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Stability of pea DDMP saponin and the mechanism of its decomposition

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Abstract

DDMP saponin can be converted to saponin B by the loss of its DDMP group (2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one). The stability of DDMP saponin from pea was investigated under various conditions (temperature, ethanol concentration, pH). DDMP saponin in water was observed to be unstable at acidic and alkaline pHs, and to have an optimal stability around pH 7. In water, DDMP saponin became unstable at temperatures >30 °C. The presence of ethanol, however, had a stabilizing effect on the DDMP group. The loss of the DDMP group at 65 °C could be completely prevented at >30% (v/v) ethanol. The breakdown reaction of DDMP saponin and the subsequent formation of saponin B was modelled using a multi-response modelling approach and was found to be best described by a first-order reaction. The activation energy was estimated to be 49 kJ/mol, indicating a chemical reaction with moderate temperature dependence. A mechanism of DDMP saponin decomposition is proposed, consisting of a fast protonation or deprotonation, followed by a rate-determining step in which maltol is the leaving group.

Keywords: Green pea(s); DDMP saponin; Saponin B; Kinetics; Ethanol; HPLC; ELSD; Modelling

1. Introduction

Saponins are non-volatile, amphiphilic, surface-active triterpene glycosides that occur in a wide variety of legume seeds, such as peas, soybeans, lentils and lupins (Lasztity, Hidvegi, & Bata, 1998). They are known to have a bitter taste (Koziol, 1991; Price, Griffiths, Curl, & Fenwick, 1985) and possess health-beneficial effects, such as lowering of cholesterol levels (Milgate & Roberts, 1995). Saponins are generally categorized into three main groups, on the basis of their aglycone (soyasapogenol) structures: groups A, B and E (Fig. 1). The aglycones of group A saponins have a hydroxyl group at the C-21 position, whereas those of group B saponins have a hydrogen atom. Group E saponins differ from group B saponins in that their aglycones have a carbonyl group at C-22. Group A saponins are *bisdesmoside* saponins, having sugar chains at the C-3 and the C-22 positions of their aglycones (soyasapogenol A), whereas Groups B and E saponins are *monodesmoside* saponins, having a sugar chain linked to only the C-3 position of their aglycones (soyasapogenol B and soyasapogenol E, respectively) (Shiraiwa, Kudo, Shimoyamada, Harada,

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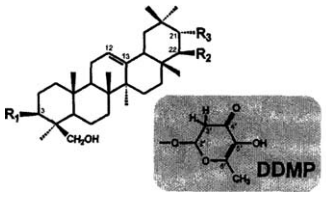

saponin type	R_1	R_2	R_3
A	$-O-(Gly)_m$	$-O-Gly-[Gly-(OAc)_n]$	$-OH$
B	$-O-(Gly)_m$	$-OH$ / $-O-DDMP$	$-H$
E	$-O-(Gly)_m$	$=O$	$-H$

Fig. 1. Structures of group A, B, E and DDMP saponins.

& Okubo, 1991a, 1991b; Yoshiki, Kudou, & Okubo, 1998). Group A saponins may contain acetyl groups attached to the terminal sugar residue of the C22 oligosaccharyl chain (Shiraiwa et al., 1991a). Group B saponins may contain a DDMP (2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one) moiety at C-22, which, upon heating, is released as maltol (Kudou et al., 1993). These B saponins are denoted as DDMP saponins.

Saponin B (soyasaponin I) has been reported as the main saponin component in green peas (Curl, Price, & Fenwick, 1985; Daveby, Aman, Betz, Musser, & Obermeyer, 1997; Kinjo et al., 1998; Price & Fenwick, 1984; Price et al., 1985, Price, Eagles, & Fenwick, 1988). However, it was not until recently that DDMP saponin (soyasaponin βg) was also identified in peas (Daveby, Aman, Betz, & Musser, 1998). DDMP saponin is widely distributed in legumes (Yoshiki, Kim, & Okubo, 1994) and is the predominating saponin in soybean (Hu, Lee, Hendrich, & Murphy, 2002; Kudou et al., 1992; Kudou et al., 1993; Lin & Wang, 2004). Saponin B is the major saponin in processed soy products and heated pea products, presumably due to the conversion of DDMP saponin to saponin B during extraction and processing (Berhow et al., 2002; Hu et al., 2002). In addition, reports have shown that DDMP saponin is converted to saponin B in acidic or basic solutions (Massiot, Dijoux, & Lavaud, 1996; Okubo & Yoshiki, 1996). These results indicate that DDMP saponin is unstable under conditions commonly applied during food processing, but detailed studies, mapping these conditions, are lacking. A systematic study of DDMP saponin stability will provide information on the effects of extraction and processing, but may also provide tools on the possible removal or masking of the undesirable bitterness of saponins in pea products. The aim of our research was to study the stability of DDMP saponin under various conditions of temperature, ethanol concentration and pH.

2. Materials and methods

2.1. Saponin extraction

Saponins were extracted from peas (*Pisum sativum, Solara* spp.) obtained from Cebeco (Vlijmen, The Netherlands). The extraction procedure is illustrated in Fig. 2. The peas were milled in a commercial blender (Waring, New Hartford, CT, USA), in the ratio 1:1 (w/w), with dry ice. Pea flour was defatted by hexane (Super gradient, Lab-scan, Dublin, Ireland), refluxing for 6 h, and subsequently the pea flour was air-dried in a fume hood overnight. Defatted pea flour (1 g) was extracted with 70% (v/v) ethanol (100 ml) for 1 h at 25 °C with constant shaking at 200 rpm in an incubator shaker (Innova 4000, New Brunswick Scientific, Nijmegen, The Netherlands). The crude extract was filtered through an ashless filter paper (White band 589^2, 110 mm, Schleicher & Schuell, Dassel, Germany). The ethanol from the clear filtrate was evaporated under vacuum at 27 °C. This evaporation step was performed in less than 15 min using a 1 l round-bottom flask. The removal of ethanol made the extract turbid, and hence the resulting extract (~30 ml) was made up to 40 ml with distilled water and was centrifuged (36,000*g*; 10 min; 10 °C). The supernatant obtained was passed through a *Sep-Pak* C18 solid phase extraction column (400 mg, Waters Plus tC18 cartridge, 37–55 μm, Waters Etten-Leur, The Netherlands), which was subsequently rinsed with 15 ml water to remove unbound material. The bound compounds were eluted with 10 ml of methanol (HPLC grade; Lab-Scan, Dublin, Ireland) and air-dried. The air-dried saponin sample was solubilized in 1 ml of 50% (v/v) ethanol and centrifuged (36,000*g*; 10 min) before HPLC analysis. For stability tests, the extraction was upscaled to 5 g of pea flour in 500 ml of 70% (v/v) ethanol, and a 5 g Waters Plus tC18 column, 37–55 μm (Waters Etten-Leur, The Netherlands) was used.

2.2. High performance liquid chromatography–mass spectrometry analysis

Reversed-phase high performance liquid chromatography (RP-HPLC) was used for the analysis of pea saponins. Evaporative light scattering detection (ELSD) was used for detection and ion trap electrospray mass spectrometry (MS) was used for component identification. A Spectra SYSTEM HPLC (Thermo Separation Products, Fremont, CA), coupled to a Sedex 55 ELS detector (S.E.D.E.R.E., Alfortville, France), was used. Separation was performed using an Aquasil reversed-phase C18 column (4.6 × 150 mm, 3 μm) (Thermo Hypersil, Bellefonte, PA, USA). The solvents used were water:acetic acid (100:0.001, v/v) (A) and acetoni-

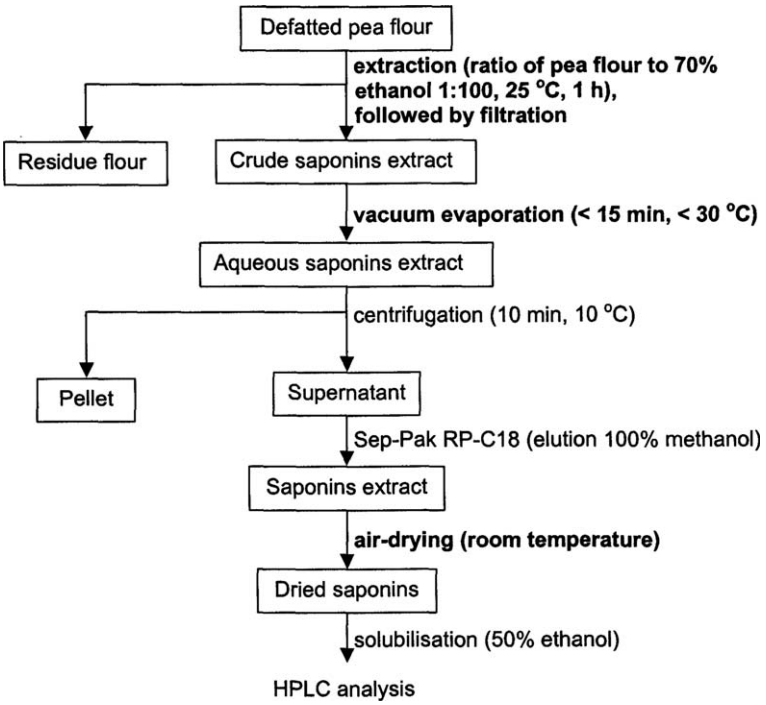

Fig. 2. Optimized extraction protocol for obtaining native saponins from pea flour. Critical steps in the procedure are in bold.

trile:acetic acid (100:0.001, v/v) (B). The gradient used was as follows: 0 → 8 min, 40 → 50% B; 8 → 10 min, 50 → 100% B; 10 → 15 min, 100% B (isocratic); 15 → 20 min, 40% B (isocratic). Samples of 20 μl were injected and a flow rate of 1 ml/min was used. The eluate from the column was split into three directions: 100 μl/min to the ELSD, 50 μl/min to the LCQ Ion-trap MS (Thermo Finnigan, San Jose, CA) and 850 μl/min to the waste. The ELSD was set at 40 °C at an air pressure of 2.3 bar and a sensitivity of 12. Quantification of DDMP and B saponins was done by means of the response factors determined by Decroos et al. (2005). The decrease in DDMP saponin concentration was obtained by integrating the area of its peak (after heat treatment) in the HPLC-chromatogram, whereas the increase in saponin B concentration was obtained by subtracting the initial saponin B area from the area of the saponin B peak obtained after heat treatment. MS analysis was performed in the positive ion mode using a spray voltage of 5.5 kV, a capillary voltage of 15 V and a capillary temperature of 200 °C. A full scan mass spectrum over a m/z (mass to charge ratio) range of 150–1500 was obtained. The mass spectra were recorded and analyzed with the use of *Xcalibur* software.

2.3. Stability tests

2.3.1. Effect of temperature

Saponin samples of 1 mg/ml were prepared in water (pH 6.7). Temperatures of 40, 50, 60, 65, 75 and 90 °C were chosen. At each temperature, the samples were heated for 15, 30, 45, 60 and 75 min with constant shaking at 400 rpm in an incubator shaker (Eppendorf AG, Thermomixer comfort, Hamburg, Germany). The samples were cooled to room temperature before an equal volume of absolute ethanol was added. The samples were centrifuged at 24,700*g* at 10 °C for 5 min, and 20 μl of the supernatant was injected into the HPLC column for analysis, as described above. All experiments were performed in triplicate.

2.3.2. Effect of ethanol

Saponin samples of 1 mg/ml were prepared in aqueous ethanol containing: 0, 5, 10, 15, 20, 30, 50 and 60% (v/v) ethanol. The samples were heated at 65 °C for 15, 30, 45, 60, 75 and 90 min with constant shaking at 400 rpm in an incubator shaker (Eppendorf AG, Thermomixer comfort, Hamburg, Germany). The samples were cooled to room temperature, centrifuged at 24,700*g* at 10 °C for 5 min, and 20 μl of the supernatant was injected into the HPLC column for analysis. All experiments were performed in triplicate.

2.3.3. Effect of pH

Saponin samples of 1 mg/ml were prepared in solutions of various pHs (2–10). Solutions at pH 2–6 were prepared in water containing HCl in molarities of 0.00125–12.5 mM. Solutions at pH 8–10 were prepared in water containing NaOH in molarities of 0.00125–1.25 mM. All solutions were stored under nitrogen. 20 μl, of a saponin stock solution of 5 mg/ml, were added to 80 μl of the solutions of various pH values. The pH of

the samples was measured and the samples were flushed with nitrogen, followed by incubation at 35 °C for 20 h. After incubation, the pH values of the samples were measured again, followed by adjustment of the pH of the samples to pH 7 with HCl or NaOH, with only negligible change in sample volume. The samples were centrifuged at 24,700*g* at 10 °C for 5 min and 20 μl of the supernatant was injected into the HPLC column for analysis. All experiments were performed in triplicate.

2.4. Modelling of the thermal stability of DDMP saponin

A first-order kinetic model was applied for characterizing DDMP saponin stability according to the following reaction:

$$\text{DDMP} \xrightarrow{k} \text{B}$$

The concentrations of both DDMP saponin and saponin B (as a function of time) were taken into account in the modelling, using the so-called multi-response modelling approach (van Boekel, 1996).

The initial concentrations of DDMP saponin and saponin B were measured, and the rate constant k, was estimated. Upon starting the experiments, both DDMP and saponin B were present, and subsequent heating caused a breakdown of DDMP. Because of the relatively large differences in the initial concentrations, it was decided to model the initial concentrations rather than fixing them. The parameters were estimated by non-linear regression in order to avoid undesirable effects of logarithmic transformations (van Boekel, 1996). The software used was Athena Visual Workbench; a programme designed for multi-response modelling (www.athenavisual.com). The activation energy was derived from the temperature-dependence of the rate constants, using the Arrhenius law.

3. Results

3.1. Optimization of extraction conditions

An extract of pea sample was analysed by reversed-phase HPLC–MS, using the gradient employed by Decroos et al. (2005). Only two peaks were obtained; each was shown to contain one component. The m/z ratios of the molecular ions $[M + H]^+$ in the mass spectra of the peaks were 1069 and 943, which correspond to DDMP saponin and saponin B, respectively. This is consistent with both saponins having the Rha(1 → 2) Gal(1 → 2)GlcUA(1 → 3) glycosyl chain. Subsequently, a short gradient was employed (see Section 2) to facilitate a higher sample throughput.

The DDMP saponin that is present in peas is heat-labile (Daveby et al., 1998) and can be converted to its corresponding saponin B (Berhow et al., 2002; Daveby et al., 1998). As the extraction conditions (temperature, time) seem to determine the DDMP saponin content (Daveby et al., 1998), the extraction temperature and time were the primary parameters to be taken into consideration when designing an extraction method. The extraction procedure described in Section 2, and as shown in Fig. 2 was obtained after careful optimization. Temperatures during extraction and drying of 20, 30 and 40 °C, extraction times of 1, 2, 3, 4 and 24 h, and extraction volumes of 100 and 300 ml per gramme of defatted pea flour were investigated. DDMP saponin was extracted as the major saponin in green peas under all extraction conditions with a small amount of saponin B. DDMP saponin was observed to remain stable at temperatures up to 30 °C, whereas a small decrease in stability was observed at 40 °C. Lin and Wang (2004) also observed that DDMP saponin remained relatively stable at temperatures ⩽30 °C. Extraction times longer than 1 h or extraction volumes larger than 100 ml per gramme of pea flour did not affect the amount of saponins extracted. Also, the ratio of saponin B to DDMP saponin (1:4) in the extract remained constant, even after 24 h of extraction, provided that the temperature was kept below 30 °C, and the evaporation step before solid phase extraction was performed in less than 15 min. The removal of ethanol resulted in a turbid extract, but the pellet obtained after centrifugation showed no traces of saponins.

Through systematic investigation of these parameters, the optimal conditions for the extraction of saponins from peas were found: the saponins from one gramme of pea flour were extracted using 100 ml of 70% (v/v) aqueous ethanol during 1 h at 25 °C (Section 2). With the use of this isolation method, DDMP saponin was obtained as the major saponin present in green peas and it was used for the systematic stability study of the effects of temperature, pH and ethanol concentration on the concentration of DDMP saponin, as a function of time.

3.2. Effect of temperature on DDMP saponin

The concentration of DDMP saponin as a function of time at various temperatures is shown in Fig. 3. The concentration of DDMP saponin remained stable in water at temperatures ⩽30 °C for a period of at least 75 min. Upon increasing the temperature, a small decrease in DDMP saponin concentration with time was observed at 40 °C. Above 40 °C, the decrease in concentration of DDMP saponin became more pronounced, with a 50% and a 75% decrease in DDMP saponin concentration after 75 min at 60 and 75 °C, respectively. At 90 °C, the decrease of DDMP saponin concentration was even faster than at 75 °C, and a decrease to 5% was reached within 75 min. At 50 and 65 °C, similar trends were observed (results not shown).

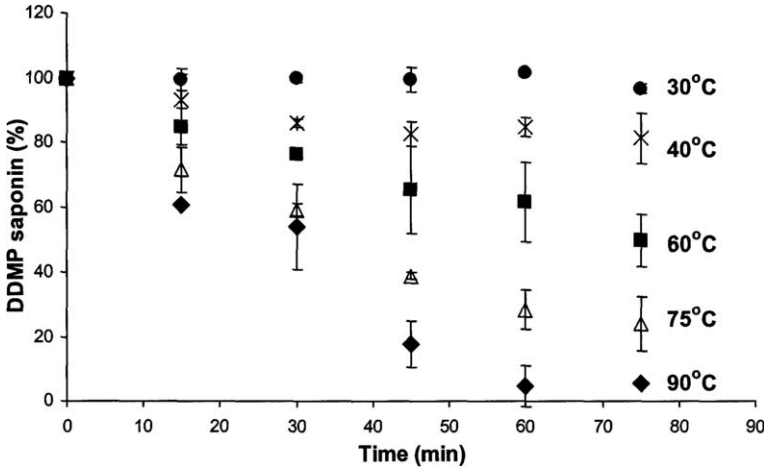

Fig. 3. Effect of temperature on the DDMP saponin concentration as a function of time.

3.3. Modelling of the (thermal) stability of DDMP saponin

With the results obtained from the thermal stability studies, a model was applied for analyzing the DDMP saponin degradation kinetics and to obtain the activation energy (E_a) of the reaction. Fig. 4 shows the course of DDMP saponin and saponin B concentrations as a function of time, and the fit of the applied model, when a temperature of 65 °C is applied. The breakdown of DDMP saponin was, at all temperatures, observed to result in the parallel formation of saponin B. Although some individual results showed a relatively high degree of variation, the molar sum of DDMP saponin and saponin B was more or less constant. The results modelled indicate that the breakdown reaction of DDMP saponin ($\text{DDMP} \xrightarrow{k} \text{B}$) follows a first-order reaction, and that there are no other parallel or consecutive reactions of quantitative importance. Table 1 shows the resulting estimates of the observed rate constant k (±95% confidence intervals) for the degradation of DDMP saponin and the formation of saponin B at various temperatures.

The E_a of the reaction was derived from the temperature-dependence of the rate constant using the Arrhenius law

$$k = k_0 \exp\left(-\frac{E_a}{RT}\right).$$

In this equation, k_0 represents the pre-exponential factor, E_a the activation energy, R the gas constant, and T the absolute temperature. In order to avoid statistical problems with nonlinear regression, due to a strong correlation between the parameters k_0 and E_a, this equation was reparameterized, as described by van Boekel (1996), to

$$k = k_{\text{ref}} \exp\left(-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right)\right).$$

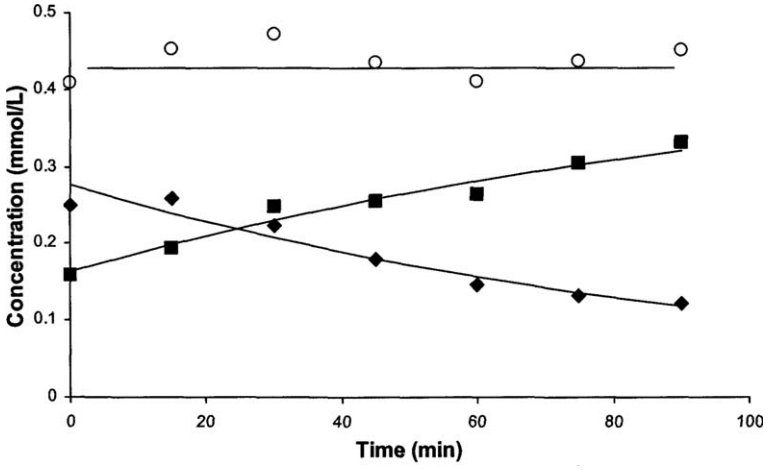

Fig. 4. Degradation of DDMP saponin (◆) and formation of saponin B (■) after heating at 65 °C. The molar sum of DDMP saponin and saponin B is also indicated (○). The solid lines represent the model fit of the proposed first-order reaction.

Table 1
The resulting estimates of the constant $k \pm 95\%$ confidence intervals for the degradation of DDMP saponin and the formation of saponin B at various temperatures

Temperature (°C)	Experiment no.	k (min^{-1})
40	1	0.0012 ± 0.0006
	2	0.0009 ± 0.0004
	3	0.0015 ± 0.0007
50	1	0.0028 ± 0.0007
	2	0.0014 ± 0.0006
60	1	0.0047 ± 0.0003
	2	0.0029 ± 0.0006
	3	0.0063 ± 0.0013
65	1	0.0067 ± 0.0009
	2	0.0056 ± 0.0003
	3	0.0094 ± 0.0008
	4	0.0067 ± 0.0009
	5	0.0056 ± 0.0003
75	1	0.0100 ± 0.0009
	2	0.0076 ± 0.0015
	3	0.0130 ± 0.0043
90	1	0.0168 ± 0.0049
	2	0.0157 ± 0.0027

In this equation, T_{ref} and k_{ref} are the reference temperature and the rate constant at the reference temperature, respectively. The reference temperature was chosen as the average of all the temperatures investigated, in this case 63.3 °C. E_a and k_{ref} were estimated using weighted non-linear regression. The weights were derived from the confidence intervals of the rate constants shown in Table 1. The estimated E_a was 49 ± 12 kJ/mol and the reference rate constant, k_{ref} was 0.0053 ± 0.0005 min^{-1} (±95% confidence interval). Fig. 5 shows the Arrhenius plot obtained using the estimates derived from the model via non-linear regression. The Arrhenius equation was applied over the whole temperature range studied. The Arrhenius plot does not appear to be completely linear: i.e., at higher temperatures, the temperature-dependence seems to be weaker than that at lower temperatures. However, the variation in the data is too high to draw valid conclusions.

3.4. Effect of ethanol

The effect of ethanol concentration on the stability of DDMP saponin treated at 65 °C for 90 min is shown in Fig. 6. The concentration of DDMP saponin at 65 °C remained stable up to 90 min at ethanol concentrations of 30% (v/v) ethanol. When the ethanol concentration was decreased to 10%, the concentration of DDMP saponin decreased with time to approximately 60% of the initial concentration after 90 min. In the absence of ethanol, however, a much faster decrease in the concentration of DDMP saponin was observed. The effects of ethanol concentrations of 5%, 15%, 20%, 50% and 60% (v/v) on the stability of DDMP saponin were also studied (results not shown). At 5%, 15% and 20% (v/v) ethanol, DDMP saponin concentration showed a similar trend with time to that at 10% (v/v) ethanol. At 50% and 60% (v/v) ethanol, the DDMP saponin concentration at 65 °C remained constant.

3.5. Effect of pH

The effect of pH on the stability of DDMP saponin, upon incubation at 35 °C for 20 h, is shown in Fig. 7. It can be seen that DDMP saponin is stable only within a relatively narrow pH range, with an optimal stability around pH 7. At acidic or basic pH, the stability of DDMP saponin decreased drastically. A change of pH by one unit may already result in a 20–60% decrease in DDMP saponin concentration under the conditions used. The degradation of DDMP saponin resulted in a proportional increase in saponin B.

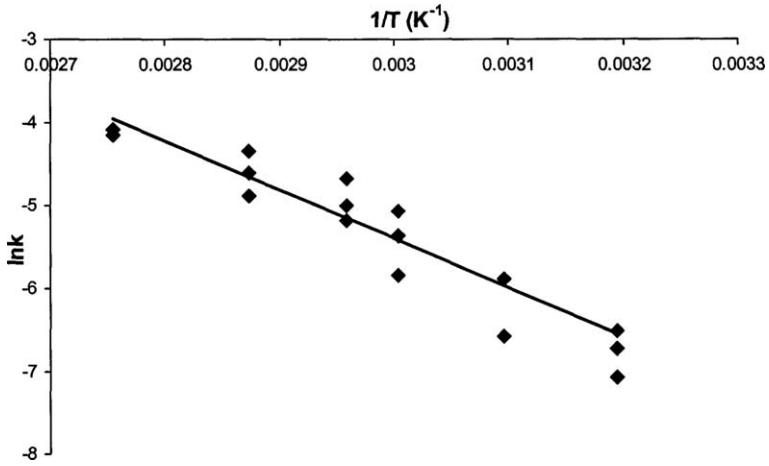

Fig. 5. Arrhenius plot for the rate constant describing the breakdown of DDMP saponin and formation of saponin B. The solid line is drawn using the parameter estimates obtained via non-linear regression (cf. $E_a = 49 \pm 12$ kJ/mol; $k_{ref} = 0.0053 \pm 0.0005$ min^{-1}; ±95% confidence interval).

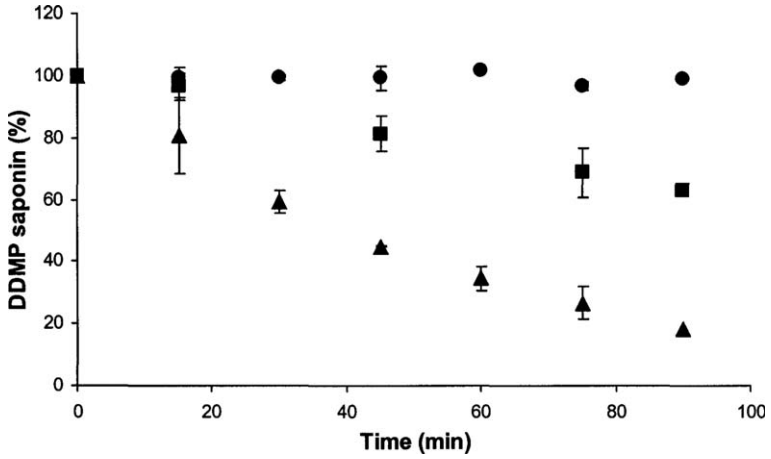

Fig. 6. DDMP saponin decomposition as a function of time (65 °C) at various ethanol concentrations of 0% (▲), 10% (■) and 30% (•).

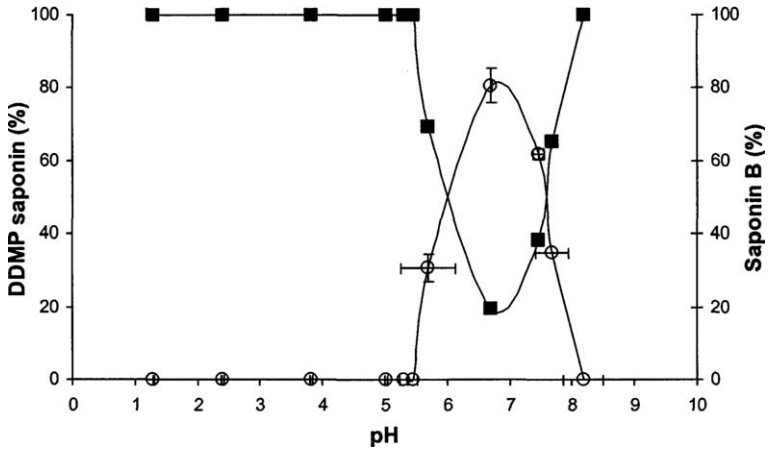

Fig. 7. Effect of pH on DDMP saponin content (○) after incubation at 35 °C for 20 h. The saponin B content after incubation is also shown (■).

4. Discussion

4.1. Effect of temperature

DDMP saponin was found to be the major saponin present in pea, but it was also found to be easily converted to saponin B. DDMP saponin was observed to be stable when extraction temperatures were kept at $\leqslant$30 °C (Daveby et al., 1998; Hu et al., 2002; Lin & Wang, 2004). Our results show that the time of exposure has a profound effect on DDMP saponin degradation but only at temperatures $\geqslant$40 °C. The estimated E_a (49 ± 12 kJ/mol) for the first-order DDMP saponin degradation reaction is relatively low for a chemical reaction, which normally has an E_a between 50 and 100 kJ/mol. This low E_a explains why the decomposition of DDMP saponin proceeds at relatively low temperatures during extraction and incubation.

4.2. Proposed mechanism for decomposition of DDMP saponin

A reaction mechanism for the decomposition of DDMP saponin is proposed in Fig. 8. The bell-shaped DDMP saponin stability profile in Fig. 7 showed that the decomposition reaction can be catalyzed by either acid or base. Under acidic conditions (Fig. 7, top reaction), the C22–O–C2′ ether linkage (see Fig. 1) is probably protonated. Such a proton transfer reaction generally proceeds very rapidly, and is diffusion-controlled. This rapid pre-equilibrium step is followed by a much slower reaction, in which the DDMP group dissociates from the C-22 position of the saponin B aglycone (denoted as 'R'), leading to the formation of the intermediate product I_A. This rate-determining step is, in principle, a mono-molecular process, which is consistent with our results, indicating that the breakdown of DDMP saponin complies with first-order kinetics. Sub-

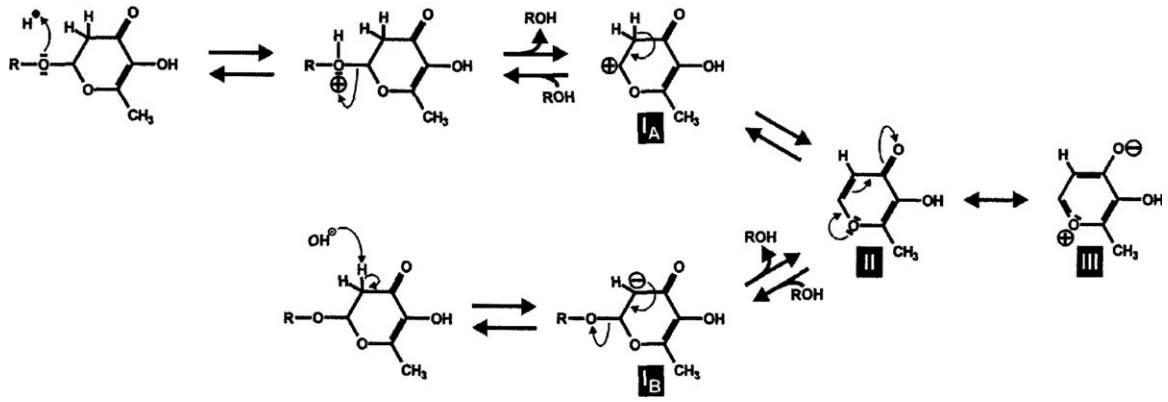

Fig. 8. Mechanism for DDMP saponin decomposition at acidic and alkaline pH. R is the saponin B aglycone as in Fig. 1. I_A and I_B are intermediate products catalyzed by acid and base, respectively.

sequently, I_A is converted to the stable product, maltol (compound II), which is resonance-stabilized (note the aromatic π-system in compound III). Under alkaline conditions, one of the acidic protons attached to C3′ is presumably abstracted (fast), followed by the formation of intermediate I_B (slow), which is subsequently converted to the same stable product, maltol.

Similar to our observations, DDMP saponin has been reported to be converted to saponin B under both acidic and basic solutions (Massiot et al., 1996). However, in contrast to this, Okubo and Yoshiki (1996) found that DDMP saponin was relatively stable in acidic solutions, but was easily hydrolyzed in alkaline solutions. It has been suggested by others (Okubo & Yoshiki, 1996) that the presence of metal ions (such as Fe^{3+}) may cause hydrolysis of DDMP saponins. In water, these metal ions may form relatively strong acids, e.g., the pK_a of $Fe(H_2O)_6^{3+}$ is 2.2 (Li, Fisher, Chen, Bashford, & Noodleman, 1996). The bell-shaped pH–[DDMP saponin] plot (Fig. 7) shows that a slight decrease of pH soon causes a considerable decrease in DDMP saponin stability, and hence, the presence of small amounts of Fe^{3+} may already result in considerable losses of DDMP saponin. However, the possibility cannot be excluded that Fe^{3+} directly binds to the DDMP saponin, and, in this way, catalyses the hydrolysis of the compound.

4.3. Ethanol as a stabilizer

The effect of ethanol became apparent in the sample clean-up step during purification of the saponins. The use of reversed-phase solid phase extraction columns, such as *Sep-Pak,* necessitated the removal of ethanol from the extract. It was observed that, during the evaporation of ethanol, a decrease in the concentration of DDMP saponin occurred. This effect became more pronounced at longer evaporation times. Together with the results in Fig. 6, this indicates that the presence of ethanol was essential at elevated temperatures to prevent decomposition of DDMP saponin.

The proposed mechanism (Fig. 8) also provides an explanation for the observed protective effect of ethanol in the decomposition reaction. Water has a much higher relative dielectric constant (82) than ethanol (24) (Shriver, Atkins, & Langford, 1990), which means that water is a much more favourable environment for the formation of charged intermediates (I_A, I_B and III) (Fig. 8) than ethanol or ethanol/water mixtures. By lowering the dielectric constant, addition of ethanol may thus shift the equilibria in such a way that DDMP saponin is stabilized. It seems that the decomposition reaction does not proceed further at ethanol concentrations of >30% (Fig. 6), i.e. below a relative dielectric constant of 60 (20 °C) (Hong, Hoshino, Kuboi, & Goto, 1999). Therefore, the evaporation time and temperature, during DDMP saponin purification, should be limited, so as to reduce the time of exposure of DDMP saponin to a too aqueous environment at elevated temperatures. Oleszek, Jurzysta, Price, and Fenwick (1990) and Hu et al. (2002) also found that saponin samples remained relatively stable in methanol at temperatures <0 °C. However, contradictory to these reports and our findings, Massiot et al. (1996) and Daveby et al. (1998) reported that DDMP saponin could decompose to saponin B in alcoholic solutions.

This study has shown that the decomposition of DDMP saponin is an acid/base-catalyzed, pseudo first-order reaction with a relatively low activation energy. The reaction can be inhibited by lowering the temperature and the dielectric constant of the solvent, and maintaining a neutral pH. These factors should be considered when performing quantitative studies on native saponins in plant extracts.

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