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Polyphenol Production via Newly Designed System Capable Of Realizing Simultaneous Extraction-Distillation Operations In A Single Column

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Abstract: *The production of polyphenols from horse chestnut shells was achieved by newly designed system containing three-column capable of realizing simultaneous extraction-distillation operations. The effects of batch and continuous operations, solvent and/or solvent combination usage on total polyphenol yield, type of polyphenol component present in the final product, and heat loss to the environment was investigated. The results showed that, high efficiency, capability of specific product design, continuous production advantages, the reduction of fresh solvent usage requirement, easier process control, reusability of pure solvent due to distillation are the possible advantages of this new design. And also, isolation of the system can minimize the main disadvantage namely heat loss by half. It was concluded that, since polyphenols in plants are frequently used in food, cosmetic and pharmaceutical industries due to their antioxidant, antimicrobial and anticancer properties, the specific solvent and design must be used for production of specific polyphenol component-rich products.*

Keywords: *Polyphenol, Extraction, Distillation, Production*

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I. Introductıon

Polyphenols contain one or more aromatic rings with one or more hydroxyl groups attached to them [1,2]. They are separated according to their chemical structure as flavonoids, phenolic acids, stilbenes, lignans and tannins [3]. The main sources of polyphenols are fruits (especially grapes, berries, apricots etc.), vegetables (onions, spinach, artichoke, broccoli etc.) and herbs (turmeric, rosemary, ginger) [4-7]. Some beverages such as green tea, beer, red wine and coffee also contain high levels of these compounds [8]. Nearly more than 8000 different types of polyphenols have been detected up to now [9]. The content and types of polyphenols in plants varies with genetic and environmental factors, cultivation and storage conditions [10]. In addition, it was determined that the amount of polyphenol in plants was higher in the outer layers than in the inner layers [11].

Polyphenols are known as secondary metabolites of plants, and are responsible for their characteristic color. They are involved in defense against different types of stress, protection against extreme conditions, UV light, pathogens, reactive oxygen species, and plant predators [12-13]. Scientists have shown that polyphenols can prevent various types of diseases due to their antioxidant properties [14-19]. They show cytoprotective effect towards normal cells and cytotoxic effects towards cancerous cells [14]. In cancer therapy, inhibition of tumor growth [15], reduction of methastatic potential [16], and suppression of the side effects of chemotheraphy and radiotheraphy [17] can be achieved with polyphenols. These molecules do not only prevent diseases, but also suppress their propagation and progression [2, 14]. They act as hormones, enzyme inhibitors, anti-microbial and anti-inflammatory agents [18-19]. In addition to cancer prevention, certain phenolic compounds also have specific properties. For example, quercetin acts as an inhibitor of lipid peroxidation, it exhibits anti-infective and anti-replicative activity against some viruses [20, 21], and gallic acid shows anti-melanogenic activity [22]. Those beneficial effects depend on consumed polyphenol concentration and absorbed amount of it in the intestine [10].

The separation of these materials from plant sources has become inevitable due to the fact that polyphenols are obtained only from foods, the concern that the increase in agricultural foods will not sufficient for the world population, and the increasing approaches to the use of polyphenols in cancer. Therefore, polyphenols have been extracted from plants by using solvents such as methanol, ethanol, water, acetonitrile, acetone or their combinations [23]. Selection of a solvent of low viscosity (methanol or ethanol) had been found to accelerate the mass transfer [24]. However, there are some concerns about organic solvent usage: possible hazardous effects on human body and possible impurities of solvents in the final product. Thus, additional time-consuming and costly purification steps (especially distillation) must be applied. Although several different types of new extraction methods including microwave, ultrasonic, supercritical extraction have been developed

[25-27] in order to decrease the used amount of organic solvent, conventional techniques have been widely used in industry due to ease of use, high efficiency and wide-range applicability [28, 29]. The most widely used conventional extraction method, known as Soxhlet extraction, has its own disadvantages: in general, batchwise operation, requirement of high amounts of organic solvent usage, product deformation due to long time heating of solvent. The advantages of the method are that there is no need for filtration after the extraction, the device cost is low, and the material to be extracted faces with fresh solvent continuously. The diffusion rate of polyphenols, thus the yield of extraction and components of polyphenol mixture, depends mainly on solvent characteristics, and extraction conditions [30-33]. Another important problem of polyphenol production in industry is characterization of the polyphenol mixture containing product. Analysis of polyphenols are achieved either by UV-Vis spectrophotometric (2,2-diphenyl-1-picrylhydrazyl (DPPH) or Folin-Ciocalteu Methods) or chromatographic methods (High Performance Liquid Chromatography; HPLC) [34, 35]. UV methods are easy to use, and have low cost but they are not capable of determining the polyphenol type. Quantification and characterization of polyphenols can be realized by chromatographic methods, but they have high cost and requires skilled staff.

In the present study, a column was designed to realize extraction and distillation operations simultaneously and polyphenol mixtures of horse chestnut was produced with this designed system. Three types of solvents and their combinations were used in the production and energy requirements of the batch and continuous systems were investigated. We further analyzed the polyphenol content of the product by spectrophotometric methods and characterize the components present in the product by HPLC. Finally, the correlation between Folin-Ciocalteu and HPLC was developed in order to minimize the HPLC usage requirements for further applications.

II. Experimental

Chemicals and materials

A non-food plant material (horse chestnut shells) was chosen as polyphenol source. Horse chestnuts were harvested from the trees in Suleyman Demirel University at sixteenth week after the beginning of the flowering period to have the highest total polyphenol content [36]. The shell of this material was shredded (0.01-0.03 m) and dried in an oven (NUVE, FN-400) at 328 K during 90 minutes. Then they were milled (SINBO, SCM-2934) up to grounded form to increase the surface area. In order to eliminate the foaming resulted from the saponin content of horse chestnut shells [36], and eliminate the filtration operation at the end of the process, 16 g of grounded material was packaged into filter paper. In the production process water (W), ethanol (E), and methanol (M), either individually or in double/triple combinations (1:1 in double; 5:3:2, 5:2:3, 2:3:5 in triple combinations, by volume) were used as solvents. All of the chemicals used in the study (Folin-Ciocalteu reactant, Na_2C0_3, methanol, ethanol, acetic acid, gallic acid, quercetin) was purchased from Sigma-Aldrich at an analytical grade.

Polyphenol analysis and characterization

Spectrophotometric analysis

The amount of total phenolics in products of a defined system was determined according to Folin-Ciocalteu procedure [35]. 0.4 ml of samples were introduced into test tubes; 0.5 ml Folin-Ciocalteu's reagent, 1.5 ml Na_2C0_3 (%20 by weight), and 5.1 ml of distilled water were added. The tubes were mixed and allowed to stand in an incubator (ILDAM) for 2 hours at 298 K. Absorption at 765 nm was measured (Cary 60 UV-VIS spectrophotometer) and the total phenolic content was expressed as gallic acid equivalents (GAE) in miligrams per gram of dry material and per ml of solution, by using the calibration curve (Absorbance = 0.01532 * Concentration of phenolics (10^{-3} mg/ml); $R^2 = 0.9989$) [37].

Chromatographic analysis

High performance liquid chromatography (HPLC) was performed with a Shimadzu HPLC equipped with LC-10ADvp pump and CTO-10Avp column oven and a DAD detector (λ_{max} = 278 nm). Mobile phase consists of water containing 3.0% acetic acid (solvent A) and methanol (solvent B). The injection volume and flow rate were adjusted as 20 µl and 0.8 ml/min, respectively. Chromatographic separation was carried out with Agilent Eclipse XDB-C18 Column (4.60 mm x 250 mm; id 5 µm) at 303 K. Gallic acid (4.8 min) and quercetin (10.7 min) were used as standards (Fig. 1).

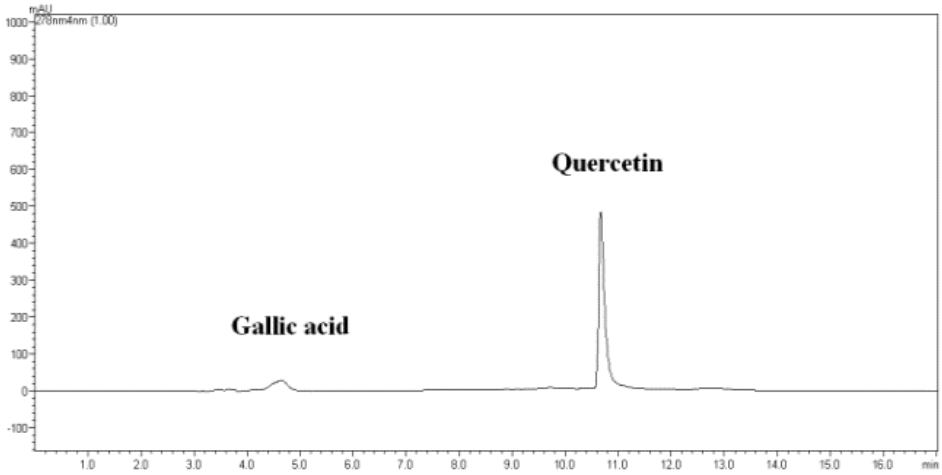


Fig. 1. HPLC chromatogram of standards

Polyphenol production system

Design of a system

In this study, a system consisting of three columns of Soxhlet apparatus which performs extraction and distillation process simultaneously was designed. This system was operated either batch or continuous manner. In both cases, the solvent or solvent mixtures in the storage tank were fed into the balloon containing the solid plant material (16 g) of the column with the help of a pump until the volume reached 400 ml, and the valve was closed, and then the balloon was started to be heated. While extraction in the balloon was carried out, the distillation was realized by accumulating the condensed solvent vapor in the Soxhlet apparatus. In the batch wise operation (Fig. 2), when the distilled solvent had reached the height of the siphon (130 ml), it was poured back into the balloon, and thus the first cycle was completed. The procedure was repeated in the same manner during three cycles in each column simultaneously. At the end of each cycle, the solid plant material and extract were removed from the medium and the new material was fed.

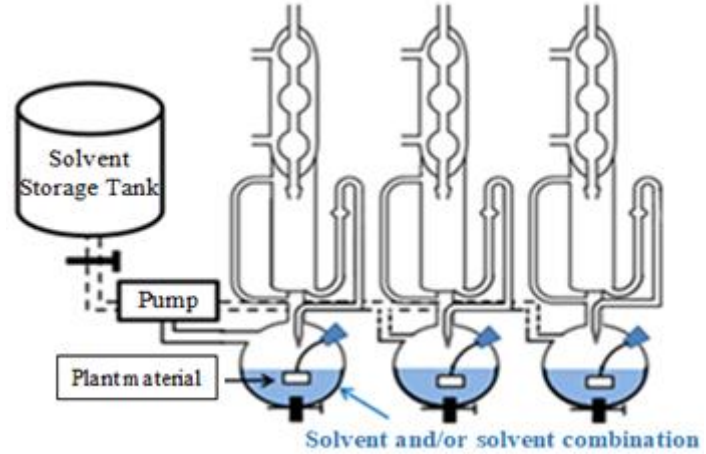


Fig. 2. Batch polyphenol production system

In the continuous system (Fig.3), the distillate (130 ml) was not discharged back into the balloon of the same column but was fed to the balloon in the next column in which 270 ml fresh solvent was pumped. The first cycle of this column was completed by removing the extract obtained at the bottom of the balloon and solid plant material from the column. In the meantime, solid material was fed to the first two columns and the second cycle for the first column and the first cycle for the second column were initiated. The system was run in the same way. The fresh solvent or solvent mixture fed from the storage tank filled the entire volume in the first balloon (400 ml), but it filled the remaining volume (270 ml) in the second and third columns. After reaching the siphon height, the distilled solvent in the first column was fed to the second column, and the solvent of the second column was fed into the third column.

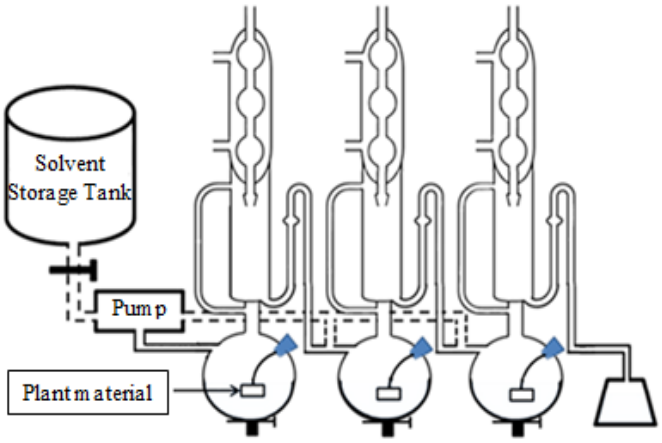


Fig. 3. Continuous polyphenol production system

Traditional Soxhlet extraction method was chosen as the control group because of its similarity in column design used in the study. The solvent or solvent mixture in which the highest amount of polyphenol was obtained in the continuous system was used as solvent of the control group. The process was carried out in three cycles as in the batch system, the volume of solvent and the amount of solid plant material were kept constant in all experiments. The time and temperatures until the completion of all cycles in the systems were continuously monitored.

Calculations

Energy loss

All of the designed systems are operated in an open atmosphere. Assuming that there was negligible vapor loss in the system and that the kinetic and potential energy changes were negligible compared to the internal energy change, the general energy balance (Eq. (1)) simplified to Eq. (2); and at steady-state conditions it expressed as Eq. 3. In other words, the work carried out with the electric heater was used for heating of polyphenols (Q_{GA}), plant material (Q_{plant}), solvent or solvent mixture ($Q_{solvent}$), and the extraction-distillation apparatus ($Q_{apparatus}$) to a certain temperature and it was also used for the heat loss from the system to the environment due to nonisolated system usage (Eq. (4)).

$$\frac{d}{dt}\left[U + m\left(\frac{v^2}{2} + gz\right)\right] = m_i\left(h + \frac{v^2}{2} + gz\right) + dQ + dW \quad (1)$$

$$\frac{dU}{dt} = dQ + dW \quad (2)$$

$$0 = Q + W \quad (3)$$

$$W_{heater}\left(\frac{J}{s}\right) \times t(s) = Q_{GA} + Q_{plant} + Q_{apparatus} + Q_{solvent} - Q_{lost} \quad (4)$$

It was assumed that all polyphenols produced in the study were gallic acid. Since the specific heat capacity value of gallic acid, a type of hydroxybenzoic acid, is not found in the literature, the molar heat capacity value of p-hydroxybenzoic acid was used [38] in calculation of the amount of heat of polyphenols (Eq. (4)).

$$Q_{GA} = (0{,}1243T_{max.} + 303{,}69)\left(\frac{J}{mol \times K}\right) \times n\,(mole) \times \Delta T(K) \quad (5)$$

16 g horse chestnut shells were used for all three cycles in the batch system and for each cycle in the continuous system. The heat capacity value of the chestnut shell in the literature is not found. Thus, because of the similarity, the heat capacity value of hazelnut (1 J/gK) [39] was used in Q_{plant} calculations [39]. Since the glass apparatus used in the study consisted of Soxhlet apparatus and balloon, total mass ($m_{Soxhlet\ aparatus+\ balloon}$ = 311 g) and the heat capacity value of glass ($c_{p,glass}$ = 840 J/kgK) were used in calculation of $Q_{apparatus}$.

In the continuous system, the initial volume fraction of the second and third columns changes, since the distillate from the previous column was supplied to the balloon with fresh solvent, Therefore, in the $Q_{solvent}$ calculations (Eq. (6)) containing solvent combinations of double and triple, it was assumed that the solvent with low boiling point always filled the distillate volume first, since it evaporated first. If this solvent was not capable of filling the whole volume, it was assumed that the component having next highest boiling point filled the

remaining part. Thus, the volume fractions (x_i) and the specific heat capacity values (c_{p_i}) at the average temperature observed in the second and third columns were calculated from Eq. (7). The density of pure solvents ($\rho_{solvent}$) and mixture of solvents ($\rho_{mixture}$) were calculated by the Antoine equation (Eq. (8)) and mole fractions, and the moles of the solvents were determined using these values.

$$Q_{solvent}(J) = n_{solvent}(mol) \times c_{p_i}\left(\frac{J}{mol*K}\right) \times (\Delta T)(K) \quad (6)$$

$$c_{p_{mixture}} = \sum_{i=1}^{3} x_i c_{p_i} \quad (7)$$

$$\rho_{solvent}\left(\frac{kg}{m^3}\right) = \frac{A}{B^{1+\left(1-\frac{Tmean.}{C}\right)^D}} \quad (8)$$

$$\rho_{mixture} = \sum_{i=1}^{3} x_i \rho_i \quad (9)$$

All calculated heat values were inserted into Eq. (4), and the heat losses emitted from the distillation surface (Soxhlet apparatus) and the condenser surface were calculated for all systems. Heat flux values were found by calculating the amount of heat lost from the surface (A = 2.π.r(m).h(m)) of these parts per unit time (Eq. (10)). The height (h) and radius (r) of the apparatus used were 0.37m and 0.025m in distillation part, and 0.55m and 0.02m in condenser part. The fluxes of heat loss in each of the system and system parts were compared.

$$q\left(\frac{kJ}{m^2}\right) = Q_{lost}(J) \times \left(\frac{1000}{Area\ (m^2) \times Time\ (s)}\right) \quad (10)$$

Folin-HPLC Correlations

In the study, HPLC analysis of the highest polyphenol containing products determined by Folin-Ciocalteu analysis was performed. Each of the phenolic compounds with different retention times were first encoded in the spectrum of the solvent type, the area fraction of the component ($x_{i,s}$) (Eq. (11)), the correlation constant (R_s) and the concentration values of that component ($C_{i,s}$) (Eq. (12)) were calculated. In these calculations, since no measurable gallic acid content was found in the products, quercetin was used as standard material and the specific value of the total amount of phenolic component for that solvent determined by Folin-Ciocalteu method were used. Then, similar procedure was followed by considering all chromatograms as a whole; the total area fraction ($x_{i,w}$) and total correlation constant (R_T) values were calculated. Obtained regression values were compared with each other. If the total component concentration obtained from the HPLC chromatograms (C_{HPLC}) deviated from the value obtained by the Folin method (C_{Folin}), the coding was corrected with the assumption that the materials with similar retention times could be the same phenolic component and the processes were repeated. Concentration values of the components were determined using the obtained correlation constants.

$$Fractional\ area\ of\ a\ component\ of\ a\ specific\ solvent\ (x_{i,s}) = \frac{Area\ of\ coded\ component\ (A_{i,s})}{Total\ area\ of\ chromatogram\ of\ that\ solvent\ (A_{T,s})} \quad (11)$$

$$C_{i,s} = R_{i,s} x_{i,s} \quad (12)$$

Statistical analysis

Results were expressed as mean of the three replicate experiments. Statistical analysis were carried out using Statistica (5.0) by applying ANOVA. Significant differences among samples were evaluated by Tukey's test at a significant level of 0.05.

III. Results And Discussion

Time requirements

Table 1 shows the time required to complete each cycle in batch operation, and the time spent by solvent and/or solvent mixtures in each column in continuous operation. In both operations, it was determined that the use of methanol in pure solvents, ethanol-methanol mixture in double solvent combinations and water-ethanol-methanol mixture containing 50% by volume methanol in triple combinations required minimum time. It has been found that the time requirements are related to the boiling point of the solvent or solvent mixtures and that the required time decreases as the amount of methanol having the lowest boiling point increases in the solvent or solvent mixtures. In the batch operation, the first cycle took longer than the second and third cycles

due to the starting of heating of the solvent from room temperature in this cycle. In the other cycles the solvent mixture started to be heated from higher temperature resulted from the partially high temperature of the distillate. In the continuous system, the fresh solvent in each column was mixed with the distillate obtained from the previous column, so that the times spent in each column were found partially closer to each other.

Table 1. Time requirements of batch and continuous operations

	Type of solvent	Time (s)								
		1st cycle	2nd cycle	3rd cycle	Total		1st column	2nd column	3rd column	Total
Batch Operation	W	7200	6600	6480	20280	Continuous Operation	7440	11030	4226	22696
	E	4107	3160	3515	10782		3157	3323	3555	10035
	M	4067	3733	3780	11580		3300	3160	2897	9357
	WE	4587	4190	4454	13231		5400	6480	5440	17320
	WM	5940	3780	3600	13320		4822	3984	4099	12905
	EM	4221	2918	2644	9783		3750	3179	2989	9918
	WEM (5:3:2)	7230	5130	4790	17150		5605	4585	4535	14725
	WEM (5:2:3)	6016	4463	4152	14631		4927	4729	4558	14214
	WEM (2:3:5)	4800	3902	3701	12403		4208	3890	3675	11773
	Control (WM)	5820	3440	3400	12660					

Temperature distributions

Polyphenol production with three cycles in the batch system generally showed a temperature drop of 0.5-5 ^{0} C between cycles. In the continuous system using three columns, it was observed that the ambient temperature in each column was close to room temperature. As an example, Fig. 4 and Fig. 5 illustrate the temperature distributions in the production by using triple solvent combination in batch and continuous operations, respectively. This was due to the high temperature of the distillate recycled to the column in batch operation and to the 67.5% by volume (270/400 ml) fresh solvent feed to each column at room temperature in continuous operation. These findings were consistent with the observations made when the time required for the completion of the process was examined.

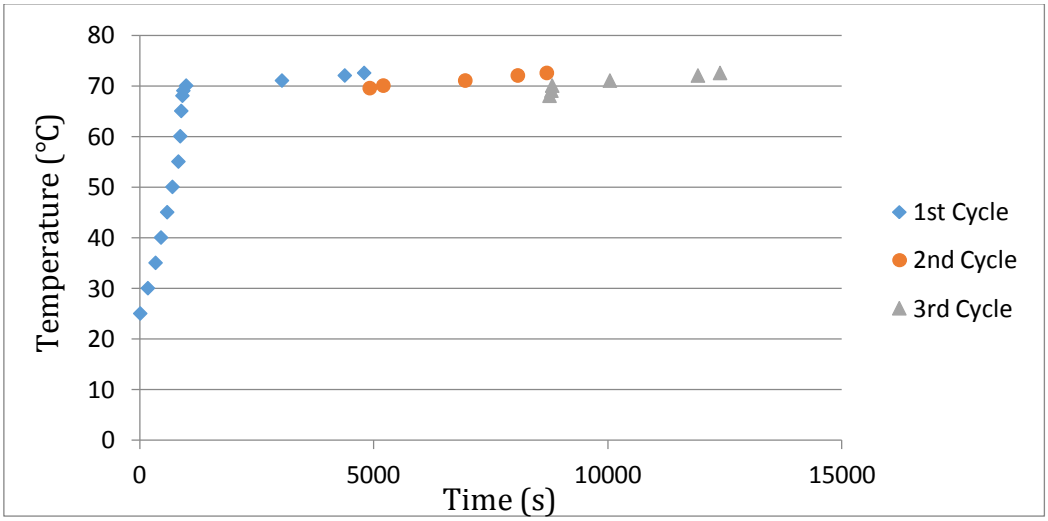


Fig. 4. Temperature distribution of medium containing WEM (2:3:5) in batch operation

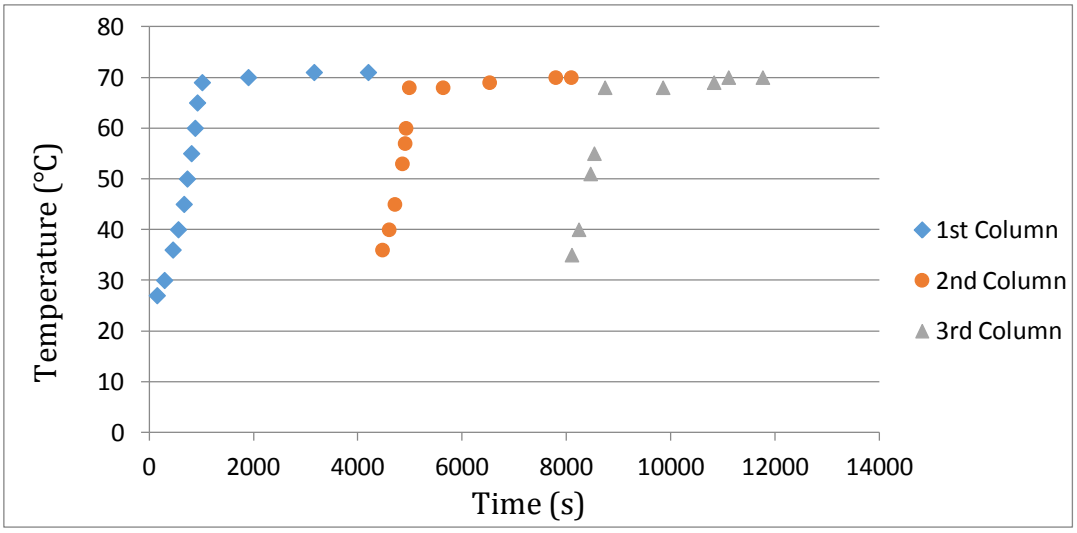


Fig. 5. Temperature distribution of medium containing WEM (2:3:5) in continuous operation

Total polyphenols

Tables 2 and 3 summarize the amounts of gallic acid equivalent total polyphenol determined by the Folin-Ciocalteu analysis in batch and continuous system products, respectively. In the batch system (Table 2) it was found that the total amount of polyphenols increased with increasing number of cycles, i.e. increasing the amount of time the material contacted with the solvent. The amount of solvent that increased momentarily by the evaporation and condensation of the solvent into the flask led to an increase in the concentration difference and thus the diffusion rate. It was found that the use of methanol in pure solvents, ethanol-water in binary solvent combinations and 5:3:2 (by volume) ratio in triple combinations caused to obtain the product with the highest polyphenol content. Addition of methanol to the pure solvent provided a 215% increase in water-methanol combination compared to water, whereas in the ethanol-methanol combination increased only 31% compared to ethanol. The increase in methanol ratio in triple solvent mixtures resulted in a decrease in the amount of polyphenol obtained in the range of 16.7-29.2%. The addition of methanol to the water-ethanol binary mixture and the addition of ethanol to the water-methanol mixture, i.e. the use of alcohols having similar chemical structures with a solvent of low polyphenol solubility such as water, had a negative effect on extraction yield. Solvents with low polarity are effective in the extraction of nonpolar components such as carotenoids, whereas solvents with high polarity provide higher extraction efficiency especially in components such as phenolic acids and flavonoids that have a partially polar structure [40]. Thus, the difference in total polyphenol amounts of the products stems from the fact that the polyphenol components present in the horse chestnut shell have different solubility in solvent and/or solvent mixtures of different polarity. When all products produced by batch system were compared, it was seen that the highest polyphenol content was obtained with water-ethanol binary mixture. Although extraction with triple solvent combination was used for the first time in this study, it has been reported in the literature that the products would have the highest polyphenol content in the extractions using water-ethanol binary combinations [41]. Finally, when the control system and the designed system were compared, it was found that the simultaneous extraction-distillation system (301.135 µg GAE/(ml x 16 g horse chestnut)) was advantageous in terms of both efficiency and cost over conventional Soxhlet extraction (193.433 µg GAE/(ml x 16 g horse chestnut)).

Table 2. Total amount of polyphenols produced in a one column-three cycle batch system

Type of solvent	Amount of polyphenols (µg GAE/(ml x 16 g horse chestnut))		
	1st column 1st cycle	1st column 2nd cycle	1st column 3rd cycle
W	77.421	107.127	138.648
E	120.124	149.993	173.903
M	149.634	183.668	186.109
WE	210.234	304.510	504.202
WM	243.479	299.699	301.135
EM	157.532	172.539	186.899
WEM (5:3:2)	215.835	283.544	339.980

WEM (5:2:3)	211.024	234.073	240.678
WEM (2:3:5)	187.976	219.138	283.257
Control (WM)	68.864	132.473	193.433

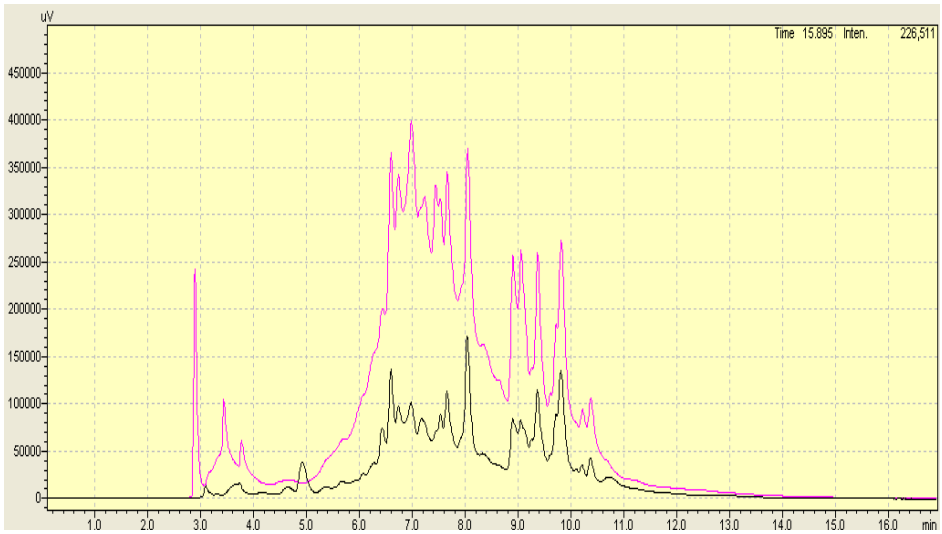
When the products obtained from the continuous system were compared in terms of the highest polyphenol content, it was determined that the solvent ranking was M>E>W in pure solvents, WM>EM>WE in binary combinations and 2:3:5>5:2:3>5:3:2 in triple combinations (Table 4). When the polyphenol content of all extracts obtained from this operation was examined, it was determined that the highest polyphenol producing solvent combination was water-methanol. Since the purpose of the study was to design an industrial production plant, this solvent combination was used as the control group. Sequencing differences between batch and continuous systems can be explained by changes in the temperature distribution in the process and consequently changes in the rate of dissolution and diffusion of the polyphenol component. Only one production unit realized by WM as a solvent produced 193.433 µg GAE/(ml x 16 g horse chestnut) polyphenol in Soxhlet extraction, (3x301.135) 903.405 µg GAE/(ml x 16 g horse chestnut) polyphenol in batch extraction-distillation system and (349.027+359.797+331.938) 1041.005 µg GAE/(ml x 16 g horse chestnut) polyphenol in continuous extraction-distillation system. Therefore, it was determined that approximately 10 times higher polyphenol was produced with continuous operation compared to Soxhlet extraction, and nearly the same yield was achieved in continuous and batch operations.

Table 3. Total amount of polyphenols produced in a three column-one cycle continuous system

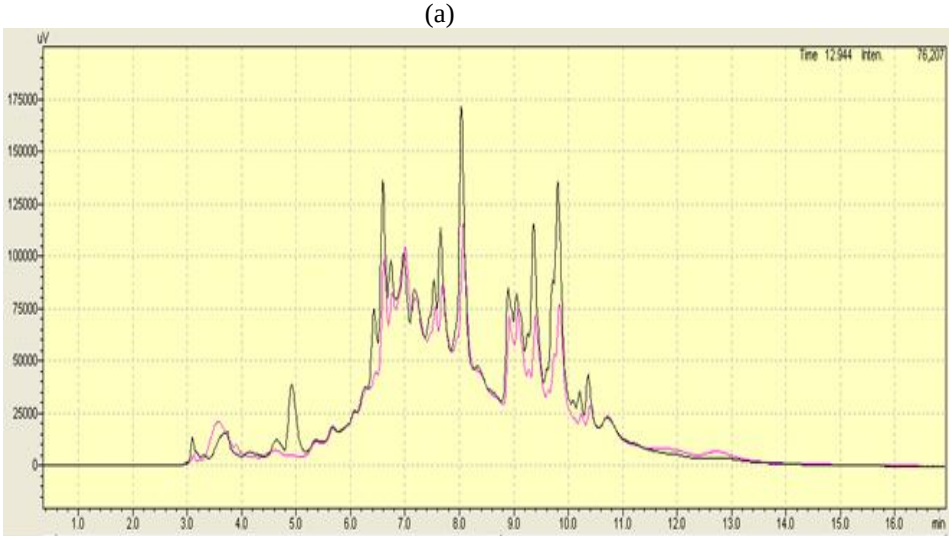
Type of solvent	Amount of polyphenols (µg GAE/(ml x 16 g horse chestnut))		
	1st column 1st cycle	1st column 2nd cycle	1st column 3rd cycle
W	77.421	36.063	9.295
E	175.770	189.556	183.812
M	229.477	262.650	202.839
WE	144.177	191.710	149.490
WM	349.027	359.797	331.938
EM	184.601	184.530	186.971
WEM (5:3:2)	271.840	309.680	246.997
WEM (5:2:3)	191.207	342.493	264.875
WEM (2:3:5)	257.911	266.312	277.010

Phenolic components

In Figure 6, HPLC analysis results of the products with the highest polyphenol content produced by both batch and continuous operations were given. As the Folin-Ciocalteu analysis showed, the WE binary combination-used batch operation produced much more polyphenols than the control group namely Soxhlet extraction (Fig. 6a). It was also determined that the continuous system performed with WM solvent combinations, which were determined to have the highest polyphenol content according to the same analysis, had almost the same polyphenol content as the control group (Fig. 6b). However, this amount was found to be much higher in Folin analysis. This difference may be due in particular to the extraction of components reacting with the Folin reactant in the WM binary combination. Previous studies also showed a significant difference when comparing the results of HPLC analysis with Folin-Ciocalteu method [42]. The reason of this was declared as follows: i) some antioxidants do not act against the Folin reagent, ii) the Folin reagent is reduced by many non-phenolic compounds (proteins, ascorbic acid, aromatic amines, Fe (II), Cu (I) and SO_2), iii) other antioxidants that are not determined by HPLC are present in the extract.



(a)



(b)

Fig. 6. HPLC chromatograms of products having the highest polyphenol content: (a) batch system with WE as solvent (b) continuous system with WM as solvent (black: control, pink: sample)

Polyphenol components and concentrations of each component calculated by applying field expansion between Folin-Ciocalteu and HPLC were given in Tables 4 and 5 for batch and continuous operations, respectively. As can be seen from the tables, the correlation constant (R_S) and component concentration ($C_{i,S}$) values obtained by field expansion in the solvent were so close to the correlation constant (R_T) and component concentration ($C_{i,T}$) values obtained by field expansion in all chromatograms (Std. dev. $3.8.10^{-5}$), which revealed the success of the method used. In the batch operation using the WE solvent (Table 4), 21 different polyphenol components were detected in the mixture containing the highest amount of total polyphenols, while 20 different components were identified in the WM solvent containing continuous operation (Table 5). Of these components, only the quercetin indicated in dark color was found to be in common. The fact that polyphenols known to have more than 8000 varieties [23], difference in solubility of each of component in each solvent makes it reasonable to observe these results.

Table 4. Polyphenol components produced by batch operation with WE as a solvent

Code	Time (s)	(A_i)	($x_{i,ç}$)x10^{-2}	($x_{i,T}$)x10^{-2}	$C_{iç}$ (µg/ml)	$C_{i,T}$ (µg/ml)
E	4.677	134984	0.241	0.070	1.2174	1.2143
P	5.684	1158765	2.072	0.060	10.4509	10.4245
Y	6.431	4989061	8.923	2.588	44.9964	44.8826
B'	6.589	3041136	0.539	1.578	27.4280	27.3587
I'	6.731	2753446	4.924	1.428	24.8334	24.7705
O'	6.974	5710006	10.212	2.962	51.4986	51.3683

Y'	7.223	4384606	7.841	2.275	39.5448	39.4448
A*	7.435	2262934	4.047	1,174	20.4094	20.3578
B*	7.52	1847478	3.304	0.958	16.6624	16.6203
J*	7.53	4096932	7.327	2.125	36.9503	36.8568
P*	8.039	5665373	10.132	2.939	51.0961	50.9668
Ü*	8.325	3859762	6,.02	2.002	34.8112	34.7232
G+	8.895	2232142	3.992	1.158	20.1317	20.0808
N+	9.05	2584556	4.622	1.341	23.3101	23.2512
U+	9.362	3112954	5.567	1.615	28.0758	28.0047
C^{Δ}	9.808	4711162	8.426	2.444	42.4900	42.3825
L^{Δ}	10.211	740972	1.325	0.384	6.6828	6.6659
R^{Δ}	10.367	1200491	2.147	0.623	10.8272	10.7999
U^{Δ}	10.634	44417	0.080	00230	0.4006	0.3996
$Ü^{\Delta}$	**10.645**	**464164**	**0.830**	**0.241**	**4.1863**	**4.1757**
E^{o}	10.975	920113	1.646	0.477	8.2985	8.2775

Table 5. Polyphenol components produced by continuous operation with WM as a solvent

Code	Time (s)	(A_i)	($x_{i,ç}$)x10^{-2}	($x_{i,T}$)x10^{-2}	$C_{iç}$ (µg/ml)	$C_{i,T}$ (µg/ml)
C	4.615	55996	0.474	0.029	0.5023	0.5038
İ	4.908	9052	0.077	0.005	0.0812	0.0814
L	5.364	86927	0.736	0.045	0.7797	0.7820
Ö	5.68	201820	1.709	0.105	1.8103	1.8156
U	6.076	332297	2.814	0.172	2.9807	2.9894
Z	6.457	685388	5.803	0.356	6.1478	6.1659
E'	6.609	813470	6.888	0.422	7.2967	7.3181
P'	6.997	1417749	12.004	0.736	12.7170	12.7544
F*	7.55	780930	6.612	0.405	7.0048	7.0254
L*	7.679	956206	8.096	0.496	8.5770	8.6022
S*	8.065	2536801	21.479	1.316	22.7547	22.8216
H+	8.914	581931	4.927	0.302	5.2198	5.2352
M+	9.071	700459	5.931	0.363	6.2830	6.3015
V+	9.391	677999	5.741	0.352	6.0816	6.0994
E^{Δ}	9.832	1201261	10.171	0.623	10.7751	10.8068
M^{Δ}	10.235	131197	1.111	0.068	1.1768	1.1803
S^{Δ}	10.393	206534	1.749	0.107	1.8526	1.8580
C^{o}	**10.715**	**306977**	**2.600**	**0.159**	**2.7535**	**2.7616**
H^{o}	11.007	51659	0.437	0.027	0.4634	0.4647
N^{o}	12.717	75786	0.642	0.039	0.6798	0.6818

Table 6 shows the results of field expansion; i.e. the number of components, R_S values and total polyphenol concentrations of mixtures produced by each solvent and/or solvent combinations used. The proximity of R_S values to R_T ($8.9996.10^{-6}$) was seen from the table. It was also confirmed by HPLC analysis that the new system designed, either batch or continuous operation, was more efficient than Soxhlet extraction (Table 6). Solvents producing the highest amount of polyphenols were WEM (2: 3: 5) in continuous operation and WE in batch operation. It was found that the amount of polyphenol was affected by the number of polyphenol components in the medium. Differences are thought to occur due to the similarity of the chemical structure of the polyphenol components, and thus reduced solubility. In addition, the order of solvent producing the highest amount of polyphenols is WEM (2: 3: 5)> M> control> WM in HPLC, whereas WM> WEM (2: 3: 5)> M> control in Folin analysis. The same sequence was observed in these two methods, except water-methanol binary combination.

Table 6. Polyphenol content and the number of component in products

Type of solvent		Number of different polyphenols	$\sum C_{HPLC}$	$R_{solvent}$ (10^{-6})
Continuous	M	23	202.839	9.07676
	WM	20	105.938	8.96986
	WEM (2:3:5)	19	262.010	8.98524
Batch	M	23	186.109	8.85664
	WE	21	504.302	9.01901
	WEM (5:3:2)	20	311.475	9.00826
Control		27	161.433	9.0001

Heat loss

When the values were compared, it was determined that the second and third cycles in the batch system, and the second and third columns in the continuous system had higher heat flux values compared to the first cycle or column (Table 7). For example, in WE-batch operation which provides the highest polyphenol production, 9.78% and 10.70% heat loss increases were observed in the second and in the third cycles compared to the first cycle, respectively. In WEM-continuous operation, heat loss increased by 1.70% in the second column and 1.36% in the third column. The higher temperatures in the second and third cycles or columns are the main reasons for the increased amount of heat transferred to the environment. When the total heat fluxes in the systems were compared with each other, it was found that the continuous system had less heat loss (2.7-6.2%, depending on solvent) than batch operation. The main reason for this is the large temperature drops resulting from mixing with the fresh solvent when the solvent is transferred from one column to the other in a continuous system, thereby reducing the temperature difference, the drive power required for heat transfer. As a result of the calculations, it was determined that approximately 46% of the heat losses occurred from the distillation surface and 54% from the condenser surface.

Table 7. Heat fluxes in batch and continuous operations

System	Type of solvent	q_{lost} (J/m^2s)			
		1st cycle	2nd cycle	3rd cycle	Total
Batch	M	-0.926	-0.975	-0.975	-0.958
	E	-0.903	-0.971	-0.973	-0.946
	W	-0.775	-0.970	-0.969	-0.900
	EW	-0.869	-0.954	-0.962	-0.927
	MW	-0.892	-0.976	-0.967	-0.936
	EW	-0.916	-0.973	-0.973	-0.949
	5:3:2	-0.898	-0.968	-0.964	-0.936
	5:2:3	-0.893	-0.963	-0.960	-0.932
	2:3:5	-0.915	-0.970	-0.967	-0.944
Continuous		1st column	2nd column	3rd column	Total
	M	-0.907	-0.935	-0.948	-0.932
	E	-0.901	-0.903	-0.916	-0.907
	W	-0.834	-0.896	-0.671	-0.844
	EW	-0.880	-0.903	-0.882	-0.889
	MW	-0.876	-0.900	-0.902	-0.892
	EM	-0.907	-0.909	-0.909	-0.908
	5:3:2	-0.877	-0.888	-0.888	-0.884
	5:2:3	-0.866	-0.897	-0.897	-0.886
	2:3:5	-0.884	-0.899	-0.896	-0.892

IV. Conclusions

A system consisting of three columns and performing simultaneous extraction-distillation processes in each column was designed in this study. Batch or continuous polyphenol production of the new system was investigated by using water, ethanol and methanol solvent and/or solvent combinations and horse chestnut shells as polyphenol source. Total polyphenol content, polyphenol components and heat losses in products of the system were compared with conventional Soxhlet extraction. It was observed that the mixtures produced in the designed system produced 10 times more polyphenol than the control group because the pure solvent from both distillate and fresh feed increased the diffusion rate, which is the driving force in extraction. In the continuous system, it was found that the extracts produced in each column were increased the yield by 80-86-72%, respectively, as a result of the comparison with the product obtained from the third cycle of the control group. Considering the production of the same quality products and the ease of system operation and control, continuous operation has significant advantages.

In the study, quercetin correlation coefficient was determined as 9.10^{-6} by applying field expansion between the results of Folin-Ciocalteu and HPLC analyzes. By the way, each polyphenol component in the product obtained from each solvent was identified and the amount of it was calculated. The polyphenol component and its amount were determined to be directly related to the type of solvent and ambient temperature used. Therefore, with the new system designed, it will be possible to realize designs with high yield and high amount of specific polyphenol component by using a suitable solvent depending on the structure of the solid plant source and the desired polyphenol component. For example, if it is desired to produce high amounts of polyphenols or quercetin from the selected source (horse chestnut) it is concluded that the water-ethanol binary solvent combination should be used in the batch operation. In the continuous operation it would be necessary to use water-ethanol binary solvent combination for polyphenol production, whereas methanol would be used to produce high amount of quercetin.

As a result of the energy analysis carried out in the study, the main disadvantage of the designed system was determined as the heat loss from the system to the environment. It was calculated that minimization of heat loss by isolating the part up to the condenser in the continuous operation would provide 46% energy saving. As a result, high efficiency, capability of specific product design, continuous production advantages, the reduction of fresh solvent usage requirement, easier process control, reusability of pure solvent due to distillation are the possible advantages of this new design.

NOMENCLATURE

$A_{i,s}$: Area of coded component
$A_{T,s}$: Total area of chromatogram of that solvent
$C_{i,S}$: Component concentration value obtained by field expansion in the solvent were so close to
$C_{i,T}$: Component concentration value obtained by field expansion in all chromatograms
C_{HPLC}: The total component concentration obtained from the HPLC chromatograms
C_{Folin}: The concentration found by the Folin- Ciocalteu method of the solvent type
$c_{p,glass}$: The heat capacity value of glass
c_{p_i}: The specific heat capacity values at the average temperature observed in the second and third columns
E: Ethanol
EM: Ethanol- Methanol
GAE: Gallic acid equivalents
Q_{GA}: Heat required to heat polyphenols
Q_{plant}: Heat required to heat plant material
$Q_{solvent}$: Heat required to heat solvent or solvent mixture
$Q_{apparatus}$: Heat required to heat the extraction-distillation apparatus
R_S: The correlation constant value obtained by field expansion in the solvent were so close to
R_T: The correlation constant value obtained by field expansion in all chromatograms
$m_{Soxhlet\ aparatus+\ balloon:}$ The extraction-distillation apparatus total mass
M: Methanol
W: Water
WM: Water- Methanol
WE: Water- Ethanol
WEM (5:3:2): %50 Water, %30 Ethanol, %20 Methanol
WEM (5:2:3): %50 Water, %20 Ethanol, %30 Methanol
WEM (2:3:5): %20 Water, %30 Ethanol, %50 Methanol
W_{heater}: The work done by the heater on the system
x_i: The volume fractions
$x_{i,s}$: Fractional area of a component of a specific solvent

$\rho_{solvent}$: The density of pure solvents
$\rho_{mixture}$: Mixture of solvents

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