Второй этап исследований по определению горьких веществ пива заключается в отборе 10 $см^3$ дегазированного пива без пены или 5 $см^3$ сусла в мерный цилиндр. Затем добавляют 1 $см^3$ HCl 3M или 0,5 $см^3$ HCl 6M и 20 $см^3$ изо-октана. Перемешивают в течение 15 минут в роторном шейкере. Затем оставляют на 20 минут, чтобы осела эмульсия или центрифугируют на протяжении 3-х минут при n = 3000 $мин^{-1}$. После того, как осела эмульсия, поверхность между слоями должна составлять не мене 5 мм. Затем отбирают изо-октановый экстракт пипеткой в кювету 10 мм. Пипетку опускают на расстояние 0,5 см выше поверхности раздела фаз. Измеряют поглощение при 275 нм относительно чистого изо-октана.

Окончательный расчет результатов определения проводили, используя формулу (1).

Единицы горечи при общей охмеленности:

$$BU = 57(114) \times D_{275}, \quad (1)$$

где 57 — коэффициент, используемый при определении горьких веществ в пиве;

114 — коэффициент, используемый при определении горьких веществ в пивном сусле;

D_{275} — оптическая плотность при λ = 275 нм.

Для 1-го образца — «Янтарь Светлое»: BU = 57 × 0,31 = 17,7.

Для 2-го образца — «Янтарь Светлое»: BU = 57 × 0,32 = 18,3.

Допускается расхождение 1,0 BU для образцов одного сорта. Расхождение в пределах нормы.

Заключительным этапом определения горьких веществ в пиве является очистка использованного изо-октана. Очистку проводят несколькими методами.

Первый метод — метод перегонки — основан на сборе верхнего слоя изо-октана в перегонную колбу. Водяную фазу не используют.

В 1 литр собранного изо-октана добавляют 50 $см^3$ 1н NaOH, 10 минут перемешивают. Оставляют на 1 сутки и удаляют водную (нижнюю) фазу с помощью всасывающей трубки или насоса. Добавляют 50 $см^3$ метанола, 10 минут перемешивают, оставляют на 1 сутки и снова удаляют водную фазу. Добавляют 250 $см^3$ метанола, 10 минут перемешивают. Отгоняют изо-октан на елочном дефлегматоре.

Второй метод — с активированным углем. Переносят остатки содержимого пробирки в делительную воронку. Сливают водную фазу и переносят органическую фазу в колбу вместимостью 6,0 $дм^3$. Собирают 2,5 л изо-октана.

Добавляют уголь Merek Art. 2586:

— 5 г, если время контакта 24 часа;

— 10 г, если время контакта 30 минут.

Периодически перемешивают колбу. Фильтруют через бумажный фильтр. Проверяют поглощение, если A > 0,01.

Третий метод — с силикагелем. После анализа переносят содержимое колбы в делительную воронку, изо-октан пропускают через силикагель 3 раза.

Выводы

В результате проведенных исследований было отмечено, что содержание горьких веществ в пиве напрямую зависит от содержания α-кислот в хмеле. Также доказано, что количество горьких веществ снижается на протяжении всего технологического процесса. Их содержание значительно уменьшается на стадии главного брожения, в конце которого образуются модифицированные α-кислоты: изо-α-кислоты, тетрагидро-изо-α-кислоты или гексагидро-изо-α-кислоты. На стадии дображивания и хранения их концентрация почти не изменяется.

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AQUEOUS ETHANOLIC EXTRACTS OF ACHILLEA MILLEFOLIUM PROA FOR INCORPORATION INTO SOFT DRINKS

The influence of ethanol concentration in the solution (from 0 to 80 % vol.) on the composition (total phenolics, phenolic acids, flavonoids, sesquiterpene lactones and total extractable compounds) and properties (radical scavenging activity and sensory characteristics) of Achillea millefolium Proa leaves, flower heads and stems extracts was studied. It was found that the sesquiterpene lactone content increased with the increase to ethanol concentration. When it varied between 40 and 70 % vol., the extracts obtained were the richest in phenolics. Under the same conditions, the leaf extracts were the richest in phenolics, and the flower head extracts were the richest in sesquiterpene lactones. The use of 20 - 60 % vol. aqueous ethanol solution yielded Achillea millefolium Proa flower head and leaf extracts with composition and properties which made them suitable ingredients for soft drinks.

Key words: A. millefolium Proa; extraction; phenolic compounds; radical scavenging activity; sesquiterpene lactones; sensory characteristics; soft drinks.

Изучено влияние концентрации спирта в растворе (от 0 до 80 %) на состав (фенольных веществ, фенольных кислот, флавоноидов, сесквитерпеновых лактонов и общих экстрактивных составляющих) и свойства (активность свободных радикалов и органолептические характеристики) экстрактов из листьев, соцветий и стеблей тысячелистника. При концентрации спирта 40-70 % были получены экстракты с наибольшим содержанием фенольных веществ. При этих же условиях, экстракты из листьев были также наиболее обогащены фенольными веществами, а экстракты из соцветий были наиболее обогащены сесквитерпеновыми лактонами. С использованием 20-60 % водно-спиртовых растворов были получены экстракты листьев и соцветий тысячелистника, состав и свойства которых позволяют использовать их в качестве ингредиентов для безалкогольных напитков.

Ключевые слова: тысячелистник, экстракция, фенольные вещества, активность свободных радикалов, сесквитерпеновые лактоны, органолептические характеристики, безалкогольные напитки.

INTRODUCTION

The utilisation of naturally-occurring bioactives as potential sources of functional food ingredients and, in particular, phytochemicals has increased in recent years because of their beneficial health effects. Consumer demand for health-related products providing immune support, energy enhancement, and healthy joint function, and promoting overall well-being has led to the development of functional foods and beverages.

A. millefolium Proa is a tetraploid cultivar of yarrow rich in proazulenes and is dominating in the field of commercially available plant material for the drug "Herba Millefolii" [2] Aqueous and alcoholic extracts of yarrow (A. millefolium L.) are widely used in European folk medicine in the treatment of inflammatory and spasmodic gastrointestinal complains, hepatobiliary disorders, as appetite enhancing drug, and externally against skin inflammations and for its wound healing effects [8, 19]. The A. millefolium extracts are also used as a commercial flavoring in beer, liqueur and non-alcoholic beverages [17, 19].

The pharmacological effects of yarrow could be due to different plant compounds. Thus, the anti-inflammatory effect is attributed to the presence of azulenogenic sesquiterpene lactones [10]. The flavonoids from yarrow mediate the spasmolytic activity [14], whereas the choleretic effects are caused by the dicaffeoylquinic acids [1]. In addition, phenolic compounds also possess antioxidant and free radical scavenging activities [5, 16, 22]. The literature survey revealed several publications concerning the analysis of total phenolic, flavonoid and sesquiterpene lactone content in A. millefolium extracts and their antioxidant activity [3, 4, 9, 11, 12, 20]. However, there is no information in literature on yarrow extraction with a view to obtaining extracts suitable for the manufacture of functional foods and beverages. Therefore, the objective of this study was to determine the effect of ethanol concentration on the content of phenolic compounds and sesquiterpene lactones as well as the sensory characteristics and radical scavenging activity of aqueous-ethanolic yarrow extracts for incorporation into soft drinks.

MATERIAL AND METHODS

Materials

Plant material

Plant material of cultivar A. millefolium Proa was obtained from Bulherba Ltd, Bulgaria (2007). The plant was sorted into flower heads, leaves and stems, then air-dried and kept in a dark place.

Solvents and reagents

The reagents, standards (except matricin) and solvents were commercially available materials of analytical grade. Folin-Ciocalteus reagent, quercetin, gallic and caffeic acids were obtained from Merck, while 1,1-diphenyl-2-picryl-hydrazyl (DPPH) from Sigma. Matricin was isolated from A. collina previously in our laboratory [21] and characterized by spectral methods. Aqueous solutions of EtOH with concentrations of 0 to 80% vol.were prepared from distilled water and ethanol (95.6 %, vol.).

Preparation of extracts

The air-dried and ground flower heads, leaves and stems (21-31 g) were placed into a flask and the corresponding solvent was added. The solid/solvent ratio was maintained at 1:10 (g dwm/ml solvent). The content in the flask was homogenized and left to macerate for 24 h at 25°C. Further, the extracts were filtered through filter paper MN 614 (Macherey-Nagel GmbH, Germany) in order to separate the plant material from the extracting solvent.

Methods for analysis

Total extractable compounds (TEC): by the weight method [7].

Determination of total phenolic (TPh) content: by the Folin-Ciocalteu method [18] and expressed as gallic acid equivalents (g/dm^3 of plant extract solution). Determination of flavonoid (F) content: by a modified Glories method [15] and expressed as quercetin equivalents (g/dm^3 of plant extract solution). Determination of phenolic acid (PhA) content: by a modified Glories method [15] and expressed as caffeic acid equivalents (g/dm^3 of plant extract solution).

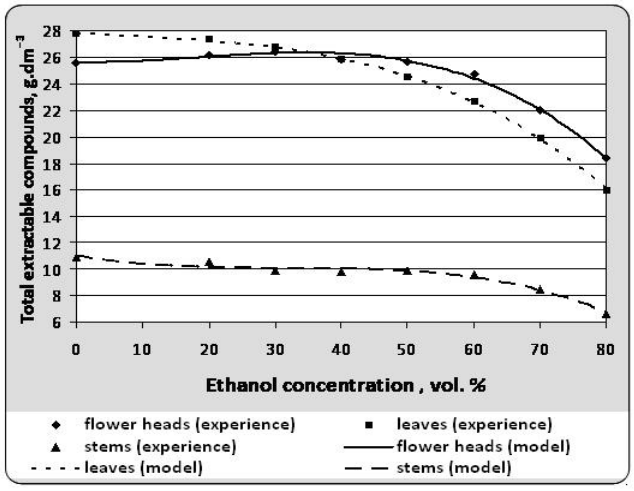

Fig. 1. Total extractable compounds (TEC) in g/dm^3 aqueous- ethanoli extracts of A.Millefolium Proa

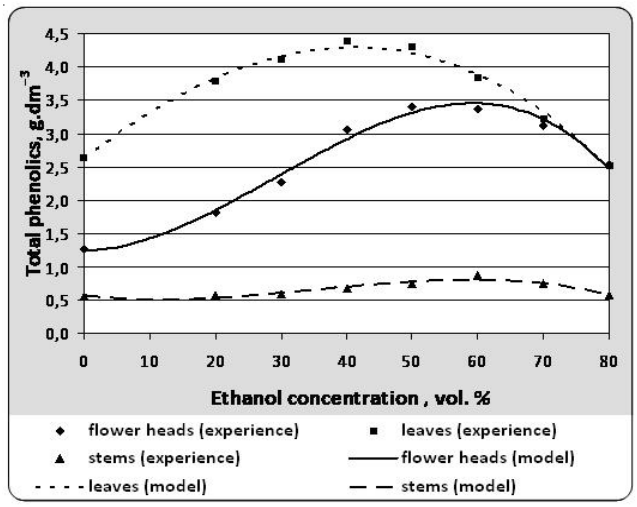

Fig. 2. Total phenolics (TPh) expressed as gallic acid in g/dm^3 aqueous- ethanolic extracts of *A.Millefolium* Proa

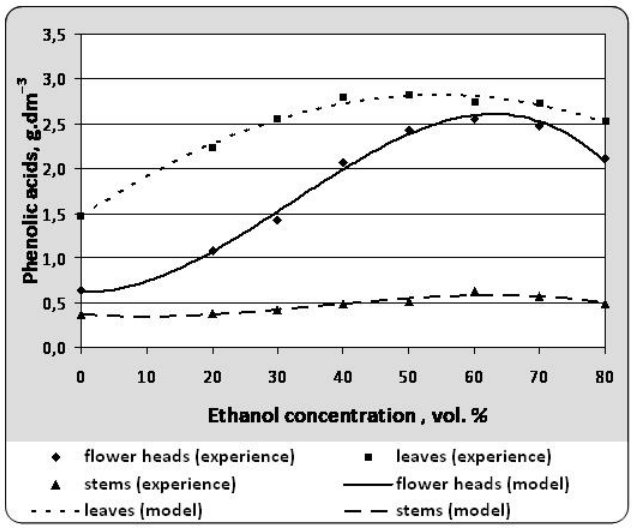

Fig. 3. Phenolic acids (PhA) expressed as caffeic acid in g/dm^3 aqueous- ethanolic extracts of *A.Millefolium* Proa

Determination of sesquiterpene lactone (STL) content: STL was determined on Bruker Tensor 27 by an IR spectroscopic method with some modifications and expressed as matricin equivalents per dm^3 of plant extract solution [20].

Radical scavenging activity (RSA): A modification method of Godow [6] was used. Ethanolic solution of DPPH radical (10^{-4} M) and A.Millefolium Proa extracts were mixed, so that the final mass ratios were $DPPH^{\cdot}$: extract dry mater = 1:1. The samples were incubated for 10 min in the dark and the decrease in absorbance at 517 nm was measured. The radical scavenging activity of the tested samples expressed as percentage reduction of $DPPH^{\cdot}$ was calculated according to the formula:

$$I = [(A_b - A_a) / A_b] .100, \ \%$$

where A_b and A_a are the absorbance values of the blank and the test samples.

Sensory analysis: The extracts, obtained from flower heads, leaves and stems with 20, 40, 60 and 80% vol. aq EtOH were used for determination of the sensory characteristics. They were added to model soft drinks and the analysis was performed using a descriptive method [13]. The maximal volume of the extract solution added to the model drinks was prepared according to the requirement for a maximum EtOH content of 0.5% in non-alcoholic beverages.

RESULTS AND DISCUSSION

Effect of ethanol concentration on the total extractable compounds

Initially, the effect of solvent concentration on the total extractable compounds (TEC) was studied (Fig. 1). As can be seen, maximal values of TEC were found in water as a solvent (0% ethanol solution). Further, TEC were almost equal with the increase in ethanol concentration up to 50-60% vol. When ethanol concentration was higher than 60% vol., the TEC decreased significantly. Fig. 1 also shows TEC in extracts obtained from different aerial parts of the plant. TEC in flower head and leaf extracts did not differ significantly and exceeded the TEC in stem extracts by 4–7 times. This was expectable because the compounds involved in the stalk construction (mainly cellulose and hemicellulose) are insoluble in water and ethanol.

Effect of ethanol concentration on the content of phenolic compounds

Next, the effect of ethanol concentration on the content of total phenolic compounds (TPh), phenolic acids (TPhA) and flavonoids (F) in extracts obtained from different aerial parts of A. millefolium Proa was investigated. As can be seen from Fig. 2-4, the amounts of total phenolic compounds, phenolic acids and flavonoids were greatly influenced by ethanol concentration. Thus, the TPh content in extracts with 20, 30 and 40% ethanol increased between 25 and 43% for flower heads, 4 – 44% for leaves and up to 14% for stems. A similar trend was observed for the content of phenolic acids (Fig. 3) and flavonoids (Fig. 4). The highest values of TPh, PhA and F were obtained at 40-60%, 40-70% and 50-70% vol. aq EtOH, respectively. It was found that water extracts were poor in phenolic compounds. The data presented in Fig. 2-4 showed that the extracts yielded by leaves were the richest in phenolic compounds. Thus, their TPh, PhA and F content was 4 to 6 times as high as that in stem extracts and twice as high as that in flower head extracts.

Furthermore, the difference in the amount of phenolic compounds in the leaves and flower heads at higher EtOH concentrations decreased and they were almost equal above 60% EtOH.

The shift in the maximal values of phenolic compounds in different aerial parts of A. millefolium Proa according to ethanol concentration is worth mentioning (Fig. 2-4). It was found that the amounts of phenolic compounds in leaf extracts reached their maximal values at lower ethanol concentrations compared to those obtained from flower heads and stems.

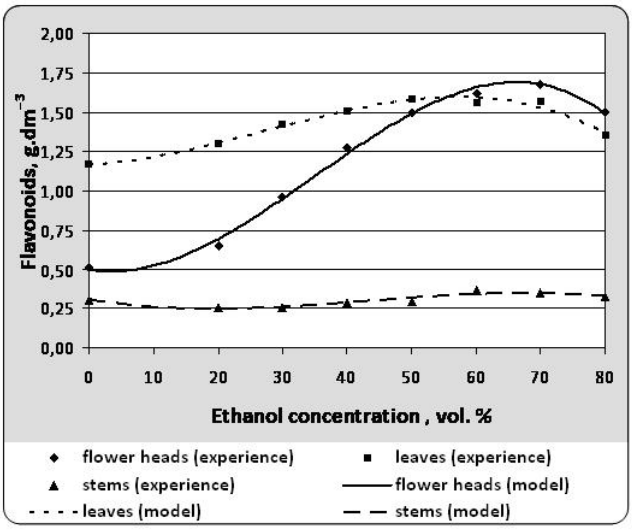

Fig. 4. Flavonoids (F) expressed as quercetin in g/dm^3 aqueous- ethanolic extracts of *A.Millefolium* Proa

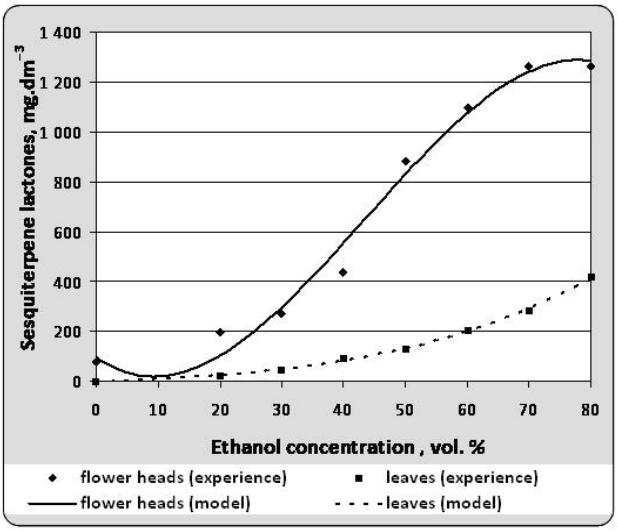

Fig. 5. Sesquiterpene lactones (STL) expressed as matricin in mg/dm^3 aqueous- ethanolic extracts of *A.Millefolium* Proa

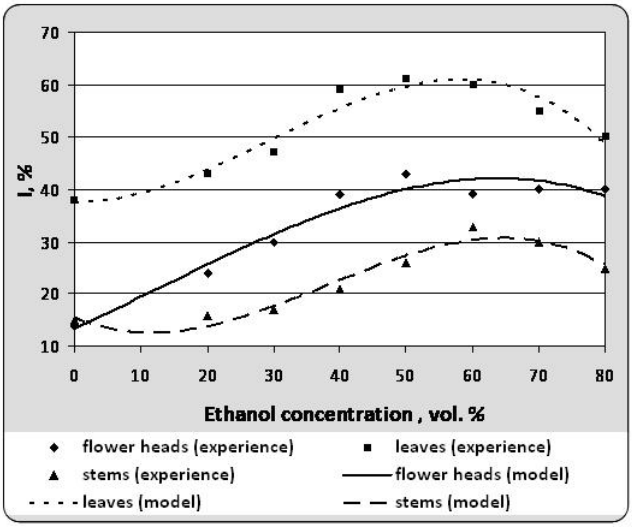

Fig. 6. Radical scavenging activity of aqueous- ethanolic extracts of *A.Millefolium* Proa expressed as % Inhibition of radical DPPH (I)

These results suggested certain differences in the phenolic content in the different aerial parts of yarrow and/or specificity in the manner and place of accumulation of these compounds in the tissues of leaves, flower heads and stems.

Effect of ethanol concentration on the content of sesquiterpene lactones

Sesquiterpene lactones (STL) were the second group of biologically active compounds included in the subject of the present investigation. The sesquiterpene lactone content was found to be much higher in the flower head extracts compared to that in leaf extracts regardless of ethanol concentration (Fig. 5). Thus, leaf extracts contained 3–8 times lower amounts of sesquiterpene lactones, while in stem extracts no sesquiterpene lactones were detected. As can be seen from Fig. 5, the content of STL in water extracts was very low: only 77.5 mg/dm^3 in flower head extracts and traces in leaf extracts. The maximal STL was obtained with 80% vol. EtOH and reached 1263 and 416 mg/dm^3 in flower head and leaf extracts, respectively.

DPPH radical scavenging activity

Fig. 6 showed the ethanol concentration effect on the radical scavenging activity (RSA) of the extracts studied. The highest RSA values were obtained at 50-70% vol. aq EtOH. The phenolic content in the extracts was found to correlate with their free radical scavenging activity (e.g. the correlation coefficient between the DPPH assay data and TPh content was 0.885), confirming that phenolic compounds were likely to contribute to the radical scavenging activity of the plant extracts. The highest DPPH scavenging activity was observed with leaf extracts, and the lowest with stem extracts.

Sensory characteristics

As a result of the sensory analysis, the model drinks prepared with stem extracts were found to be the poorest in taste and flavoring, and not balanced. This could be explained by the significantly low content of extractable substances in the corresponding extracts. The series of model drinks prepared with leaf extracts was more interesting. They possessed grassy and spicy notes in their taste and aroma. The taste was characterized by the appearance of astringent notes. It was not accidental since the extracts of leaves were the richest in phenolic compounds. Astringent taste nuances were also felt in some samples with flower head extracts. It is worth noting that the bitter taste typical of yarrow was only detected in the variants prepared from flower head extracts with 60 and 80% EtOH. The bitterness in the latter case, feeling as a final taste was not so pleasant. Fruit tones predominated in the model drinks from extracts with 20% ethanol. The model drinks prepared from extracts with 20% EtOH were found to be richest in phenolic compounds as compared to those obtained with 30-80% EtOH. They were distinguished by a balanced, mouthcoating, fresh taste with fruit notes and a pleasant aroma, except for the model drink with stem extracts. The model drink from a flower head extract with 60% EtOH was the richest in sesquiterpene lactones and possessed pleasant sensory characteristics.

CONCLUSIONS

The results obtained showed that extracts prepared from the yarrow (*Achillea millefolium* Proa) leaves were richest in phenolic compounds and possessed highest DPPH radical scavenging activity. Maximal amounts of sesquiterpene lactones were observed in flower head extracts. It was also found that extracts from yarrow leaves and flower heads with 20 – 60 % aq EtOH were suitable for use in soft drink formulations. The drinks would possess a pleasant taste and contain some of the biologically active substances of yarrow.

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