A novel process for extraction of natural sweetener from licorice (*Glycyrrhiza glabra*) roots

Mamata Mukhopadhyay, Palash Panja

Department of Chemical Engineering, I.I.T. Bombay, Mumbai 400076, India

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A B S T R A C T

Pressurized hot water extraction (PHWE) is a very useful technique for recovering bioactive molecules from natural materials using subcritical compressed liquid water in the temperature range of 50–150 °C. A novel process has been developed for making a natural sweetener from licorice (*Glycyrrhiza glabra*) roots involving selective chemical reaction and easy separation, for recovering mono-ammonium glycyrrhizate (MAG) using hot water with dissolved ammonia and pressurized with carbon dioxide. The performance of the novel PHWE process has been evaluated to ascertain the optimum process parameters to maximize the recovery of MAG by varying the parameters, such as temperature (30–120 °C), pressure (1–10 atm), extraction time (60–120 min), water-to-feed ratio (20–40 ml/g), number of stages (1–3), stirring rate (0–350 rpm), ammonia concentration (0.01–4%, w/v), and grinding. The quantification of MAG recovered in the extract has been based on the vanillin sulfuric acid method using UV spectrophotometer. The maximum recovery of MAG from licorice roots has been achieved at 110 °C and 5 atm with the ratio of 40 ml/g of 0.01% (w/v) ammonia solution to powdered feed after 90 min of extraction.

1. Introduction

Licorice (*Glycyrrhiza glabra)* roots and rhizomes are extensively used in herbal medicines for their emollient, anti-inflammatory, anti-viral, anti-allergic, anti-oxidant, gastro-protective, and anti-cancerous properties. It is widely used worldwide in food, confectionery and pharmaceutical products, such as cough syrups, herbal supplements, chewing gums, drinks, and candy. It is a powerful natural sweetener, 50–170 times sweeter than sucrose. The chemical constituents of the roots include several bioactive compounds, such as glycyrrhizin (∼16%), different sugars (up to 18%) flavonoids, saponoids, sterols, starches, amino acids, gums and essential oils. Kitagawa [1] reported the detailed structures of 33 constituents in licorice roots and their sweetness. Glycyrrhizin is a water-soluble pentacyclic triterpenoid glycoside responsible for the sweetness of licorice and its aglycone is responsible for various medicinal attributes and clinical applications in the treatment of spleen, sore throat, bronchitis, liver, kidney, and ulcer. The glycoside usually occurs in a combined calcium or potassium salt form of glycyrrhizic acid (GA) which is a weak acid containing three carboxyl and five hydroxyl groups. The acid form of glycyrrhizic acid is not particularly water-soluble, but its ammonium salt is soluble in water at pH greater than 4.5. The mono-ammonium salt of GA is used as an anti-inflammatory and anti-allergic remedy for the treatment of bronchial asthma, eczemas and other diseases.

Methods of preparation of glycyrrhizic acid (GA) from licorice roots were investigated by different researchers and some important methods are summarized in Table 1. Most accepted technologies of the extraction of GA from the roots include extraction with hot water at ambient pressure in the presence of various additives like alkalis, mineral acids, ethyl alcohol, etc., such as, aqueous ammonia [2–4], methanol [5–7], ethanol [8]. The primary aqueous extract of licorice roots contains GA and many other water-soluble substances and then it is subjected to further processing for more purified products. Pure glycyrrhizic acid (GA) may be prepared from the licorice root by using alcohol as the solvent for extraction [9] using an ultrasonic device followed by purification. The most common purification procedure involves acidification of the extract by adding acids such as H_2SO_4 or HCl acids [4,5] for the formation of the solid product of salt of GA (at pH 1–2). The molecules of GA can also be extracted into the organic phase from aqueous solution by interacting with the polar functional groups of organic extractants through hydrogen bonding. So the nature of organic extractant and pH are the crucial factors for purification of GA. Several procedures are reported in the literature on solvent extraction

Table 1
Summary of important processes for extraction of glycyrrhizin (or mono-ammonium glycyrrhizate) from licorice roots

Ref. no.	Title	Inventor	Year	Method used
[2]	Separation of major components in licorice using high-performance liquid chromatography	Beasley et al.	1979	Aqueous extract is dissolved in 4% ammonium hydroxide solution for separation of at least eight major components in licorice extract using a gradient, reversed-phase HPLC system
[3]	Separation of glycyrrhizic acid and liquiritin from *Glycyrrhiza uralensis* Fisch extract by three-liquid-phase extraction systems	Shen et al.	2007	Dried licorice slices are extracted with aqueous NH_3 solution (0.5 vol%) by sonication. Three-liquid-phase systems contained four components that are organic solvent, inorganic salt, polymer and the treated licorice extract.
[4]	Purification of glycyrrhizin from *G. uralensis* Fisch with ethanol/phosphate aqueous two phase system	Tianwei et al.	2002	Powder is extracted by 0.01% NH_4OH at 90–100 °C, filtered, concentrated, dried under vacuum then added in mixture of EtOH, water and K_2HPO_4. After settling, upper portion collected and precipitation by H_2SO_4
[5]	Preparative purification of glycyrrhizin extracted from the root of licorice using high-speed counter-current chromatography	Jiang et al.	2004	Powder of licorice roots is extracted by 70% aqueous-methanol solution, then filtered and HCl is added for precipitation. Precipitation is dried by freeze-drying and separation of components is carried out by HSCCC
[6]	Separation and analysis of glycyrrhizin, 18β-glycyrrhetic acid and 18β-glycyrrhetic acid in licorice roots by means of capillary zone electrophoresis	Sabbioni et al.	2005	Powder of root is extracted by a solution of methanol and water 1:1 at 60 °C for 25 min under stirring, and then centrifuged for 10 min at 3000 rpm. The supernatant is filtered through a cellulose acetate syringe filter. Then analysis of G was performed
[7]	Determination of nine flavonoids and coumarins in licorice root by high-performance liquid chromatography	Zeng et al	1990	100-mg into a micro-Soxhlet is refluxed with 10 ml of MeOH for 2 h in a water-bath (70 °C). The methanolic extract is concentrated at 70 °C to less than 3 ml, after cooling to room temperature, is made up to 5 ml with methanol then used for HPLC
[8]	Microwave-assisted extraction of glycyrrhizic acid from licorice root	Xuejun et al.	2000	Extraction is performed in microwave-assisted extractor by water, EtOH, EtOH–water, ammonia solution (different concentrations) or ethanol–water–ammonia and analyzed by HPLC
[9]	Determination of three active principles in licorice extract by reversed-phase high-performance liquid chromatography	Tung-Hu and Chieh-Fu	1991	Extract of coarse roots with solution (water, 0.05 (M) NaOH, 50% ethanol, 95% ethanol or methanol). The solvent filtrates, condensed under reduced pressure, dried by lyophilization and after preparing suitable solution it is subjected to HPLC
[10]	The application of macro-porous resins in the separation of licorice flavonoids and glycyrrhizic acid	Boqiang et al.	2005	Powder is extracted with EtOH/H_2O (70:30, v/v) by sonication in an ultrasonic bath, solution was centrifuged and supernatant is concentrated to 1/10 of original volume in a rotary evaporator. Then solution is passed though different type of resins
[11]	Isolation and purification of inflacoumarin A and licochalcone A from licorice by high-speed counter-current chromatography	Wang et al.	2004	Roots are extracted with ethanol–water (95:5) by sonication, concentrated by vacuum rotary evaporator; residue is dissolved in 2% NaOH and then filtered. Precipitation takes place by adding HCl in filtrate. Precipitate is washed by water and freeze-dried

by various organic solvents, purification by ion-exchange and polymeric resins, adsorption, chromatographic separation, supercritical fluid extraction, foam separation [5,10], microwave assisted extraction (MAE) [7] and multi-stage counter-current extraction (MCE) [11] to extract GA. Most of the existing processes for the extraction and purification of the sweet ingredients from the licorice roots involve a number of steps and large amounts of solvents/chemicals. In order to obviate these difficulties, a novel process has been developed in the present work using hot water pressurized with carbon dioxide and dissolved ammonia for production of the natural sweetener in the form of mono-ammonium glycyrrhizate (MAG). The objective of this work was to evaluate the performance of the PHWE process and to ascertain the optimum process parameters to maximize the recovery of MAG by varying the process parameters, such as temperature, pressure, ammonia concentration, extraction time, solution-to-feed ratio, number of stages of extraction, stirring, and grinding.

2. Experimental

2.1. Materials and chemicals

Two types of feed namely licorice roots (1–1.5 cm diameter and 4–5 cm length) and powdered roots (mesh size 250) have been supplied by pharmaceutical shop, Jadavji Lallubhai & Co. in Mumbai, India. Milli-Q water purified on a Milli-Q® Ultrapure Water Purification Systems has been used for extraction. Sucrose with 99.94% purity from Sisco Research Laboratories Pvt. Ltd., 98.4% concentrated H_2SO_4 from Ranboxy Fine Chemicals Limited, vanillin with 99% purity from Sd Fine-Chem Limited, ethanol with 99.9% purity from Hayman Limited, 25% aqueous NH_3 from Merck have been used in the present work. CO_2 gas has been supplied by Sicgil Industrial Gases, India, with 99.99% purity. For making 0.01% (w/v) NH_3 solution, 0.88 ml 25% NH_3 solution (density of 25% ammonia solution = 0.9106 gm/ml) was added in 2000-ml distilled water. Similarly other solutions were prepared to have 0.01–4.0% (w/v) NH_3 solution.

2.2. Pretreatment

Licorice roots are cut into small pieces (1–1.5 cm diameter and 1–1.5 cm length), soaked with cold water and kept for 24 h for softening which leads to easier extraction later. Dry powder is also pretreated with absolute ethanol to remove undesired constituents. Pretreatment with ethanol (at 1 atm, 60 °C), methanol (at 1 atm, 50 °C) and *n*-hexane (at 1 atm, 40 °C) have also been tried. But nothing was extracted when *n*-hexane was used for pretreatment. As the powdered licorice roots pretreated with ethanol and untreated powder have resulted in the same recovery and color of the aqueous extract, pretreatment with ethanol is considered redundant.

2.3. Pressurized hot water extraction

All PHWE experiments in the present work have been conducted in batch mode. The experimental set-up consists of a 2.5-L autoclave made of SS 316 which can be operated at a pressure up to

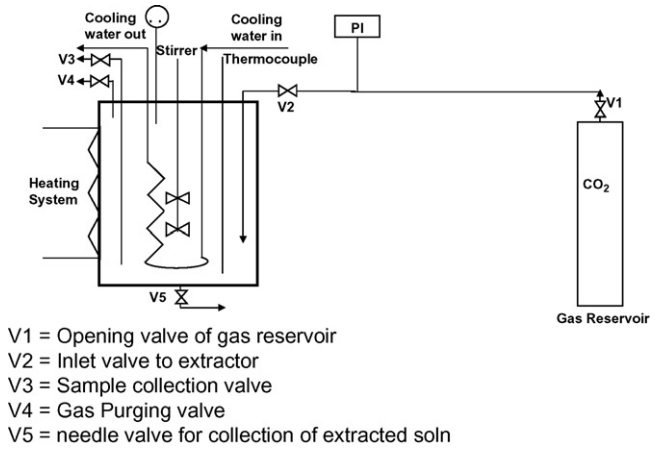

Fig. 1. Experimental set-up of PHWE.

200 bar and a temperature up to 250 °C. A schematic diagram of the experimental set-up is shown in Fig. 1.

For MAG to be formed from GA as its salt during PHWE, water with ammonia (0.01–4.0% w/v) is used. Two types of feed are used here namely small pieces of licorice roots and dry powder. A known amount of roots is charged in the PHW extractor with known amount of the NH_3 solution of different concentration. CO_2 cylinder is used to pressurize the system. The solution is filtered after extraction using the vacuum filter and the residue is again extracted at the same condition. The solutions obtained from two consecutive extractions are mixed together and the mixture is processed for purification.

2.4. Purification

The higher is the concentration of ammonia in water, the higher is the number of ammonium group in the salt produced. For extraction of MAG, the optimum concentration is found to be 0.01% NH_3 (w/v). The primary aqueous extract (with 0.01% NH_3 w/v) of licorice roots contains, in addition to mono-ammonium glycyrrhizate (MAG), other water-soluble undesirable substances, and further processing is needed for its purification. The process flow diagram for extraction and purification of MAG is shown Fig. 2. The filtrate is concentrated in vacuum rotary evaporator and then 70% ethanol is added (3:1) to this. The precipitate is separated from the upper portion of the solution and discarded. The upper portion of the solution is concentrated to remove ethanol which is again recycled back. The concentrated solution free from ethanol is then taken for acidification. Drop-wise H_2SO_4 is added in the solution and pH is monitored. White precipitates of MAG are formed when pH becomes 1–2 and no further precipitation takes place. With a higher concentration of aqueous NH_3 for PHWE, dark colored precipitates of di- and tri-ammonium glycyrrhizate are formed. The precipitates are separated out from the solution and washed with absolute ethanol to remove sulfuric acid and other undesirable substances. White precipitated material is then dried under vacuum and is characterized as the crystal of mono-ammonium glycyrrhizate (MAG) which is the desired natural sweetener.

2.5. Characterization

The quantification of the yield of MAG is done by colorimetric method followed by a UV measurement, as given by [12] with some modification. As the authors have well established the procedure for quantification of active ingredients in roots and leaves by spectrophotometric method, this method was used in the present work.

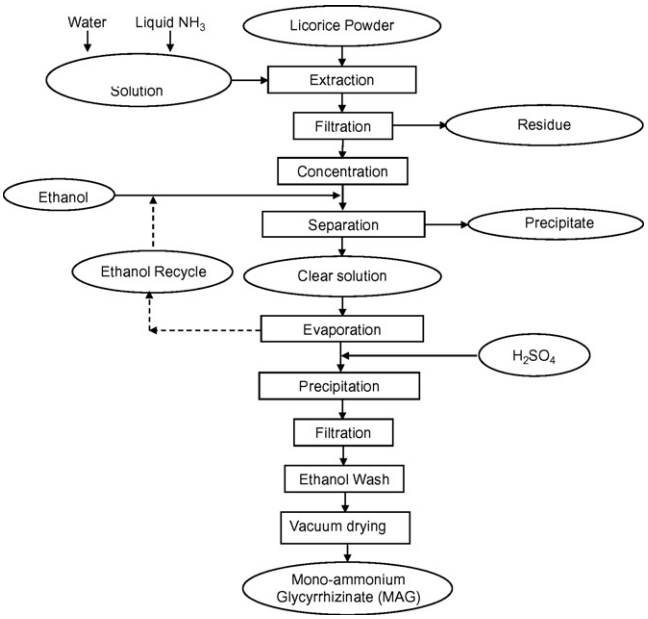

Fig. 2. Process flow diagram of extraction and purification of MAG.

TLC and HPLC could not be conducted in the present work for quantitative and qualitative determinations due to the nonavailability of pure components as reference samples which are rarely available in the market.

The colorimetric method used here is known as the vanillin sulfuric acid method. The sample is prepared by diluting (1:50 to 1:100) the aqueous extract with distilled water. For this method 8% vanillin solution in absolute ethyl alcohol and 72% sulfuric acid solution are prepared. 1 ml of the dilute solution is put into a 25-ml test tube placed in an ice bath and then 1 ml of 8% solution of vanillin in absolute alcohol and 5 ml of 72% solution of sulfuric acid are added. The mixture is shaken to obtain a homogeneous solution. Then the test tube is heated in a water bath at 60 ± 0.5 °C for 10 min and then cooled in an ice bath for 2–5 min. The blank solution is prepared similarly, but instead of 1 ml of the dilute solution, 1 ml of distilled water is added to the test tube. The absorbance of the colored (greenish brown) solution is measured on a spectrophotometer (Helios Alpha UV–Vis spectrophotometer model, Thermo Electron Corporation) at a wavelength of 470 nm. After getting the absorbance, the concentration of the sweet components equivalent to sucrose is calculated from the calibration plot. The calibration curve is prepared from samples of sucrose of different known concentrations ranging from 5 to 60 μg/ml and their absorbance at 470 nm as plotted in Fig. 3. The error of measurement and calculation of yield is ±3.5% maximum.

3. Results and discussion

PHWE from licorice roots has been performed for 60–120 min with constant stirring up to 350 rpm at temperatures ranging from 30 to 120 °C and pressure ranging from 1 to 10 atm as shown in Table 2. Experiments have been conducted with different ratios of solution-to-licorice roots and different percentages of NH_3 in water. Two types of feed namely small pieces of licorice roots and powdered licorice roots have been studied. During extraction by aqueous ammonia solution, a chemical reaction between ammonia and glycyrrhizic acid produces mono-ammonium glycyrrhizate (MAG), as shown in Fig. 4.

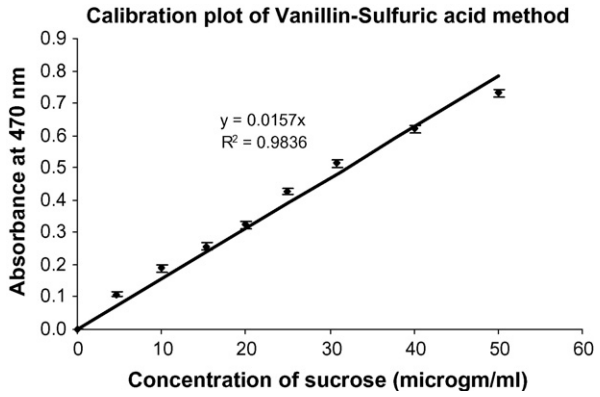

Fig. 3. Calibration plot of sucrose according to vanillin sulfuric acid method.

For pressurization of hot water, gaseous CO_2 has been used at a moderate (2–20 bar) pressure eliminating the need for steam injection or a pump. The role of CO_2 in the innovative process is multi-fold. One of them is lowering of pH enabling selective separation of major components. It also removes air from the system and provides inert environment, so that no degradation or oxidative reactions/conversions take place. Even an "inert" gas (e.g., nitrogen) is used for flushing the system off from oxygen (from air). This step is simultaneously avoided. Presence of slight residual CO_2 after extraction helps in slowing down degradation before and during subsequent steps. Thirdly it provides an easy means to pressurize the system without using a steam boiler and for easy controlling of the CO_2 solubility for the above two purposes. Fourthly, CO_2 is a gas and is removed from the aqueous slurry unlike HCl or other acids. This eliminates the subsequent steps for neutralization of pH after extraction and other additional steps, if a mineral acid is used. Finally usage of CO_2 reduces cost of production, as CO_2 is cheaper than nitrogen plus acid and chemicals used for other steps. Accordingly usage of CO_2 is not equivalent to addition of acid. This is one innovative aspect of the process, namely, the usage of CO_2 in extraction of natural sweetener from licorice along with pressurized hot water, which is distinct from the available prior art.

3.1. Parametric study

3.1.1. Effect of temperature

Fig. 5 shows the variation of the yield of MAG with temperature varying from 30 to 120 °C with 40 ml/g of water. Table 3 shows that the yield in the first extract is 7.8% at 30 °C whereas it is 14% at 120 °C and at temperatures above 60 °C, the total MAG recovered increases with rise in temperature up to 110 °C after that it is almost constant. It can be observed that the effect of temperature on the recovery is very significant. As temperature increases, properties of water like viscosity, density, surface tension, and polarity decrease leading to a decrease in resistance to mass transfer. So at a higher temperature more MAG is recovered from the licorice roots.

3.1.2. Effect of pressure

Fig. 6 shows the effect of pressure on the yield of MAG at 90 °C and the values are also presented in Table 4. The recovery is almost invariant with pressure at pressures above 5 atm. A higher pressure

Table 2
Various experiments with licorice roots for extraction of MAG

Feed type	Solvent		Temperature (°C)	Pressure (atm)	Time (min)
	Name	Solvent: feed (ml/gm)			
Powder	Water (0.01% (w/v) NH_3)	40	30, 60, 90	1	120
Powder	Water (0.01% (w/v) NH_3)	40	90, 110, 120	5	120
Powder	Water (0.01% (w/v) NH_3)	40	90	10	120
Pieces	Water (0.01% (w/v) NH_3)	40	90	1	120
Powder	Water (0.01% (w/v) NH_3)	20	90	1	120
Powder	Water (0.01% (w/v) NH_3)	60	90	1	120
Powder	Water (0.01% (w/v) NH_3)	80	90	1	120
Powder	Water	40	90	1	120
Powder	Water (0.1% (w/v) NH_3)	40	90	1	120
Powder	Water (0.15% (w/v) NH_3)	40	90	1	120
Powder	Water, methanol (1:1, v/v)	40	60	1	60
Powder	Water, Ethanol (1:1, v/v)	40	60	1	60
Powder	*n*-Hexane	20	40	1	60
Powder	Water (1% (w/v) NaOH)	40	60	1	60
Powder	Water, EtOH (25%, v/v), NH_3 (4%, w/v)	40	60	1	60
Powder	EtOH	20	60	1	60

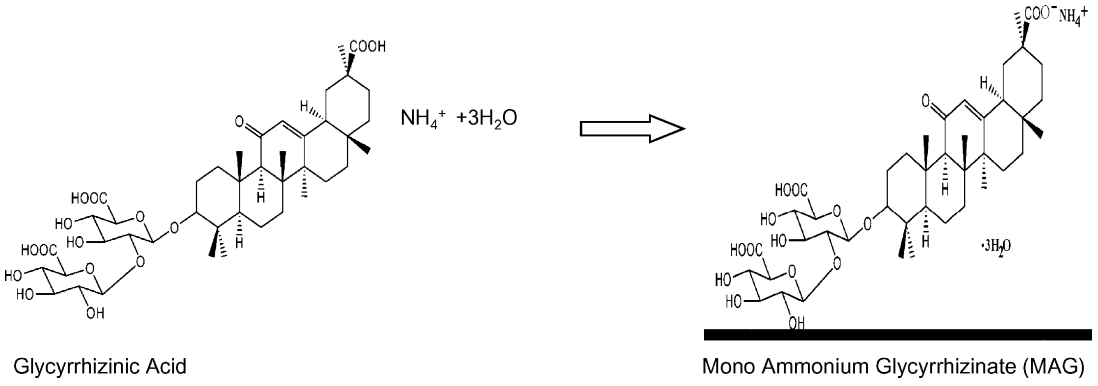

Fig. 4. Reaction between glycyrrhizic acid and aqueous ammonia to produce MAG.

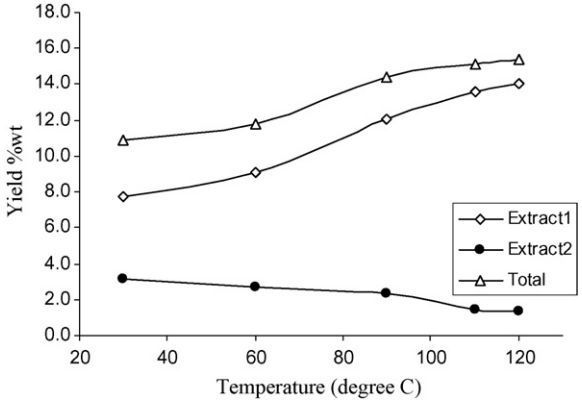

Fig. 5. Yield of MAG of aqueous extract from powdered licorice roots at different temperatures.

Table 3
Yield of MAG from licorice roots at different temperatures

Temperature (°C)	Yield (%, w/w)		
	Extract 1	Extract 2	Total
30	7.8	3.1	10.9
60	9.1	2.7	11.8
90	12.1	2.3	14.4
110	13.6	1.5	15.1
120	14.0	1.4	15.4

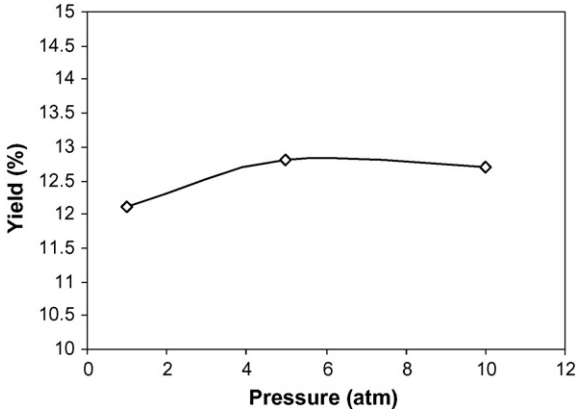

Fig. 6. Effect of pressure on yield of MAG of aqueous extract at 90 °C.

is maintained in the extractor to keep the water in liquid form and it also reduces evaporation loss. Solubility of ammonia in water increases with pressure and so sufficient ammonia is available in the solution to react with glycyrrhizic acid to produce MAG. When pressure is raised to above 5 atm, evaporation of NH_3 is suppressed and yield is increased.

3.1.3. Effect of extraction time

Fig. 7 shows the effect of extraction time on the yield of MAG from licorice root powder. At 60 °C, the yield increases sharply with

Table 4
Yield of MAG from licorice roots at different pressures at 90 °C

Pressure (atm)	Yield (%)
1	12.1
5	12.8
10	12.7

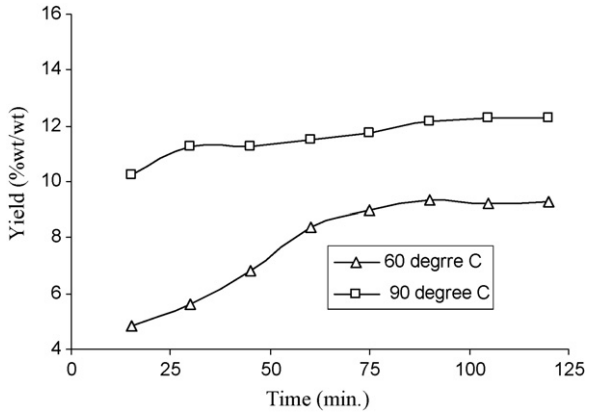

Fig. 7. Variation of yield of MAG with 40 ml/gm water with time.

time and it reaches a fixed value after 75 min whereas at 90 °C, the variation of the yield is not much with time. It can be seen that after 100 min, the yield of MAG is almost constant. Because initially, when there is no MAG in water, yield increases sharply with time. As more and more MAG is formed, the driving force for mass transfer decreases and the rate of mass transfer also decreases accordingly. After 90 min 40 ml/g of water gets saturated with MAG from 5 g of roots and the yield reaches a fixed value.

3.1.4. Effect of grinding

PHWE experiments have been performed using small pieces (1–1.5 cm diameter and 1–1.5 cm length) of licorice roots with 40 ml/g water (with 0.01% NH_3 w/v) at 90 °C for 2 h. This extraction gives 5.7% yield whereas powdered licorice roots yields 12.1% at the same condition. Very large surface area provides more mass transfer from powdered licorice roots whereas for the solid pieces of roots, the surface area is very less and the most parts of the roots are not accessible for extraction of the acid.

3.1.5. Effect of water-to-feed ratio

The values of yield of MAG with different water-to-feed ratios are shown in Fig. 8 for PHWE from 5 g powdered roots at 90 °C at 1 atm for 120 min. It can be seen that an increase in the water-to-feed ratio for PHWE increases the yield. However there is no significant change if the water-to-feed ratio goes beyond 60 ml/g of powdered feed. So the optimum water-to-feed ratio is considered as 6 ml/g.

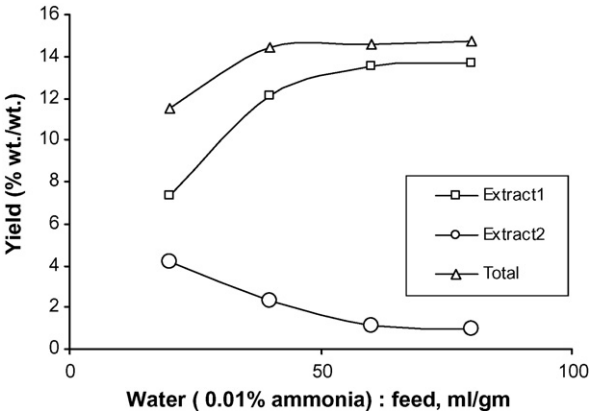

Fig. 8. Variation of yield of MAG with water-to-feed ratio at 90 °C.

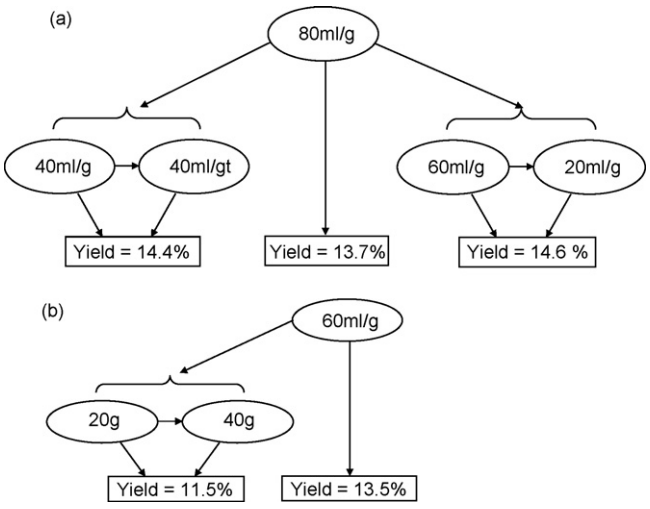

Fig. 9. (a) Comparison of yield with combination of 80 ml/gm water-to-feed ratio at 90 °C. (b) Comparison of yield with combination of 60 ml/gm water-to-feed ratio at 90 °C.

3.1.6. *Effect of number of stages*

The effect of number of stages is illustrated in Fig. 9a and b. From the values of yields obtained at 90 °C as compared in Table 5, one can note that the total yield is 14.4% by the water-to-feed ratio of 40 ml/g (for the 1st stage) and 40 ml/g (for the 2nd stage), whereas the total yield is 13.7% by the water-to-feed ratio of 80 ml/g (for the 1st stage). The total yield is 14.6% by the water-to-feed ratio of 60 ml/g (for the 1st stage) and 20 ml/g (for the 2nd stage) whereas the total yield is 11.5% by the water-to-feed ratio of 20 ml/g (for the 1st stage) and 40 ml/g (for the 2nd stage). The total yield is 13.5% by the water-to-feed ratio of 60 ml/g (for the 1st stage). From Fig. 9a and b, it is thus noted that the combination of 60 ml/g for the 1st stage of extraction followed by 20 ml/g for the 2nd stage extraction is the best combination so far as the yield is concerned.

3.1.7. *Effect of stirring*

At a temperature lower than 60 °C, the powdered roots of licorice have a tendency to settle down and accumulate on the bottom of the extractor and so the solid to water ratio becomes very high there. At a temperature more than 90 °C, the circulation of particles takes place throughout the solution due to convective flow of liquid when stirrer is off. So for a temperature higher than 60 °C, stirring is not necessary. Otherwise, extraction without stirring is very inefficient as most of the materials are not accessible to water. However the yield increases by increasing the rpm and less time of extraction is needed for the same yield [13].

3.1.8. *Effect of NH_3 concentration*

NH_3 concentration in the extraction medium, i.e., water, is a crucial factor for extraction of glycyrrhizic acid from licorice roots for production of mono-ammonium glycyrrhizate (MAG). The effect of ammonia concentration on the yield of MAG by PHWE at 90 °C is shown in Fig. 10. The values of the yields are also shown in Table 6. A controlled amount of ammonia is required for production of MAG which is white in color. Otherwise it leads to production of tri-ammonium glycyrrhizate which is bitter in taste. As the concentration of ammonia increases in the aqueous solution, the color of the extract becomes dark brown. So the formation of MAG can be visually monitored.

Table 5
Effect of number of stages on extraction yield

Extract 1		Extract 2		Total yield (%)
Water: feed (ml/gm)	Yield (%)	Water: feed (ml/gm)	Yield (%)	
20	7.3	40	4.2	11.5
40	12.1	40	2.3	14.4
60	13.5	20	1.1	14.6
80	13.7	20	1.0	14.7

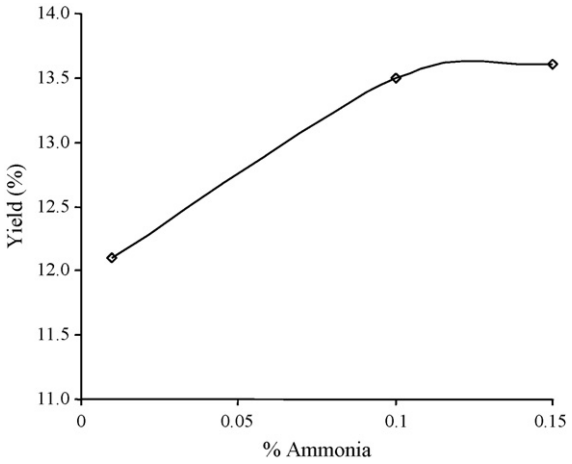

Fig. 10. Variation of yield of MAG with ammonia concentration at 90 °C.

Table 6
Yield of MAG with different concentrations of ammonia at 90 °C

NH_3 (%, w/v)	Yield (%, w/w)
0.01	12.1
0.1	13.5
0.15	13.6

Table 7
Summary of optimized parameters for PWHE and maximum yield

Material	Licorice
Parts used	Powdered roots
Water (ml): feed (g)	60
Temperature (°C)	110
Pressure (atm)	5
No. of stages	2
NH_3 (%, w/v)	0.01
Time (min)	90
Maximum yield (%, w/w)	13.6

4. Conclusions

A novel process has been developed for the production of a natural sweetener in the form of MAG from licorice roots involving chemical reaction and selective separation by the PHWE technique using subcritical CO_2 for pressurization and with dissolved ammonia for the ease of subsequent purification. A systematic parametric study has been undertaken to evaluate the performance of the process at different process conditions and to optimize the process conditions for the maximum yield of the sweet active ingredient and easier purification. Two types of feed have been investigated, namely the roots cut into pieces and ground roots and PHWE has been carried out at temperatures (30–120 °C), pressures (1–10 atm), extraction time (60–120 min), water-to-feed ratio (20–40 ml/g), number of stages (1–3), stirring rate (0–350 rpm), ammonia concentration (0.01–4.0%, w/v) with and without stirring. The colorimetric method namely the vanillin sulfuric acid method

using a UV spectrophotometer has been used to analyze MAG. The maximum amount of MAG is recovered from licorice roots at 110 °C and 5 atm with the ratio of 60 ml/g in 1st stage and 20 ml/g in 2nd stage of 0.01% (w/v) ammonia solution to powdered feed after 90 min of extraction, as summarized in Table 7.

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