration of ginsenosi de Ro reaching a value of 4.08 mg/mL at a bidesmoside concentration of 1.4 mg/mL (Kimata et al., 1985). A significant decrease in the solubilizing effect on saikosaponin-a was observed upon methylation or reduction of the glucuronide carboxyl group of ginsenoside-Ro indicating the role of the glucuronide moiety in the observed effect (Tanaka, 1987). A greater extent of enhancement was obtained for *Hemsleya macrosperma* (cucurbitaceae) bidesmosides Ma2 and Ma3, which are structurally similar to ginsenoside Ro with similar cmc values, at a concentration of 0.1% resulting in saikosaponin-a solubilities of 5–8.7 mg/mL compared to 3.4 mg/mL for ginsenoside Ro (Morita et al., 1986). The solubility enhancement of saikosaponin-a became apparent near the cmc of these bidesmosides (Kimata et al., 1985; Morita et al., 1986; Nakayama et al., 1986).

The solubility of diene saponin saikosaponin-b1 produced by heating or mild-acid treatment of saikosaponin-a was increased by malonyl-ginsenosides and to a lesser extent by ginsenoside Ro (Zhou et al., 1991). The effect of malonyl-ginsenosides on saikosaponin-a has also been demonstrated (Zhou et al., 1991). While neutral dammarane ginsenosides did not have a solubilizing effect on saikosaponins by themselves, they enhanced the solubilizing effect of ginsenoside Ro (Watanabe et al., 1988) and dammarane ginsenosides (Zhou et al., 1991).

Solubility enhancement of saikosaponin-a has also been observed in the presence of glycyrrhizic acid, which is the glucuronide monodesmoside saponin of licorice (Sasaki et al., 1988). The decrease in the degree of enhancement observed at high glycyrrhizic acid concentrations was attributed to the increase in solution viscosity (Sasaki et al., 1988). A solubilizing effect was also observed for the 30-β-glucoside (isolated

from licorice roots) and glucuronide esters of glycyrrhizic acid at higher concentrations (Sasaki et al., 1988). In addition to bidesmosides, co-occuring compounds such as acyclic sesquiterpene oligoglycosides have also been shown to have a solubilizing effect on monodesmosides of *Sapindus mukurossi* (Kasai et al., 1986a) and *Sapindus delavayi* (Wong et al., 1991).

Solubility enhancement may have important implications for the bioactivity and processing of saponins. Monodesmosides, while poorly soluble in water in purified form, can be extracted readily due to the solubilizing effect of co-occuring compounds (Kimata et al., 1983). Micellar solubilization by saponins can be exploited for the development of micellar extraction processes or to affect the solubilization of ingredients in cosmetic, pharmaceutical or food formulations (Shirakawa et al., 1986).

Solubility of saponins is also affected by the properties of the solvent (as affected by temperature, composition, and pH). While water, alcohols (methanol, ethanol) and aqueous alcohols are the most common extraction solvents for saponins, solubility of some saponins in ether, chloroform, benzene, ethyl acetate, or glacial acetic acid has also been reported (Hostettmann and Marston, 1995). In the ethanol concentration range of 30–100%, solubility of soyasaponin Bb (soyasaponin I) was maximum in 60% ethanol (Shimoyamada et al., 1993). Solubility of gypsophia saponin in water increased with temperature from 7.4 g/100 mL at 30°C to 18.0 g/100 mL at 70°C (Biran and Baykut, 1975). A sharp increase was observed in the solubility of soyasaponin Bb, which was very low in the acidic region, in the pH range 6.5–7.3 (Shimoyamada et al., 1993). The degree of partitioning of components of crude 70% ethanol extract of soybeans between water and butanol was dependent on the concentration of the extract and pH of the aqueous phase (Shimoyamada et al., 1995). The highest recovery of soyasaponin I in the butanol layer was obtained using 0.04 g/mL of crude extract in the acidic region (about pH 4) (Shimoyamada et al., 1995).

While bitterness is the most common sensory attribute associated with saponins (Price et al., 1985), the occurrence of sweet saponins is also well known (Kennelly et al., 1996). For example, the sweetness of licorice is attributed to its main saponin, glycyrrhizic acid (Figure 1), which is 50 times sweeter than sugar (Muller and Morris, 1966).

The complex structure of saponins may undergo chemical transformations during storage or processing which in turn may modify their properties/activity. The glycosidic bond (between the sugar chain and the aglycone), and the interglycosidic bonds between the sugar residues can undergo hydrolysis in the presence of acids/alkali, due to hydrothermolysis (heating in presence of water) or enzymatic/microbial activity resulting in the formation of aglycones, prosapogenins, sugar residues or monosaccharides depending on the hydrolysis method and conditions (Hostettmann and Marston, 1995). Complete acid hydrolysis yields the constituent aglycone and monosaccharides, whereas under basic hydrolysis conditions, cleavage of O-acylglycosidic sugar chains results in the formation of prosapogenins (Hostettmann and Marston, 1995). The solubility behavior of the parent aglycone can be markedly different than the saponin due to its lipophilic nature (Table 3). DDMP (2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one) conjugated saponins, which were determined to be the genuine saponins in intact soybeans, are hydrolyzed into Group B and E saponins upon heating, in alkaline solutions, and in the presence of iron (Kudou et al., 1993; Okubo and Yoshiki, 1995). Soyasaponin βg, which was stable in acidic solution and at temperatures $< 90°$C, was converted into soyasaponin Bb at basic pH and upon heating at 90–100°C (Okubo and Yoshiki, 1995). In the presence of $FeCl_3$, it was degraded into soyasaponin Be and Bb in a ratio of 3:2 (Okubo and Yoshiki, 1995). Deacylation of quillaja saponins was observed upon storage in aqueous solution at pH > 6 (Okubo and Yoshiki, 1995).

The interaction of sterols (Gestetner et al., 1971, 1972; Walter et al., 1954; Shany et al., 1970), minerals (West et al., 1978), and proteins (Potter et al., 1993; Tanaka et al., 1995) with saponins may result in the modification of the physicochemical properties and biological activity of these compounds. Steroid saponins (such as digitonin (Gestetner et al., 1972), alfalfa saponins (Walter et al., 1954)), and triterpenoid saponins (such as lucerne (Gestetner et al., 1971, 1972; Shany et al., 1970)) form water-insoluble addition products with cholesterol and phytosterols such as sitosterol and stigmasterol. Interaction of sterols and lucerne saponins was dependent on the structure of the saponin and sterols (Gestetner et al., 1971, 1972). While cholesterol and β-sitosterol formed complexes with lucerne saponins containing medicagenic acid, which possess carboxyl groups at C23 and C28 positions, saponins with soyasapogenol aglycones did not precipitate (Gestetner et al., 1971). Insoluble complexes were also formed between ammoniated glycyrrhizic acid and alfalfa root saponins and the minerals zinc and iron (West et al., 1978).

The nature and effect of the saponin-protein interaction were dependent on the type of protein (Potter et al., 1993) and the type of the saponin mixture (Tanaka et al., 1995). Upon heating at 78°C (upto 26 min) quillaja saponin interacted with casein to form high molecular weight complexes, whereas soybean proteins formed insoluble aggregates independent of saponin addition (Potter et al., 1993). Similarly, while heating salt soluble proteins from walleye pollack meat at 40–100°C for upto 10 min in the presence of quillaja saponins increased protein aggregation, tea seed saponins inhibited the aggregation of the protein (Tanaka et al., 1995). Complex formation between beet saponin and protein (as evidenced by turbidity and interfacial tension measurements) and destabilization of a model dispersion of sucrose, oil, saponin, and protein in acidic conditions point to the role of beet saponin and protein in the formation of acid beverage floc in sucrose-sweetened carbonated soft drinks and acidified syrups (Morton and Murray, 2001).

The interaction of saponins and proteins also resulted in modifications of protein properties such as heat and enzyme stability (Ikedo et al., 1996; Shimoyamada et al., 1998), and surface properties (Chauhan et al., 1999). Heat stability of bovine serum albumin (BSA) (Ikedo et al., 1996), and resistance of BSA (Ikedo et al., 1996) and soybean protein (Shimoyamada et al., 1998) to chymotryptic hydrolyses improved upon addition of soybean

saponins. The stability of whey proteins to chymotryptic hydrolyses however decreased upon addition of soybean saponins (Shimoyamada et al., 2000). Similarly, unlike soybean protein whose sensitivity to tryptic hydrolysis improved, whey proteins showed higher sensitivity in the presence of soya saponins (Shimoyamada et al., 2000). The influence of soybean saponin on the trypsin hydrolysis of bovine milk α-lactalbumin was attributed to the modification of the protein's tertiary structure (Shimoyamada et al., 2005). Desaponization of quinoa protein increased water hydration capacity and lowered the fat binding and buffer capacity, and total nitrogen solubility (Chauhan et al., 1999). Removal of saponins reduced the emulsion and foaming capacity of the proteins but increased the stability of the foams and emulsions (Chauhan et al., 1999).

Biological Activity

Saponins have been reported to possess a wide range of biological activities, which are summarized and listed alphabetically in Table 4 (Hostettmann and Marston, 1995; Lacaille-Dubois and Wagner, 1996; Milgate and Roberts, 1995; Francis et al., 2002). While crude isolates, extracts, and saponin-containing plants have been utilized in the investigation of biological activity, especially in the earlier studies, developments in the isolation/purification and characterization techniques have enabled the investigation of the bioactivity of well characterized saponins and led to the emergence of structure and bioactivity relationships (Oda et al., 2000; Gurfinkel and Rao, 2003).

The ability of saponins to swell and rupture erythrocytes causing a release of haemoglobin (the *in vitro* haemolytic activity) has been one of the most investigated properties of saponins (Oda et al., 2000). However, even for this activity, which has been related to the saponin structure (type of aglycone and the presence of sugar side chains), there is no apparent consistency between members of this diverse group (Oda et al., 2000).

The toxicity of saponins to insects (insecticidal activity), parasite worms (anthelmintic activity), molluscs (molluscicidal), and fish (piscidal activity) and their antifungal, antiviral, and antibacterial activity are well documented (Lacaille-Dubois and Wagner, 1996; Milgate and Roberts, 1995; Francis et al., 2002). Toxicity of saponins to warm blooded animals is dependent on the method of administration, source, composition, and concentration of the saponin mixture (George, 1965; Oakenfull and Sidhu, 1990). While they show toxicity when given intravenously, their toxicity is much lower when administered orally which has been attributed to their low absorption and the much reduced haemolytic activity in the presence of plasma constituents (Fenwick et al., 1991; George, 1965; Oakenfull and Sidhu, 1990). The results of *in vivo* studies with rats (Yoshikoshi et al., 1995; Gestetner et al., 1968), mice (Gestetner et al., 1968), and rabbits (Gestetner et al., 1968) suggested that saponins are not absorbed in the alimentary channel but hydrolyzed to sapogenins by enzymatic action. A study on the bioavailability of soyasaponins in humans showed that ingested soyasaponins had low absorbability in human intestinal cells and seem to be metabolized to soyasapogenol B by human intestinal microorganisms *in vivo* and excreted in the feces (Hu et al., 2004).

Table 4 Reported biological activities of saponins (Hostettmann and Marston, 1995; Lacaille-Dubois and Wagner, 1996; Milgate and Roberts, 1995; Francis et al., 2002)

Biological Activity
Adaptogenic
Adjuvant
Analgesic activity
Antiallergic
Antiedematous
Antiexudative
Antifeedant
Antifungal
Antigenotoxic
Antihepatotoxic inhibitory effect on ethanol absorption
Anti-inflammatory
Antimicrobial
Antimutagenic
Antiobesity
Antioxidant
Antiparasitic
Antiphlogistic
Antiprotozoal
Antipsoriatic
Antipyretic
Antispasmodic
Antithrombotic (effect on blood coagulability)
Antitussive (relieving or preventing cough)
Antiulcer
Antiviral
Chemopreventive
Cytotoxic
Diuretic
Effect on absorption of minerals and vitamins
Effect on animal growth (growth impairment), reproduction
Effect on cognitive behavior
Effect on ethanol induced amnesia
Effect on morphine/nicotine induced hyperactivity
Effects on ruminal fermentation
Expectorant
Haemolytic
Hepaprotective
Hypocholesterolemic
Hypoglemic
Immunostimulatory effects
Increase permeability of intestinal mucosa cells
Inhibit active nutrient transport
Molluscicidal
Neuroprotective
Reduction in fat absorption
Reduction in ruminal ammonia concentrations
Reductions in stillbirths in swine
Ruminant bloat
Sedative

The safety of saponins of commonly used food and feedstuffs such as soybeans (Ishaaya et al., 1969), and alfalfa (Malinow et al., 1981) has been established by animal toxicology studies. The safety of saponins (such as glycyrrhizic acid) or saponin-containing extracts (such as quillaja extracts) that are used as

Table 5 Lethality of quillaja saponins to CD-1 mice (Kensil and Marciani, 1991)

Dose (μg)	Quil-A	QS-7	QS-18	QS-21
125	1/5	0/5	4/5	0/5
250	2/5	0/5	5/5	0/5
500	4/5	0/5	5/5	1/5

Results are expressed as number of deaths per group of five mice within 72 h after intradermal injection of saponins.

food additives has been the subject of thorough reviews (Joint FAO/WHO Expert Committee on Food Additives, 2004, 2005a; Eastwood et al., 2005). Toxicological recommendations for glycyrrhizic acid are based on its effect of increasing mineralocorticoid activity, which in turn results in electrolyte imbalance due to sodium retention and potassium excretion, and water retention. This effect though reversible can lead to elevated blood pressure if sustained (Joint FAO/WHO Expert Committee on Food Additives, 2005a). Safety evaluation of quillaja extracts takes into consideration the chemical composition of the extracts (such as saponin content, qualitative, and quantitative information on non-saponin constituents) (Joint FAO/WHO Expert Committee on Food Additives, 2004). Quillaja extracts are classified as type 1 and type 2 based on their saponin content, 20–26% and 75–90% respectively (Joint FAO/WHO Expert Committee on Food Additives, 2004), and Acceptable Daily Intake (ADI) values are based on the saponin content of the extracts (Joint FAO/WHO Expert Committee on Food Additives, 2005b). Purification of a saponin extract may result in production of highly potent saponin fractions with varying degrees of toxicities as observed for quillaja saponins (QS-7, QS-18, and QS-21) produced by the purification of an aqueous quillaja extract (Quil-A) (Table 5) (Kensil and Marciani, 1991).

Saponins can impact the immune system through their adjuvant activity, their ability to improve effectiveness of orally administered vaccines by facilitating the absorption of large molecules, and their immunostimulatory effects (Cheeke, 1999). The ability of saponins to act as immunological adjuvants by enhancing the immune response to antigens has been recognized since 1940s (Bomford et al., 1992; Francis et al., 2002). In addition to quillaja saponins, which have been almost exclusively used in the production of saponin adjuvants (Bomford et al., 1992), adjuvant activity of soyasaponins, lablabosides, jujubosides, quinoa, gypsophila, and saponaria saponins has also been reported (Bomford et al., 1992; Oda et al., 2000; Estrada et al., 1998).

Cholesterol-lowering activity of saponins, which has been demonstrated in animal (Oakenfull and Sidhu, 1990; Matsuura, 2001) and human trials (Oakenfull and Sidhu, 1990; Kim et al., 2003b; Bingham et al., 1978), has been attributed to inhibition of the absorption of cholesterol from the small intestine, or the reabsorption of bile acids (Oakenfull and Sidhu, 1990). Feeding animals (poultry, rats, monkeys) diets containing purified saponins or concentrated extracts containing saponins such as digitonin (a steroid saponin obtained from *Digitalis purpurea*), saikosaponin (triterpenoid saponins obtained from roots of *Bupleurum falcatum L.* and related plants), saponaria, soya, chick pea, yucca, alfalfa, fenugreek, quillaja, gypsohila, and garlic saponins resulted in reductions in the plasma and in some cases liver cholesterol concentrations (Oakenfull and Sidhu, 1990; Matsuura, 2001). Recent research highlighted the role of saponins in addition to isoflavones on the hypocholesterolemic effect of soy protein (Lucas et al., 2001; Oakenfull, 2001). The cholesterol lowering effect of dietary saponins in humans is also supported by ecological studies (Chapman et al., 1997). The low incidence of heart disease in the Batemi and Maasai populations of East Africa despite a saturated fat/cholesterol diet, has in part been attributed to the use of plant dietary additives containing saponins in addition to polyphenols, phytosteroids and water-soluble dietary fibre (Chapman et al., 1997).

Anticancer activity has been reported for a number of triterpene and steroid saponins including but not limited to soyasaponins (Rao and Sung, 1995; Kerwin, 2004; Berhow et al., 2000; Plewa et al., 1998), ginsenosides (Huang and Jia, 2005; Liu et al., 2000), saikosaponin-d (Hsu et al., 2004), diosgenin (Raju et al., 2004), and glycyrrhizic acid (Hsiang et al., 2002). Although the potential of soybean saponins as anticarcinogens has been studied in recent years, animal studies are rather limited and most of the evidence comes from cell culture studies (Kerwin, 2004). Methyl protoneogracillin (Hu and Yao, 2003), methyl protogracillin (Hu and Yao, 2001) (steroidal saponins isolated from the rhizomes of *Dioscorea collettii*), protoneodioscin (Hu and Yao, 2002a), and protodioscin (Hu and Yao, 2002b) (furastanol saponins isolated from the rhizomes *of Dioscorea collettii*) have been identified as potential anticancer agents by the National Cancer Institute's (NCI) anticancer drug screen program. Anticancer activities of saponin containing plants such as ginseng and licorice are also being investigated (Wang and Nixon, 2001; Yun and Choi, 1998; Shin et al., 2000). While the cancer preventive effects of ginseng have been demonstrated in experimental models and in epidemiological studies, the evidence on its effect on humans is not conclusive (Shin et al., 2000).

The aglycones, which might be naturally present in the plants or formed by hydrolysis of saponins *in vivo* or during storage and/or processing of the plant material, may have biological activity which is absent or present in a lower degree in their corresponding saponins. The study of the relationship between chemical structure and colon anticancer activity of soybean saponins (as indicated by their ability to suppress the growth of a colon cancer cell line) revealed that the soyasapogenols were more bioactive than the glycosidic saponins (Gurfinkel and Rao, 2003). Other aglycones with anticancer activity include dammarane sapogenins from ginseng (Huang and Qi, 2005), betulinic acid (Yogeeswari and Sriram, 2005; Wick et al., 1999), and oleanolic acid (Liu, 1995; Hsu et al., 1997). Oleanolic acid, one of the most common triterpene saponin aglycone, has also been reported to possess anti-viral (anti-HIV), anti-inflammatory, hepatoprotective, anti-ulcer, antibacterial,

hypoglycaemic, anti-fertility, and anticariogenic activity (Liu, 1995). Anti-viral (anti-HIV), anticancer, antibacterial, antimalarial, anti-inflammatory, anthelmintic, and antioxidant properties have been demonstrated for betulinic acid and its derivatives (Yogeeswari and Sriram, 2005). The conversion of saponins to their aglycones may also result in the loss of activity. For example the hydrolysis of saponins by ruminal bacteria results in the loss of antiprotozoal activity, which requires the intact saponin structure (Cheeke, 1999). Similarly, the deacylation of quillaja saponins decreased their adjuvant activity (Marciani et al., 2002).

COMMERCIAL APPLICATIONS

The diverse physicochemical and biological properties of saponins have been successfully exploited in a number of commercial applications in food, cosmetics, agricultural and pharmaceutical sectors. Market trends towards the use of natural ingredients, and increasing evidence of their biological activity have increased the demand for saponins in recent years (Brown, 1998; Malcolm, 1995). As natural non-ionic surfactants, they find widespread use as emulsification and foaming agents, and detergents (San Martin and Briones, 1999; Balandrin, 1996). Other investigated/proposed applications of saponins and saponin containing plants include as feed additives (Cheeke, 1999; Zhan, 1999; Aoun et al., 2003; Jensen and Elgaard, 2001), as bacterial (Henderson, 2001) and vegetable growth regulators (Yamauchi et al., 2000), and for soil remediation (Roy et al., 1997). While the two major commercial sources of saponins are *Quillaja saponaria* and *Yucca schidigera* extracts (San Martin and Briones, 1999; Balandrin, 1996), a number of other plant materials such as horse chestnut (Indena, 2005), tea seed (Zhan, 1999), and soybeans (Organic Technologies, 2005) are being utilized/evaluated for use as commercial sources of saponins. Pharmaceutical applications of saponins include as raw materials for production of hormones (Blunden et al., 1975), immunological adjuvants (Kensil et al., 2004), and as drugs (Panagin Pharmaceuticals Inc., 2005; Panacos, 2005). Saponins have also been reported to be the active ingredients in various natural health products, such as herbal extracts (Balandrin, 1996).

Food Applications

Yucca (Mohave yucca, *Yucca schidigera* Roezl Fla) and quillaja (quillaia, soap bark, *Quillaja saponaria* Mol Fla) are classified as food additives in the US under section 172.50 (Natural Flavoring Substances and Natural Substances Used in Conjuction with Flavors) (US Food and Drug Administration, 2003). The food additives from natural origins containing saponins used in Japan include enzymatically modified soybean saponin, *Pfaffia paniculata* extract, quillaja extract, tea seed saponins, and yucca foam extract (Japanese Ministry of Health and Welfare, 2005). Quillaja extract is classified by the European Union as a foaming agent for use in water-based, flavored non-alcoholic drinks (E 999; 200 mg/liter calculated as anhydrous extract) (Office for Official Publications of the European Communities, 1996).

Although quillaja and yucca are not considered Generally Recognized As Safe (GRAS) by the US Food and Drug Administration (FDA), they have been given GRAS designation by Flavor and Extract Manufacturers' Association (FEMA) (FEMA #2973, and 3120 respectively) (Ash and Ash, 2002). There is a pending GRAS notice (GRN #165) received by FDA in 2005 from the American Beverage Association for quillaja extract (type 2) to be used as a foaming agent in semi-frozen carbonated and non-carbonated beverages at levels not exceeding 500 milligrams dry weight per kilogram beverage (US Food and Drug Administration, 2005a).

Quillaja extract (type 1) is used in foods and beverages mainly for its foaming properties at concentrations of 100 ppm (dry basis, undiluted extract) in soft drinks, and at concentrations up to 250 ppm in frozen carbonated beverages (Joint FAO/WHO Expert Committee on Food Additives, 2004). Quillaja extract, type 2 is used in Japan as an emulsifier for preparations containing lipophilic colors or flavors that are added to soft drinks, fermented vegetables, and dressing (at claimed concentrations <10 ppm) (Joint FAO/WHO Expert Committee on Food Additives, 2004). Licorice and licorice derivatives, which are considered as GRAS by FDA, are used in foods such as baked foods, beverages, chewing gum, candy, herbs and seasonings, plant protein products, and vitamin and mineral dietary supplements as a flavoring agent only with specific limitations (U.S. Food and Drug Administration, 2005b). Saponins have also been proposed for use in foods as antimicrobial (Sogabe et al., 2003) and anti-yeast agents (Ashida and Matsuda, 1999). Other commercial saponin products for food applications include soybean concentrates marketed as functional food ingredients and nutraceuticals (Organic Technologies, 2005), and a Korean ginseng extract called saponia (Godwithus Co Ltd., 2005).

The presumed health benefits of oleanolic acid led to the development of methods to fortify food products (such as olive oil) with oleanolic acid (van Putte, 2002). Proposed applications for oleanolic acid include as a flavoring agent to modify the aftertaste/taste of the artificial sweetener (Kang et al., 1999) and in fat blends as crystal modifier (Bhaggan et al., 2001).

The physicohemical properties of saponins can also be utilized in food processing applications. Thus, while complex formation of saponins with cholesterol has been used for the removal of cholesterol from dairy products such as butter oil (Micich et al., 1992; Richardson and Jimenez-Flores, 1994), the interaction of saponins with cell membranes has been considered for the selective precipitation of fat globule membranes from cheese whey (Hwang and Damodaran, 1994). In this last application, saponins are used to increase the hydrophobicity of the fat membrane to facilitate flocculation and precipitation of the formed complexes (Hwang and Damodaran, 1994).

Cosmetics

Due to their surface active properties, saponins are being utilized as natural surfactants in cleansing products in the personal care sector such as shower gels, shampoos, foam baths, hair conditioners and lotions, bath/shower detergents, liquid soaps, baby care products, mouth washes, and toothpastes (Indena, 2005; Olmstead, 2002; Brand and Brand, 2004). Natural surfactants containing saponins available commercially include Juazarine from the bark of *Zizyphus joazeiro* tree (Anonymous, 2004), horse chestnut saponins (Indena, 2005) and mixture of plant saponins (Bio-Saponins, Bio-Botanica, Inc., 2005). Saponins and sapogenins are also marketed as bioactive ingredients in cosmetic formulations with claims to delay the aging process of the skin (Yoo et al., 2003; Bonte et al., 1998), and prevent acne (Bombardelli et al., 2001).

Pharmaceutical/Health Applications

Steroid saponin-containing plant materials gained commercial significance in 1950s as raw materials for the production of steroid hormones and drugs. The synthesis of progesterone from the sapogenin diosgenin (Figure 1A) obtained from Mexican yam by Marker et al. in 1940s (Marker et al., 1947) was the beginning of a remarkable era in steroid research culminating in the synthesis of the first oral contraceptive in 1951. Diosgenin isolated from *Dioscorea* species and to a lesser extent structurally similar sapogenins such as hecogenin from *Agave* species have been widely used as raw materials by the steroid industry (Blunden et al., 1975).

Saponins have been used as immunological adjuvants in veterinary vaccine formulations due to their immune enhancing properties since 1950s (Dalsgaard, 1974). Their use in human vaccines, however, has been limited by their complexity and toxicity. Purification of the quillaja extract to yield fractions with differing chemical and biological properties enabled the characterization and thus reproducible production of the fractions for optimal adjuvant activity and minimal haemolytic activity and toxicity (Cox et al., 2002; Kensil and Marciani, 1991). Consequently, there have been significant advances in the development of saponins as human vaccine adjuvants in the last decade leading to the development of a new generation of vaccines against cancer and infectious diseases which are at various phases of clinical trials (Kensil et al., 2004). The use of quillaja extracts (even at concentrations commonly used in foods) as oral adjuvants in human clinical tests requires supporting toxicology and general safety data due to their non-GRAS status (Dirk and Webb, 2005).

The wealth of information on the biological activity of saponins and aglycones from a variety of sources is providing leads for the development of drugs. The chemopreventive and chemotherapeutic activities of ginseng dammarane sapogenins have prompted the development of anticancer drugs which are at various stages of development (Panagin Pharmaceuticals Inc., 2005). A new class of HIV drugs called Maturation Inhibitors (PA-457, in Phase 2 clinical trials) are being developed using betulinic acid derivatives (Panacos, 2005).

Pharmaceutical compositions or plant extracts containing saponins have been patented for the prevention and/or treatment of a variety of conditions such as inflammation (Forse and Chavali, 1997; Bombardelli et al., 2001), infection (Forse and Chavali, 1997), alcoholism (Bombardelli and Gabetta, 2001), pre- and post-menopausal symptoms (Bombardelli and Gabetta, 2001), cardiovascular and cerebrovascular diseases such as coronary heart disease and hypertension (Yao et al., 2005; Hidvegi, 1994), prophylaxis and dementia (Ma et al., 2003), ultraviolet damage including cataract, and carcinoma cutaneum (Satoshi et al., 2004), gastritis, gastric ulcer, and duodenal ulcer (Kim et al., 2003a). The use of saponins in pharmaceutical preparations as adjuvants to enhance absorption of pharmacologically active substances or drugs has also been patented (Kensil et al., 1996; Tanaka and Yata, 1985).

Saponin-containing plants such as ginseng, yucca, horse chestnut, sarsaparilla, and licorice have been used in traditional medicine by various cultures for centuries for the prevention/ treatment of various ailments (Liu and Henkel, 2002; Hostettmann and Marston, 1995). Characterization of the medicinal plants and their extracts points to the role of saponins in conjuction with other bioactive components such as polyphenols in the observed health effects (Liu and Henkel, 2002; Alice et al., 1991). Over 85% of the herbs most commonly used in Traditional Chinese Medicine were observed to contain saponins (in addition to polyphenols) in significant detectable amounts, while the herbal products in the eight best known and most commonly used formulae were explicitly rich in these components (Liu and Henkel, 2002). It should be noted that while some of the health benefits associated with these plants have been supported by clinical data or described in pharmacopeias and in traditional systems of medicine, a variety of uses attributed to these medicinal plants have not been substantiated (Table 6).

EXTRACTION AND PURIFICATION OF SAPONINS AND SAPOGENINS

The recognition of the commercial significance of saponins with expanding applications and increasing evidence of their health benefits have prompted research on process development for the production of saponins on a commercial-scale from natural sources. Existing food processing methods, such as soy protein production, are also being re-evaluated to obtain information on the partitioning of saponins between different process streams (Rickert et al., 2004a, 2004b), which is used to recover saponins as separate fractions (Haokui, 2001), to maximize their retention in the final product (Singh, 2004), and to identify potential raw materials for the production of saponins (Rickert et al., 2004a).

Due to the abundance of saponins in nature, a wide range of plant materials can be used as raw materials for commercial production of saponins. A significant commercial opportunity lies

Table 6 Medicinal uses of licorice (*Radix glycyrrhizae*), ginseng (*Radix ginseng*), and horse chest nut (*Semen hippocastani*) (World Health Organization, 1999a, 1999b, 2001)

MEDICINAL USES	Radix glycyrrhizae	Radix ginseng	Semen hippocastani
Supported by clinical data		As a prophylactic and restorative agent for enhancement of mental and physical capacities, in case of weakness, exhaustion, tiredness, and loss of concentration, and during convalescence.	For treatment of symptoms of chronic venous insufficiency, including pain, feeling of heaviness in legs, nocturnal calf-muscle spasms, itching and oedema. For the symptomatic treatment of chronic venous insufficiency, sprains and bruises.
Described in pharmacopeias or in traditional medicines	As a demulcent in the treatment of sore throats, and as an expectorant in the treatment of coughs and bronchial catarrh. In the prophylaxis and treatment of gastric and duodenal ulcers and dyspepsia. As an anti-inflammatory agent in the treatment of allergic reactions, rheumatism and arthiritis, to prevent liver toxicity, and to treat tuberculosis and adrenocorticoid insufficiency.	Treatment of diabetes, impotence, prevention of hepatoxicity, and gastrointestinal disorders such as gastritis and ulcer.	Treatment of coronary heart disease.
Described in folk medicine, not supported by experimental or clinical data	As a laxative, emmenagogue, contraceptive, galactagogue, antiasthmatic drug, and antiviral agent. In the treatment of dental caries, kidney stones, heart disease, consumption, epilepsy, loss of appetite, appendicitis, dizziness, tetanus, diphtheria, snake bite and haemorrhoids.	Treatment of liver disease, coughs, fever, tuberculosis, rheumatism, vomiting of pregnancy, hypothermia, dyspnoea, and nervous disorders.	Treatment of bacillary dysentery and fevers. Also as a haemostat for excessive menstrual or other gynaecological bleeding, and as a tonic.

in the value-added processing of by-products for the concentration of saponins and/or aglycones such as soybean oil extraction residue (Yoshiki et al., 2005), soy molasses (Dobbins, 2002), asparagus waste (Schwarzbach et al., 2004), and sugarbeet pulp (Sasazuka et al., 1995).

The development of an effective processing methodology starts with the identification of process objectives/product specifications, which is in turn determined by end-product use. The spectrum of saponins with commercial applications ranges from crude plant extracts, which are commonly used for their foaming properties, to high purity saponins with health applications such as vaccine adjuvants, production of which requires a sequence of purification steps. In addition to well-established analytical methodologies, new technologies and approaches are also being investigated to overcome processing challenges posed by the complex nature and diversity of this unique class of compounds. While common trends can be identified, process development is carried out for each raw material as the composition of the plant material and the saponin mixture will affect the process considerably.

Extraction of Saponins

The first step in the processing of saponins involves their extraction from the plant matrix. As in any extraction process, the extraction solvent, extraction conditions (such as temperature, time, pH, solvent to feed ratio), and the properties of the feed material (such as composition and particle size) are the main factors that determine process efficiency and the properties of the end product.

If a purified product is desired, the efficiency of the purification steps needs to be considered while optimizing extraction parameters. For example, conditions maximizing the extraction yield can decrease the selectivity and thus, the purity of the saponins, complicating further purification steps (Wanezaki et al., 2005). The finding that malonyl isoflavones could be separated from soybean saponins easier than other soybean isoflavones due to their higher polarity led to the optimization of the extraction of soybean saponins to be based on malonyl isoflavone content of the extract (Wanezaki et al., 2005).

Sample Pretreatment

Pretreatment steps, which are carried out to increase the efficiency of the extraction, include drying, particle size reduction, and defatting (using a lipophilic solvent such as ethyl acetate or hexane). Defatting can also be carried out after the extraction of saponins. Particle size reduction (grinding) is usually carried out to increase the mass transfer efficiency of the extraction. The variable qualitative and quantitative distribution of saponins in plants enables the selection of the plant part to be used as raw material considering efficiency of the process

and/or extract properties. The efficiency of the separation is improved by using part of the plant with the highest saponin concentration. Selection of the raw material can also be used to overcome processing challenges posed by the other components present. For example, the use of quinoa hulls as raw material for saponin extraction eliminated the problems associated with swelling of starch during extraction of whole seeds (Muir et al., 2002).

Extraction Methods

While traditional solvent extraction methods are commonly used for the production of saponin extracts, recent research focuses on technologies that improve the extraction efficiency by reducing extraction time and solvent consumption/waste without compromising sample quality. Microwave (Vongsangnak, 2004; Kwon et al., 2003a,b,c) and ultrasound (Wu et al., 2001) assisted extractions involve disruption of the internal cell structure and release of intracellular product to facilitate mass transfer, which is achieved by rapid and selective heating of the raw material in a solvent which is (partially) transparent to microwave energy (in microwave extractions) (Kwon et al., 2003a,b,c; Vongsangnak, 2004) and the mechanical effects of acoustic cavitation (in ultrasonic extractions) (Wu et al., 2001).

Commercial applications of Microwave-Assisted Processes (MAP™, microwave technologies patented by Environment Canada) are being currently developed for extraction of natural products such as oilseeds (in collaboration with Bunge Canada, formerly CanAmera Foods, and BC Research) (Environment Canada, 2005) and high value, low volume, natural active ingredients for the pharmaceutical and nutraceutical markets (Radient Technologies Inc., 2005). Ultrasonic liquid processing devices are being used at production level in the pharmaceutical, chemical, petrochemical, and paint industry as well as in the bioprocessing and food industries (Hielscher GmbH, 2005).

Lab-scale microwave and ultrasonic extractions were investigated for the extraction of ginsenosides from ginseng (Kwon et al., 2003b; Vongsangnak, 2004), saponins from chickpeas (Kerem et al., 2005) and glycyrrhizic acid from licorice root (Pan et al., 2000). The ginsenoside yield and composition of a 80% methanol (50 mL) extract obtained from ginseng powder (5 g) using MAP™ for 30 s (4×) (at 72.2°C) were comparable to those of a 12 hr conventional reflux extraction carried out under similar conditions (Kwon et al., 2003b). Similarly, a maximum saponin yield of 7.4 mg/100 mg DW could be obtained in 6 min by microwave-assisted extraction of ginseng (100 mg sample: 15 mL water-saturated n-butanol, 50°C) compared to 8 hr for soxhlet extraction (7.7 mg/100 mg DW; 100 mg sample: 80 mL methanol, 70°C), 6 hr for heat reflux extraction (6.7 mg/100 mg DW; 100 mg sample: 15 mL methanol, 70°C), and 2 hr for ultrasonic extraction (7.6 mg/100 mg DW; 100 mg sample: 15 mL water-saturated n-butanol) (Vong sangnak, 2004). Savings in time and solvent consumption compared to traditional methods such as heat reflux, ultrasonic, Soxhlet extractions, and extraction at room temperature were also achieved by microwave assisted extraction of glycyrrhizic acid from licorice root (Pan et al., 2000). Multi-stage counter-current extraction has also been investigated to improve the efficiency of extraction of glycyrrhizic acid from licorice (Wang et al., 2004).

Pressurized liquid extraction (PLE) involves the use of pressurized solvents at high temperatures. The high temperatures made possible by the application of pressure results in improvements in mass transfer properties of the solvent, hence improving extracting efficiency. The change in solvent polarity hence solubility with temperature of the pressurized solvent coupled with enhanced mass transfer properties makes PLE an attractive method for saponin processing; however, the applications up to date have largely been limited to analytical procedures. In their study on the PLE of medicinal plants, Benthin et al. (1999) compared PLE of escin from CH_2Cl_2-defatted horse chestnut using aqueous methanol (65%) at 140 bar and 100°C with traditional extraction procedure and achieved a higher escin concentration in the pressurized liquid extract (3.73%) than in the traditional extract (2.63%). Extraction efficiency of ginsenosides from *Panax ginseng*, American ginseng and health supplement products using PLE (25–30 bar, 20 minute extraction, 20–25 mL solvent used, 140°C) was comparable to Soxhlet extraction (Lee et al., 2002). The ginsenoside yield of aqueous non-ionic surfactants was higher than that of water (at concentrations higher than critical micelle concentration (0.01%)) and methanol at lower temperatures (Choi et al., 2003). Efficiency of extraction of glycyrrhizic acid from licorice using pressurized methanol (Ong, 2002) (at 100°C, 20 min, 20–25 mL solvent) and pressurized water (Ong and Len, 2003) (at 95°C) was comparable to or higher than that obtained with a multiple step ultrasonic extraction using 70% methanol.

Extraction Solvent

Water, lower alcohols (methanol and ethanol), or water: alcohol mixtures have been widely used for extraction of saponins from plant matrices (Kitagawa, 1986; Bombardelli and Gabetta, 2001). Other solvents investigated for extraction of saponins include aqueous (Choi et al., 2003; Fang et al., 2000) and alcoholic surfactant solutions (Choi et al., 2003), and glycerine (Gafner et al., 2004). The addition of ammonia to solvents for glycyrrhizic acid extraction is based on chemical complexation of glycyrrhizic acid with ammonia, which results in an increase in its extraction yield (Pan et al., 2000).

Supercritical CO_2 has been demonstrated to be a viable alternative to organic solvents for the processing of natural materials with advantages such as ease of solvent removal, solvent free products, and an oxygen free environment. However, the application of $SCCO_2$ technology to the processing of polar solutes such as polyphenolic and glycosidic compounds has been limited by the low solvent power of $SCCO_2$ for these solutes, which can be improved by the addition of cosolvents (Hamburger et al., 2004). The use of cosolvents, however, overrides one of the major advantages of $SCCO_2$ processing: solvent-free processing.

Supercritical CO_2 extraction of ginsenosides from ginseng (Wang et al., 2001), saikosaponins from *Bupleurum chinense DC* (Ge et al., 2000) and glycyrrhizic acid from licorice (Chuanjing et al., 2000; Kim et al., 2004) using cosolvents (ethanol (Wang et al., 2001; Ge et al., 2000), methanol (Chuanjing et al., 2000), and aqueous methanol (Chuanjing et al., 2000)) has been reported. Wang et al (Wang et al., 2001) obtained an oil product containing ginsenosides using $SCCO_2$ extraction of ginseng root hair at 308–333 K and 10.4–31.2 MPa with ethanol. The addition of ethanol to CO_2 (6 mol%) increased the SFE yield of ginsenosides in ginseng oil by a factor of 10 while increasing the yield of the oil by a factor of 4 at 333 K and 31.2 MPa. The enrichment of saponins in plant oils offer interesting product formulations, and may warrant further research. Optimum conditions for recovery of glycyrrhizic acid from licorice were 30 MPa and 60°C for 60 min using $SCCO_2$ + 70% methanol (15% by volume) (Kim et al., 2004).

Effect on Extraction Yield. The choice of solvent for a particular application will be based on the effect of solvent on saponin yield and purity, and the composition of the saponin mixture. Differences between yield and composition of extracts arise from the varying selectivities of the solvents towards individual saponins and other feed components.

The saponin recovery obtained by aqueous alcohol extraction (40–80%) of quinoa hulls was higher than that obtained by pure water or alcohol extractions (Muir et al., 2002). Ultrasound-assisted and Soxhlet extraction of ginseng using water-saturated *n*-butanol gave higher ginsenoside yields than pure and 10% methanol (Figure 2) (Wu et al., 2001). DDMP-saponin yield of 80% ethanol extraction of dehulled peas was higher than that of pure methanol extraction, which was very low (Daveby et al., 1998).

The yield of crude extract of *Glinus lotoides* seeds decreased with the methanol content of aqueous methanol, and the highest crude extract yield (16.5%) was observed with pure water. The highest yield of the *n*-butanol fraction (obtained by the partitioning of the crude extract between water and *n*-butanol) and the purified saponins, however, was achieved by 20 and 60% methanol, respectively (Figure 3) (Endale et al., 2004). The highest total extract yield of MAP^{TM} extraction of ginseng was obtained using 45–60% ethanol, whereas the saponin content increased with ethanol concentration reaching a maximum at 60–75% ethanol (Kwon et al., 2003c). In red ginseng extraction (at 80°C, 5×8 hr), solids yield decreased whereas recovery of ginsenosides increased with ethanol concentration (optimum composition with 70% ethanol) (Sung and Yang, 1985).

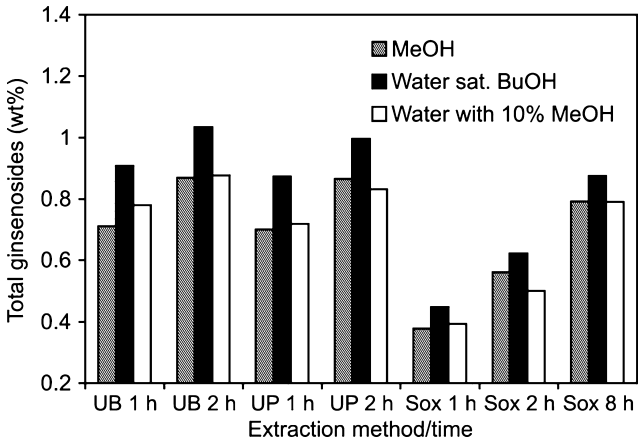


Figure 2 Total ginsenoside yield obtained by extraction of Chinese ginseng root with water, water-saturated butanol, and 10% methanol in UB-ultrasound cleaning bath, UP-ultrasound probe horn, and Sox-Soxhlet extractor (from Wu et al., 2001, Copyright (2001), with permission from Elsevier).

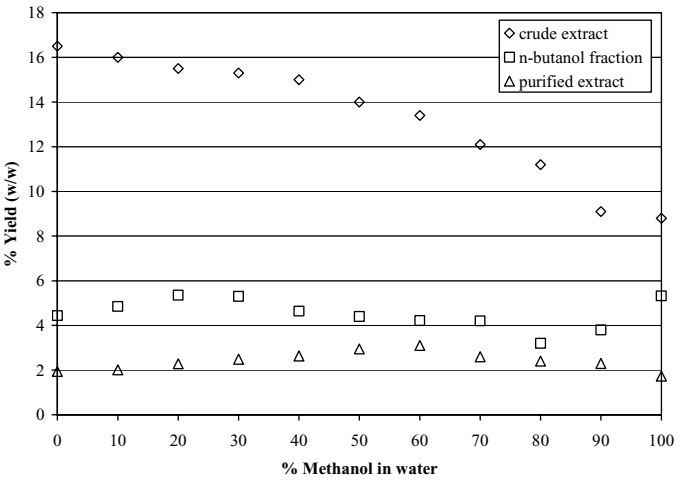


Figure 3 Yield of crude extract, *n*-butanol fraction, and purified extract obtained by extraction of *Glinus lotoides* seeds as a function of solvent composition (Data from Endale et al., 2004).

The recovery of glycyrrhizic acid from licorice using microwave assisted extraction reached a maximum at 50–60% ethanol (Pan et al., 2000). Addition of ammonia to the extraction solvent, which reacts with glycyrrhizic acid to form glycyrrhizic ammoniate, resulted in higher recoveries which were independent of ethanol concentration in 0–60% ethanol range (Pan et al., 2000). No significant difference in glycyrrhizic acid yield was observed between the solvents pure water, 10% ethanol, and 0.5 wt% ammonia in water (Wang et al., 2004).

Effect on Composition of Saponins and Properties of Extracts. The extraction solvent will also affect the composition of the saponin extract. The ratio of neutral to malonyl ginsenosides in aqueous ethanol extract of American ginseng increased with the proportion of ethanol in the solvent (Du et al., 2004). While maximum extraction of neutral ginsenosides was obtained with 70% ethanol, the highest yield of malonyl ginsenosides was achieved using 40% ethanol resulting in the highest total ginsenoside yield with 60% ethanol (Du et al., 2004). Differential extraction of saponins from quinoa bran using pure water and alcohol solvents was reflected in the differences in the saponin composition of the extracts (Muir et al., 2002).

Extraction solvent has also been found to affect the physicochemical properties of the saponin extracts, including particle size, size distribution, morphology, water uptake profiles, sorption isotherms, densities, flow properties, and compaction

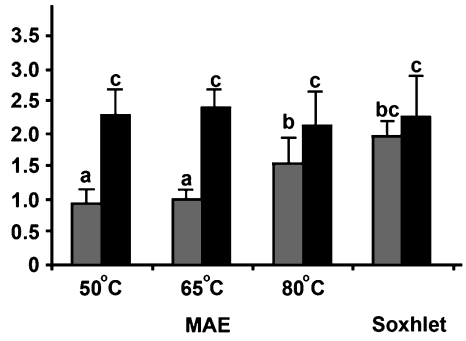


Figure 4 Recovery (g/100 g seed dry weight) of DDMP-saponin obtained by extraction of ground chickpea using microwave assisted extraction (three serial 5-min extractions) and Soxhlet extraction with methanol (black), and 70% ethanol (gray). Bars represent means ± standard deviation ($n = 5$); different letters represent statistical significance level of $p \leq 0.01$ (from Kerem et al., 2005, Copyright Society of Chemical Industry. Reproduced with permission. Permission is granted by John Wiley & Sons Ltd. on behalf of the SCI).

profiles, which are of great significance in pharmaceutical applications (Endale et al., 2004).

Effect of Temperature and Solvent:Feed Ratio on Extraction Efficiency. While temperature was found to have no effect on the microwave-assisted methanol extraction of chickpea saponins, the saponin yield of ethanol:water extracts increased with temperature (Figure 4) (Kerem et al., 2005). Solids (total extract) yield of red ginseng extraction increased while saponin recovery decreased with temperature (particularly at 100°C) (Sung et al., 1985). The multi-stage counter-current extraction yield and glycyrrhizic acid concentration both increased with temperature in the range 30–70°C (Wang et al., 2004). The temperature effect on the composition of aqueous licorice extract was reflected in its flavor characteristics (Vora and Testa, 1997). The low temperature (65.6–82.2°C) extracts had significantly higher glycyrrhizic acid, sugar content, and inorganic salt content, with a mild, sweet flavor, whereas higher temperatures resulted in stronger licorice character with balanced sweetness (Vora and Testa, 1997).

Glycyrrhizic acid concentration in the ethanol extract of licorice decreased with increasing solvent/feed ratio from 339 mg/mL at 6 mL/g to 245 mg/mL at 10 mg/mL while extraction yield stayed in the range of 75–83% (Wang et al., 2004). An increase in recovery of glycyrrhizic acid (%) with microwave assisted extraction was observed with solvent/feed ratio (from 1.88% at 5:1 to 2.58% at 20:1) (Pan et al., 2000). The optimum ratio for quinoa saponin extraction was determined to be 10–15:1 considering extraction yield and practical considerations such as ease of stirring (Muir et al., 2002).

Purification of Saponins

Purification of the crude saponin extract usually requires a sequential approach. A common method for the preliminary purification of saponins after the extraction step involves the partitioning of saponins between aqueous extracts and a water immiscible solvent such as *n*-butanol (Kitagawa, 1986). Further purification can be carried out using solvent precipitation (Kitagawa, 1986; Nozomi et al., 1986), adsorption (Giichi, 1987), ultrafiltration (Muir et al., 2002), and/or chromatography (Kensil and Marciani, 1991). While chromatographic procedures such as open column chromatography, thin layer chromatography, flash chromatography, liquid chromatography (low, medium and high pressure), and countercurrent chromatography have been well established and widely used for analytical scale purification of saponins (Hostettmann and Marston, 1995), their feasibility for commercial scale processing of saponins needs to be evaluated. The purification techniques used in the production of saponins for a variety of applications are discussed below with specific examples.

An aqueous extract of *Quillaja saponaria* bark was separated into 22 fractions (QA1-22) with different adjuvant activity and toxicity using a purification procedure involving methanol extraction followed by silica gel and reverse phase high pressure liquid chromatography (RP-HPLC) (Figure 5) (Kensil and Marciani, 1991).

Due to their high volume of production and increasing evidence on the biological activity of soyasaponins, soybeans (Dobbins, 2002; Giichi, 1987; Bombardelli and Gabetta, 2001), and by-products of soybean processing (Yoshiki et al., 2005) have great potential as raw materials for commercial saponin production. The full realization of this potential in the marketplace however requires development of processing schemes to effectively tackle the associated processing challenges.

The patent "Process for isolating saponins from soybean-derived materials" (Dobbins, 2002) exploits the temperature dependence of solubility behavior of saponins in water:acetone mixtures for the production of a soyasaponin concentrate. An acetone:water (4:1) extraction step (56°C at atmospheric pressure at pH >6.5) followed by cooling the extract led to the precipitation of saponins resulting in a 70% saponin concentrate. Further purification up to 90% was achieved by crystallization.

A soya extract containing 22.5% group B soyasaponin and 15% isoflavones was obtained by reflux extraction with pure or aqueous aliphatic alcohols followed by hexane extraction (for defatting purposes) (Figure 6) (Bombardelli and Gabetta, 2001). In an alternative approach, the defatted soya extract was treated with polyethoxylated castor oil to dissolve the resinous residues and adsorbed onto a polystyrene-based resin. Soya extract containing the isoflavones and saponins were then eluted using 95% ethanol (Figure 6). The soya extract was fractionated into group B saponins and isoflavones using solvent precipitation with aqueous alcohol and a water immiscible protic solvent (such as ethyl acetate) (Figure 6). The fractionation of soya extracts into isoflavone and saponin fractions can also be achieved using an adsorption step (Giichi, 1987; Bombardelli and Gabetta, 2001). The saponin fraction can be further purified using gel filtration and partition chromatography (Giichi, 1987).

Due to the unstable structure of soyasaponin βg, which adds to the complexity and cost of the purification process, group B and E saponins were identified as target compounds in the

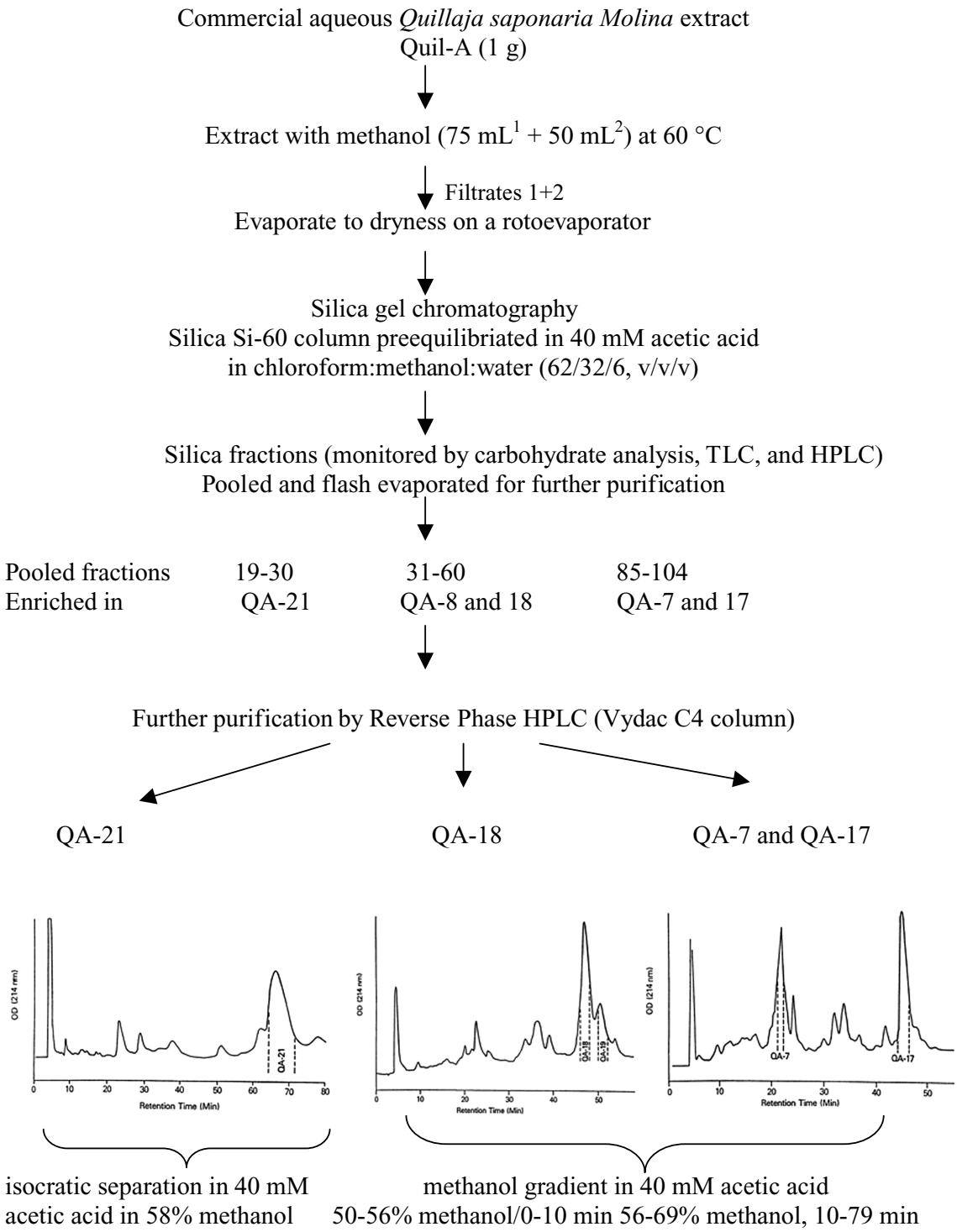


Figure 5 Purification of quillaja saponins for use as adjuvants (adapted from Kensil and Marciani, 1991).

processing of a soybean by-product, the residue of oil extraction, for the isolation of functional soybean saponins (Yoshiki et al., 2005). A fractionation procedure for the production of Group B and E saponin fractions was developed based on information on the chemical characteristics of soyasaponin βg (Figure 7). The soybean glycosides obtained by acidic precipitation were further fractionated into an isoflavone-rich (supernatant) and a DDMP saponin-Fe_2 complex rich fraction (precipitate) by dissolving them in ethanol, mixing with $FeCl_2$ and allowing them to stand overnight. Saponins were further purified by alkaline hydrolysis to remove Fe-DDMP complex, followed by acidic precipitation and partitioning of the precipitate between water and *n*-butanol.

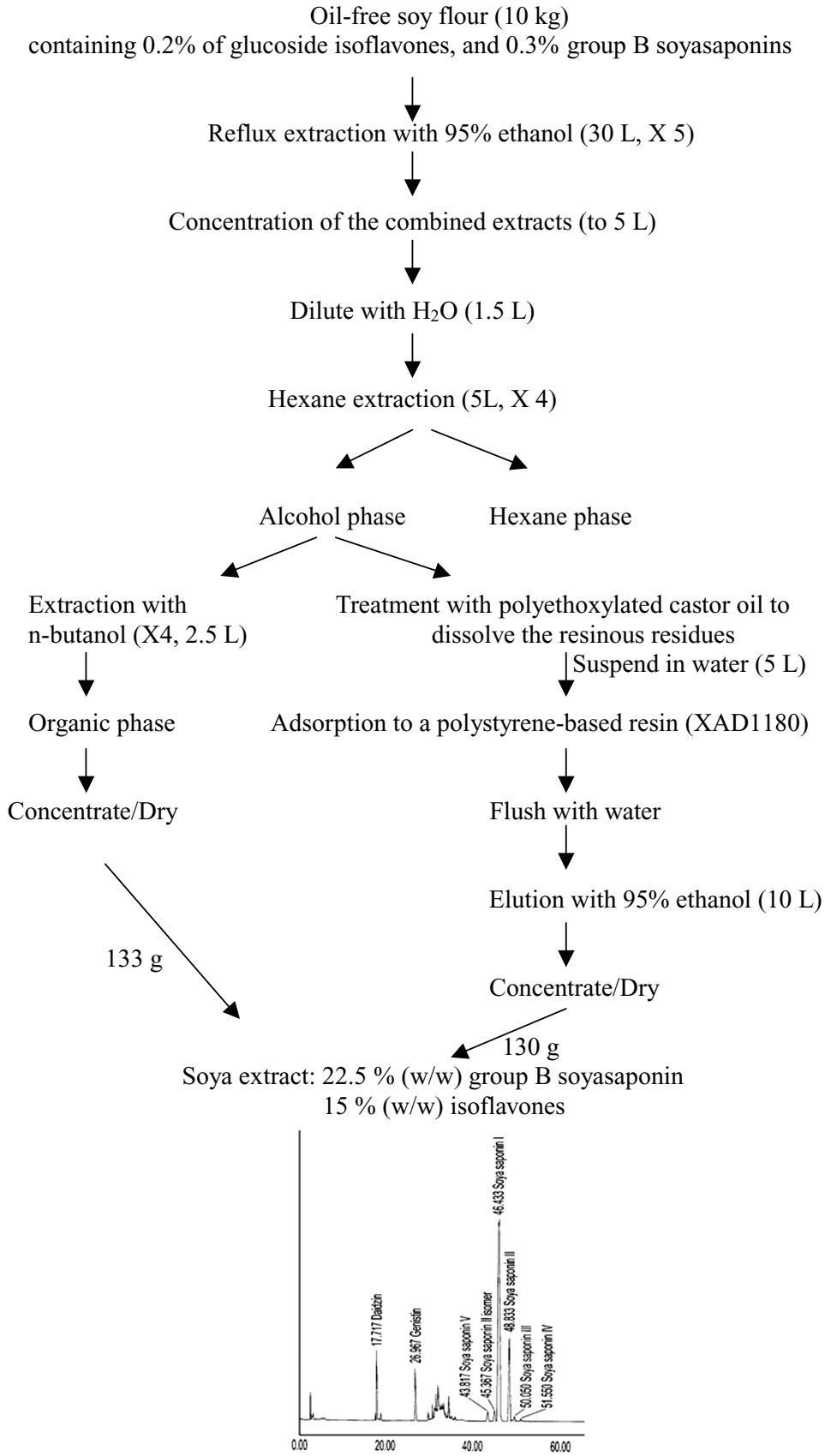


Figure 6 Production and purification of soya extract containing saponins and isoflavones (adapted from Bombardelli and Gabetta, 2001).

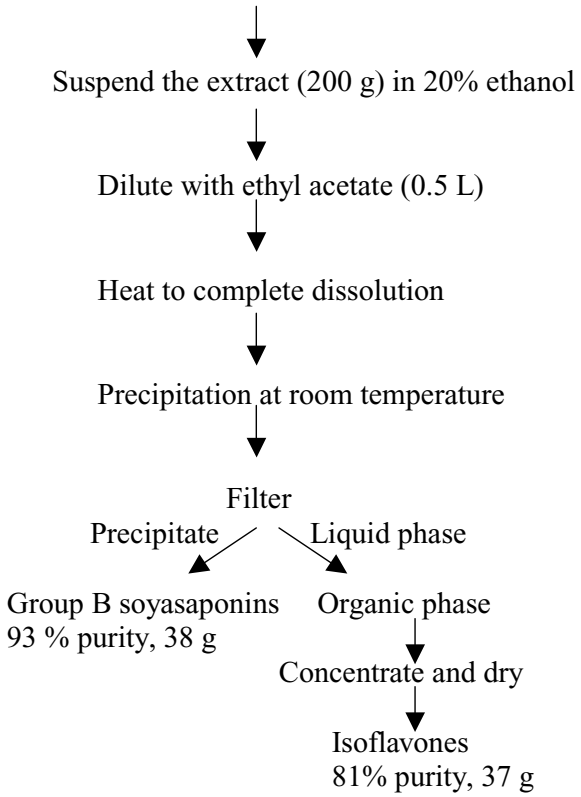


Figure 6 (Continued)

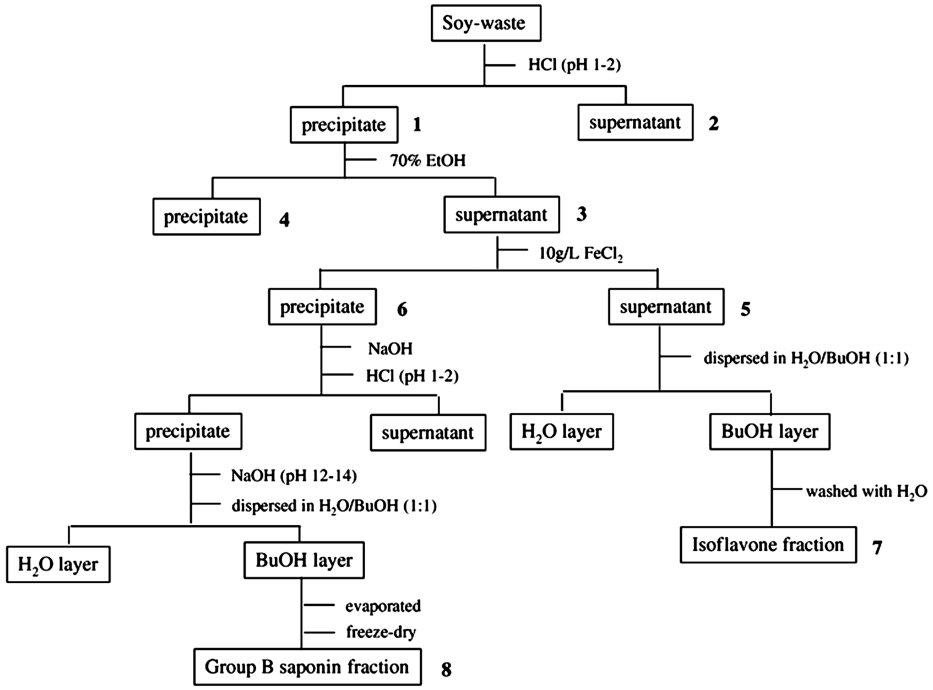


Figure 7 Fractionation of soybean glycosides based on chemical characteristics of soybean saponin βg (from Yoshiki et al., 2005, Copyright (2005), with permission from Elsevier).

The evaporated and freeze-dried *n*-butanol fraction contained Group B (>90%) and E saponins (>10%).

Soyasaponin-I has also been isolated from other legumes including red and white clover, alfalfa, and lucerne using solvent precipitation, adsorption, and heat treatment in an aqueous lower aliphatic solution of an alkali hydroxide (Kitagawa, 1986). One approach involved adsorption of the concentrated extract in water or water:alcohol mixture (≤30%) using a porous, crosslinked polystyrene resin, followed by subsequent elution (with alcohol or alcohol:water mixture), concentration and purification of the saponins by column chromatography on silica gel. Alternatively, the crude saponins were recovered in *n*-butanol by distributional extraction of the concentrated extract. The isolation of soyasaponin-I from the *n*-butanol fraction was then achieved by solvent precipitation (using a soyasaponin soluble and insoluble solvent pair such as methanol and ethyl acetate) followed by treatment with activated charcoal and crystallization from a solvent mixture of chloroform:methanol:water. Alternatively, the *n*-butanol extract was heated in an aqueous lower aliphatic alcohol solution of an alkali hydroxide under reflux, neutralized (by passing it through a column of an ion exchange resin of strong acid type), concentrated, and further purified by column chromatography on silica gel to obtain soyasaponin-I (Kitagawa, 1986).

Quinoa saponin concentrates containing up to 85–90% saponins were produced by ultrafiltration of aqueous alcohol extracts (Muir et al., 2002). Individual saponins were then recovered by Reversed Phase Solid Phase Extraction and preparative RP-HPLC with 98% purity. Solvent (water-*n*-butanol) partitioning, dialysis, and membrane filtration have also been investigated for the recovery of saponins from quinoa (Muir et al., 2002).

A patented process for the isolation of escin from horse chestnut uses the ether extraction of the cholesterol-saponin adduct obtained by treating an aqueous-alcoholic horse chestnut extract with cholesterol and separation of the resulting precipitate (Wagner and Bosse, 1964). Further fractionation of escin into its two isomers of high purity is achieved by converting it into free acid form (by treating it with a cation exchange agent) and heating (50–90°C) until one of the isomers is precipitated due to low solubility in water (Wagner and Bosse, 1963).

Foaming properties of saponins have also been used for the concentration of saponins from unfermented aqueous mixtures (Barbour and Dibb, 1976). A 10–50 fold saponin concentration in the aqueous extract was achieved by foam fractionation with a suitable gas (air, nitrogen and carbon dioxide) (Barbour and Dibb, 1976).

Effect of Processing on Saponin Structure/Properties

As the processing focus shifts from elimination of saponins to their extraction/concentration or retention, information on the effect of processing conditions (such as heat treatment) on the content, structure and properties of saponins becomes a key element in process development. Chemical modification of saponins, as outlined in the section on their physicochemical properties, can take place during processing and/or storage resulting in a change in their total content, composition, and properties/biological activity which may or may not be desirable. Information on the effect of processing conditions on saponins is not only essential to product quality but can also be exploited to customize the saponin properties for a specific application.

Earlier research on the effect of processing conditions on saponins concentrated on the effects of food processing methods such as cooking, soaking, canning, and fermentation on the saponin content of food plants or foods. The decrease in saponin content of foods caused by these processes has been well-documented for a variety of foods such as legumes and quinoa (Anderson and Wolf, 1995; Zhou and Erdman, 1997; Ridout et al., 1991).

The most widely investigated saponin group has been the ginsenosides with a wealth of information available on the effect of various processes such as drying (Du et al., 2004; Popovich et al., 2005), microwave and conventional heating (Ren and Chen, 1999), steaming (Kim et al., 2000), chemical treatment (Kim et al., 1998a), extraction parameters (Du et al., 2004), irradiation (Kwon et al., 1990), and storage (Du et al., 2004) on the concentration of individual ginsenosides and/or their biological activity. The effects of heating, extraction, and storage on oat saponins (Önning et al., 1994), alfalfa saponins (Tava et al., 2003), and soyasaponins (Daveby et al., 1998) have also been documented.

The thermal stability of selected saponins has been investigated by process conditions (time, temperature, pH) and the properties of the saponin. Oat saponins (avenacosides A and B) were heated at 100 and 140°C at different pH to study the degradation during heat processing (Önning et al., 1994). While they were stable up to 100°C for 3 hr at pH 4–7, heating at 140°C especially at pH 4 lead to partial degradation. The degradation rate was significantly increased at pH 4–6 in the presence of catalytic amounts of iron and stainless steel. Drying of American ginseng at temperatures above 40°C resulted in a decrease in the total ginsenoside content (Reynolds, 1998; Du et al., 2004) with a corresponding increase in the ratio of neutral/malonly ginsenosides, which was attributed to the hydrolysis of malonyl to neutral ginsenosides (Figure 8) (Du et al., 2004). The lower thermal stability of malonyl ginsenosides was also documented during heating of American ginseng in 50% ethanol and aqueous extracts (Ren and Chen, 1999). The effect of microwave heating on ginsenoside degradation was the same as conventional heating (Ren and Chen, 1999). A relatively lower thermal stability was also observed for protopanaxadiol than protopanaxatriol saponin (Sung et al., 1985).

Degradation can also occur during extraction and storage as affected by time and temperature (Daveby et al., 1998; Tava et al., 2003). Extraction temperature will be limited by the thermal stability of the target compounds. For example, extraction of glycyrrhizic acid using pressurized methanol was carried out at 100°C as the stability was impaired at temperatures higher than 120°C (Ong, 2002). The malonyl saikosaponins a and d

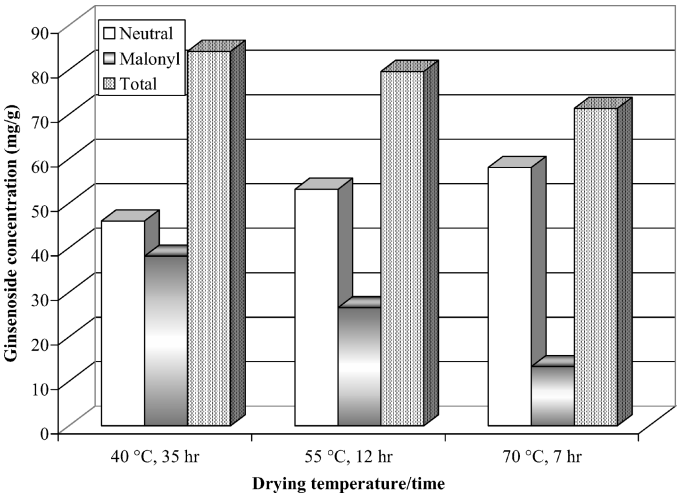


Figure 8 Concentration of ginsenosides in ethanolic extracts obtained from dried ginseng root powder (data from Du et al., 2004).

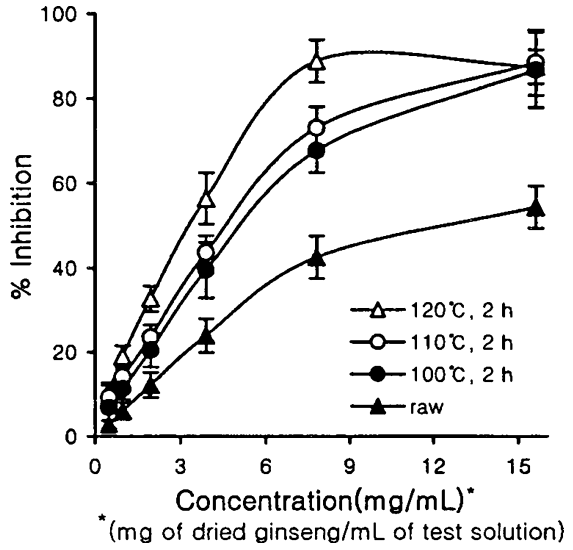
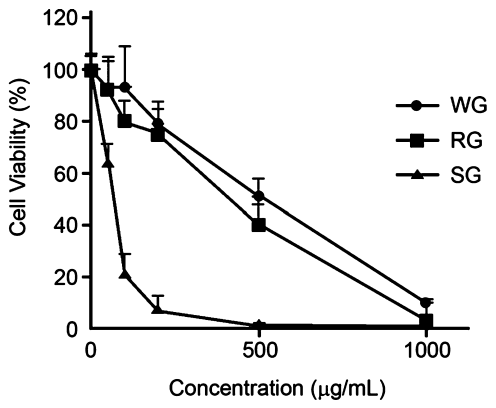


Figure 9 Radical scavenging activity of raw and steamed ginsengs (mean ± sem, $n = 5$) (from Kim et al., 2000. Copyright (2000) American Chemical Society, and American Society of Pharmacognosy).

were hydrolyzed by heat and/or acid and the saikosaponins were converted into hydroxylsaikosaponins during the decoction of *Bupleurim falcatum* roots (Ebata et al., 1996). DDMP-conjugated soyasaponin I was converted into soyasaponin I during extraction and storage of dehulled peas (Daveby et al., 1998). Prolonged storage of *Medicago sativa L.* saponins in ethanol resulted in artefact formation due to esterification of acidic saponins with alcohol (Tava et al., 2003). Extraction solvent also affects the properties of the product through its effect on the content and composition of saponins as outlined in the section on extraction solvent.

In the majority of the studies, the effect of processing on saponins has been monitored using the total content or composition of the saponin mixture. Changes in the saponin content/composition, resulting from degradation of saponins present in the raw material and production of new saponins, in turn affect their properties such as bioactivity with significant implications for product quality and product development.

The realization of the enhanced biological activity (antioxidant, anticancer activity) of heat-treated ginseng (such as red ginseng produced by steaming and drying) has put the research focus on the identification of trace compounds formed during heating (Rh_2, Rg_3, Rg_5, Rh_1) and the investigation of their biological activity (Yun et al., 2001; Kim et al., 2000). Steaming of raw ginseng at temperatures $>100^{\circ}C$ enhanced its biological properties such as its vasodilating (Kim et al., 2000), radical scavenging activity (Figure 9, Kim et al., 2000) and cytotoxicity (Figure 10, Park et al., 2002). The enhanced activity was in turn attributed to the changes in the composition of the ginsenoside mixture induced by the heat treatment (Figure 11). These findings have led to the use of processing as a means to enhance the biological activity of ginsenosides (Park, 2005; Kim et al., 1998b; An et al., 2005). A prodecure containing a series of drying and steaming steps has been used to improve the content of ginsenosides with anticancer activity such as Rg_1, Rg_2, Rg_3, and Rf (An et al., 2005). A ginseng product (sun ginseng) (with a ra-



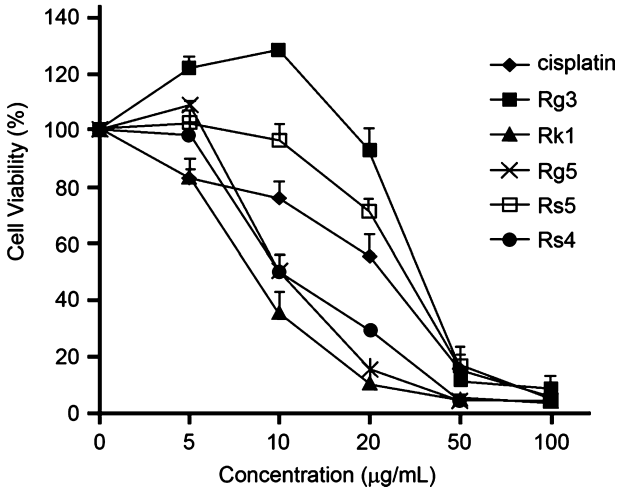


Figure 10 Cytotoxicity of (a) Methanol extracts of white ginseng (WG), red ginseng (RG), processed ginseng (SG, 120°C, 3 h), and (b) Purified ginsenosides (from Park et al., 2002, with permission).

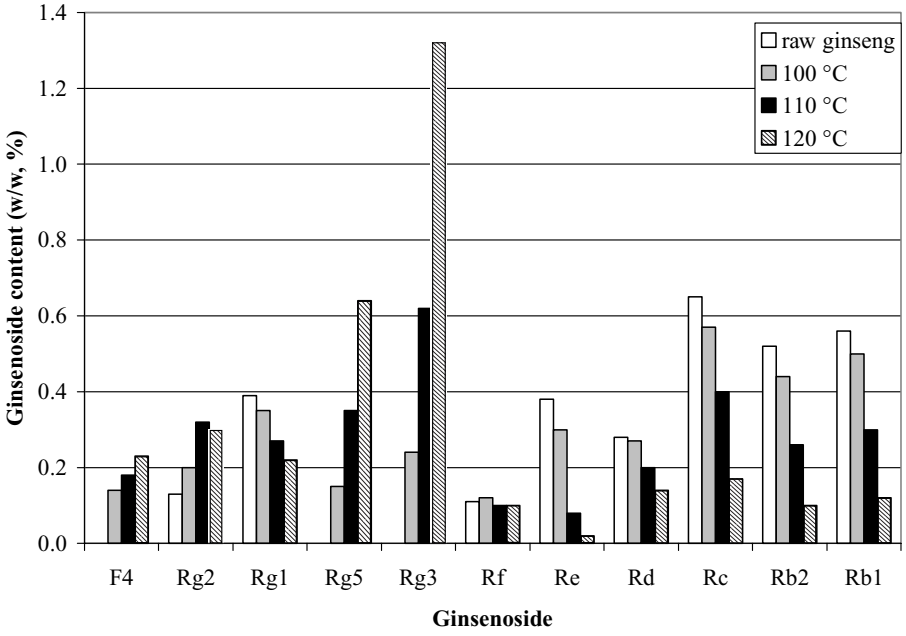


Figure 11 Content (w/w%) of ginsenosides in raw ginseng and ginsengs steamed at 100, 110, and 120°C (data from Kim et al., 2000).

tio of ginsenoside (Rg_3+Rg_5) to (Rc+Rd+Rb_1+Rb_2) above 1) produced by heat treatment at 120–180°C for 0.5–20 hours with enhanced pharmacological effects such as antioxidant and vasodilation activity has also been patented (Kim et al., 1998b).

The hydrolysis of saponins to sapogenins can also modify their bioactivity (as discussed in the section on biological activity). Ginseng sapogenins have been shown to have potent anticancer activity making them the focus of drug development efforts as discussed in the next section.

The biological activity of saponins can also be modified by structural changes induced by activity of enzymes naturally present in the plant material. Enzymatic hydrolysis of bidesmodic saponins retained in the fruit pulp of *Phytolacca dodecandra* berries upon crushing of the berries during aqueous extraction resulted in the formation of monodesmosides with high molluscicidal activity (Ndamba et al., 1994). Similarly, the fungitoxicities of the oat avenacosides were activated by the cleavage of their C-26 bound glucose moiety by α-glucosidase (avenacosidase) contained in oat leaves (Grunweller and Kesselmeier, 1985).

Chemical modification of DDMP-conjugated soyasaponins in soybeans can lead to changes in the quality of soybean foods. For example, while hydrolysis of DDMP saponins can lead to changes in flavor characteristics, the color of the product can be modified by the formation of an insoluble brown complex in the presence of iron (Okubo and Yoshiki, 1994).

Extraction and Purification of Sapogenins

The isolation of sapogenins from plant materials has been widely investigated due to their commercial significance as steroid precursors (Marker et al., 1947; Rothrock et al., 1957). There is renewed interest on production of sapogenins arising from evidence on their biological activities, which are being exploited in a number of applications including pharmaceuticals and cosmetics as described in the section on commercial applications.

Sapogenins can be produced using chemical (Muir et al., 2002; Rothrock et al., 1957), enzymatic (Isaac, 1977), or hydrothermal (Wilkins and Holt, 1958; Wilkins and Holt, 1961) hydrolysis of saponins present in the plant material followed by extraction with organic solvents (such as methanol, ether, ethylene chloride, benzene, carbon tetrachloride, and ethyl acetate) (Rothrock et al., 1957; Hershberg and Gould, 1956; Spensley, 1955) or supercritical fluids (Inada et al., 1990; De Crosta et al., 1993). Alternatively, the hydrolysis can be carried out after solvent extraction of saponins (Wall et al., 1952; Muir et al., 2002) or after expressing the juice containing saponins (Löken, 1975; Miramontes, 1959). Hydrolysis and extraction can take place simultaneously utilizing supercritical fluids (De Crosta et al., 1993; Inada et al., 1990). In their patent on the extraction of plant materials using supercritical fluids, De Crosta et al. (De Crosta et al., 1993) describe a procedure for the extraction of steroid aglycones such as diosgenin and sarsapogenin from plants (barbasco root and Yucca seed, respectively) using CO_2 modified with 10% chloroform and a pressure gradient of 100–300 atm at 250°C, which employs a hydrolysis step during or prior to the supercritical fluid extraction.

Sample pretreatment steps such as incubation with (Miramontes, 1959) or without (Gould and Hershberg, 1956) enzymes (carbohydrases such as cellulase and pectinase) and/or the addition of steroid precursors, saturated hydrocarbons, and plant growth regulators (Hardman, 1971) have been shown to increase

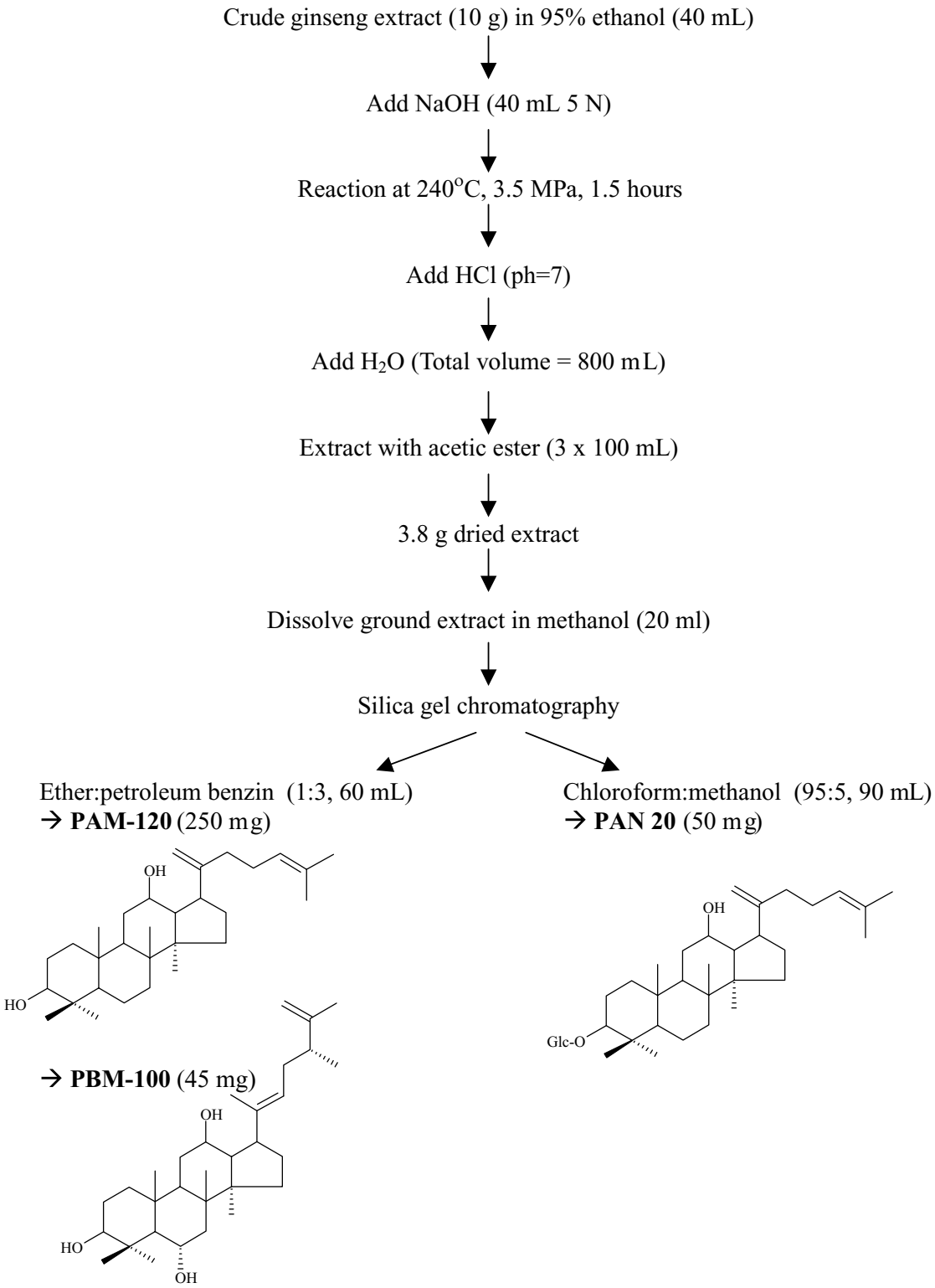


Figure 12 Production of dammarane sapogenins from ginseng (adapted from Huang and Qi, 2005).

the yield of steroid sapogenins. Heating the fermented slurry obtained as waste juice arising from decortication of leaves of the plant *Agave sisalan* to temperatures above 140°C under pressure facilitated the separation of the solids by filtration or centrifugation (Wilkins and Holt, 1958; Wilkins and Holt, 1961).

A recent patent (Huang and Qi, 2005) describes the production of sapogenins from ginseng by reacting a crude ginseng extract with water and a short chain alkali metal alcoholate solution or hydroxide ethanol solution at high temperature (150–300°C) and pressure (2.5–8.4 MPa) (Figure 12). Further purification of the reaction mixture was achieved using silica gel column chromatography to yield novel sapogenins with anti-cancer activity including PAM-120, PBM-110, PBM-100, PAN-20, and PAN-30 (Huang and Qi, 2005).

The aglycones of saponin molecules, such as betulinic acid and oleanolic acid, are also present in nature as isolated molecules. In those cases, their isolation from the plant material only necessitates extraction and purification steps. For example, betulinic acid was extracted from the bark of trees such as *Platanus acerifolia* species using medium polarity solvents such as dichloromethane, chloroform or diethylether followed by crystallization from methanol (Draeger et al., 2001). An herbal extract containing betulinic acid with anticancer activity was produced from ground bark of *Zizyphus jujuba* by macerating the bark in solvent (10–50% aqueous ethanol) (Mukherjee et al., 2004). The recognition of the health benefits of oleanolic acid resulted in the development of processes for the production of extracts containing oleanolic acid from skins of fruits such as apples, pears, cranberries, cherries, and prunes using organic solvents for use in food formulations (Beindorff et al., 2001) and for the fortification of food products such as olive oil with oleanolic acid (van Putte, 2002).

CONCLUSIONS

Saponins include a diverse group of compounds characterized by their structure containing a steroid or triterpenoid aglycone and one or more sugar chains. Their physicochemical and biological properties, few of which are common to all members of this diverse group, are increasingly being exploited in food, cosmetics and pharmaceutical sectors. The full realization of their commercial potential, which is driven by consumer demand for natural products and increasing evidence of their health benefits, requires development of commercially feasible processes that can address processing challenges posed by their complex nature, including their stability. Information on the composition (qualitative and quantitative) and properties of the saponins present in the raw material, and the effects of processing on their composition and properties are key elements of successful process design. The abundance of saponins in nature and their presence in significant quantities in processing by-products (such as by-products of soybean processing) result in a wide range of natural materials that can be exploited for commercial production.

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