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Research Article

A Study on nutritional and technological Valorisation of wild fruits

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ABSTRACT

Wild pears, plums, berberis and hawthorn, harvested in from different regions in Bekaa: Arsal, Nabha and Ham, were analyzed to determine its nutritional and technological properties. Studies on the vitamin C, anthocyanins, pectins, lipids, fibers and minerals were conducted. The obtained results showed that pears of Arsal and Nabha are the richest in fibers; plums of Nabha are the richest in vitamin C; berberis are the richest in proteins and lipids, and hawthorns are the richest in minerals. Thus, those wild fruits were found to be an important nutritional source. Moreover, the extracted pectins were highly methylated and can have a promising high gelification capacity. Fortunately, these findings will definitely encourage people to benefit from these fruits in its fresh or cooked state, which emphasizes the importance of the biodiversity's conservation.

Key words: Wild fruits, chemical composition, pectin and fibers.

INTRODUCTION

Fruits contain various bioactive molecules, most of which have the antioxidant properties [1]. This is due to the presence of vitamin C, vitamin E, β-carotene, and other polyphenols (flavonoids, anthocyannes) which have a very important antioxidant activity. Wild fruit trees play a major role in ensuring raw resources for humans, animals and environmental stability [2]. They are important for traditional consumption and medicinal uses. Several studies have been conducted on wild fruits such as hawthorn [3], plums [4], and roses [5] to promote their chemical and physical compositions.

Being an integral part of the Middle East, one of the 8 major centers of diversity identified by Vavilov, Lebanon had high wildlife species diversity [6]. These wild species are limited by the reduction of biodiversity, which emphasizes the importance of its conservations by traditional methods.

The loss of this biodiversity has a direct effect on humans, through affecting food security and the economic benefits associated with the use of those wild plants. It is important to note that only Thirty species out of 500.000 are being currently cultivated [6].The objective of this study is to determine, for the first time, the chemical composition of some wild fruits (*Crategus azarolus azarolus*, *Crategus azarolus aronia*, *Pysus syriaca*, *Prunus ursina*) located in different regions in Bekaa as Arsal, Nabha and Ham, in order to evaluate their traditional value and technological aptitude. It also aims to encourage people to use the fruit as an additional food source, and protect the Lebanese biodiversity.

MATERIALS AND METHODS

Wild fruits are harvested from the regions: Arsal, Nabha and Ham, and kept in well-closed bags, in the freezer (at - 20˚C) until the experiment date.

Extraction of pectin

The extraction of pectin was made according to the method of Pagan et al[7]. 100 g of fruit pulps are dissolved in 500 mL distilled water (acidified with nitric acid to pH 1, 2) and were heated to 80˚C for 1 h.

Each suspension was centrifuged for 15 min at 1700 x g to remove impurities. The resulting supernatant is concentrated at 40 °C to reduce the volume to 1/5. The obtained concentrate was then centrifuged to remove the insoluble parts. A volume of ethanol 96 % (w/w) was added to an equal volume of the supernatant, inducing the precipitation of pectin that

will be collected separately. Then, the separated pectins were washed with ethanol 70 % (w/w) to remove mono- and disaccharides. Finally, they were dried at 50 °C for 24 h.

The degree of methylation of pectins

The degree of methylation was determined by the method of Iglesias and Lozano [8].

A pectin solution is prepared at a concentration of 8.33 mg/mL in distilled water. Methylated and esterified pectins are then hydrolyzed by adding 1 mL of NaOH to 12 mL of the pectin solution. Next, the solution was incubated for 30 min at room temperature, and a dilution with distilled water was made up to a weight of 20 g. After a filtration on a membrane 0.22 µm, 20 µL of the solution were injected onto a Supelcosil LC-18 column (25 cm * 4.6 cm, 5 µm). The mobile phase used was a solution of H_2SO_4 (pH = 2.2) at a flow rate of 0.5 mL/min.

A visible UV detector was used for the detection of the acetic acid: Shimadzu SPD-10A at 210 nm and the methanol was detected using detector Shimadzu RID-10A.

The degree of esterification

The degree of esterification (DE) was determined by the titrimetric method. 500 mg of dry sample were placed in a 250 mL flask with 2 mL of ethanol and dissolved with 100 mL of deionized water. After dissolving completely the sample, 5 drops of phenolphthalein were added. Then the sample was titrated with NaOH (0.5 M) and the result was recorded as an initial titration. Next, 10 mL of NaOH (0.5 M) were added and stirred vigorously, and the solution was maintained for 15 min. After that, 10 mL of HCl (0.5 M) were added and the mixture was stirred until the pink color disappears. 5 drops of phenolphthalein were added and the solution was again titrated with NaOH (0.5 M) until the color changes to pink (end point). This volume was selected as the final titration.

DE was calculated according to the formula: **[final volume / (initial volume + final volume)] *100**

Galacturonic acid

The galacturonic acid was determined using the method cited by Southgate [9]. The hydrolyzate was prepared by adding 12 mL of sulfuric acid (12 M) to 100 mg of dry pectin. The mixture was dispersed by a vortex and left at 35 °C for 1 h with periodic agitation to disperse the cellulose. After adding 22 mL of water, the tubes were closed and shaken, and then were placed in boiling water for 2 hours. After that, the tubes were cooled to reach the room temperature. Then, a volume of 0.3 mL of the hydrolyzate was added to 0.3 mL of a solution of NaCl/H_3BO_3 (containing 2 g NaCl and 3 g H_3BO_3 in a volumetric flask of 100 mL) and 5 mL H_2SO_4 (98%). The mixture was stirred with the vortex and placed at 70 °C for 40 min. After cooling in water, 0.2 mL of dimethyl phenol (prepared with 0.01 g in 100 mL of glacial acetic acid) was added, and the solution was again stirred with the vortex. After 10 min, the absorbance was read at 400 nm and 450 nm on a spectrophotometer using water as a blank. The difference in readings between 400 and 450 nm was correlated to the concentration of the sample. A standard curve was established using sequential concentrations of galacturonic acid (25, 50, 75, 100, 125 µg/mL).

Vitamin C

The extraction and dosage of vitamin C was performed according to AOAC [10]. This method is based on the reduction of 2, 6 dichlorophenol indophenol (DCPIP) by ascorbic acid. A volume of 15 mL of ionized water, boiled then cooled, were added to 2 mL of the standard solution of vitamin C (containing 0.05 g of ascorbic acid in 100 mL HPO_3, 3%). The mixture was titrated with DCPIP reagent prepared from 42 mg $NaHCO_3$ and 50 mg DCPIP in a 200 mL volumetric flask until a persistent pink color for 15 seconds. The volume of DCPIP used was noted (V_1).

Vitamin C in the wild fruit juice is determined by the calibrated dye. A mass of 5 g of grinded fruits was mixed with 25 mL HPO_3 (3%). The mixture was centrifuged at 1700xg for 15 min. The supernatant with a pink color undergoes a purification process by solid-liquid extraction on tubes LC-18 (3 mL). After preparation of the tube with 4 mL methanol followed by 10 mL H_2O, 4 mL of supernatant were treated. The first 2 mL were discarded and the last 2 mL were titrated with a volume V_2 of DCPIP dye.

Extraction of anthocyanins

The method used was adapted from the AOAC [11]. A volume of 10 mL of fruit juice with 10 mL of $Pb(OAc)_2$ and 5 mL of NH_4OH, was centrifuged at 1700xg for 15 min to obtain a clear supernatant (if not $Pb(OAc)_2$ was added with a new centrifuge).

Two washes were done with 25 mL of ethanol (80%), followed each time by a centrifugation (where the pellet is retained). After the second wash, the tube was inverted for 5 min to drain the fluid.

10 mL of n-butanol and 1 mL of HCl were added to change the color of the $PbCl_2$ solution, and then a centrifugation was carried out. The supernatant was retained in a separatory funnel and the remaining lower phase from this centrifugation was washed

again with n-butanol and centrifuged. The resulting supernatant was added to the first. Next, the addition of 100 mL of petroleum ether and 1 mL of water was made to the supernatant to extract the butanol from the solution and transfer anthocyanin bottom of the separatory funnel. This anthocyanin was collected to a volume of 2.5 mL.

Determination of proteins

Proteins were determined using the method AOAC [12]. A sample of 1 g of fruit, dried then grinded, was placed in specific tubes (500 mL) with a spoon of catalyst (containing 5g K_2SO_4 and 0.25 g $CuSO_4$). 12-15 mL H_2SO_4 (96-98%) and 10 mL H_2O_2 (30-35%) were added to the sample. Sample digestion was done for 20 min. After cooling, the distillation took place automatically by adding 50 mL of water and 50 mL NaOH (35%) for 5 min. The released NH_3 was gathered in a 200 mL erlenmeyer flask containing 25 mL of boric acid (4%). Titration of the ammonium ion was made using a solution of HCl (0.1M) in the presence of 3-5 drops of Tashiro indicator (1g red methylene with 0.25 g of blue methylene/100mL water + alcohol).

The protein content was calculated by multiplying the mineral nitrogen content by 6.25.

Extraction of lipids

The method was performed with a Soxhlet extractor. A mass of 1-2 g of each sample, dry then grinded, was weighed into the filter papers. After being closed, these papers were placed in the Soxhlet apparatus where they were washed by petroleum ether for 24 hours. The evaporated ether passes through the sample taking with it fats. These were quantified after drying of the solvent in an oven for 2 hours.

Determination of minerals

Mineralization was carried out according to the method AOAC [13]. For each fruit, a sample (1 g) of dry and grinded fruit, were put into a porcelain crucible, and then were placed in an oven at 500 °C for 2 h. After cooling, the ash was wetted with 10 drops of water, and 3-4 mL HNO_3 (50%) was added carefully. Excess of HNO_3 was then evaporated on a hotplate. The crucibles were returned to the oven at 500 °C for 1 h. After cooling, the ash was dissolved in 10 mL HCl (50%) and the volumes were adjusted to 50 mL with distilled water. The dosage of mineral components was conducted by atomic absorption. Na and K were determined by flame photometry.

Extraction of fibers

This was carried out according to the method cited by Southgate [9]. 1 g of sample, dried then grinded, was placed in crucibles pyrex. A volume of 150 mL H_2SO_4 (1.25%) was added with 2-3 drops of n-octanol, as an anti-foam. After boiling for 30 min, H_2SO_4 was drained by suction. 3 washes were done with hot distilled water. After drainage of the final wash, 150 mL of hot KOH (1.25%) were added with 3-5 drops of n-octanol. The same procedure, followed for the sulfuric acid, was repeated for the KOH. The 3 washes with hot distilled water were carried out, followed by an additional wash with 25 mL of acetone. Crucibles, dried at 105°C for 1 h, were weighed and the weight obtained represents the mass of fiber and ash. The fibers were removed by placing the crucible in the oven at 550 °C. After weighing the crucibles, the weight of the fibers was then obtained by taking the difference between the first and the second weight.

RESULTS AND DISCUSSION

Pectin

Table 1 shows that both, the quantity and the quality of pectin, was affected by two factors: the origin and the degree of ripeness of the fruit. Among the varieties studied of the same species, the amount of pectin in hawthorns of Nabha (11.69 % of the dry matter (DM)) was higher than that in Ham (5.7 % DM) and in Arsal (11.25 % DM). The same was observed in the varieties of different species. For example, the pectin content of pears (6.83% DM of Nabha and 1.41 % DM of Arsal) which is lower than that of hawthorns. This variability in the amount of pectin between different varieties was also noticed in domestic fruits, such as citrus and apple pomace with a respective pectin content of 25 % and 15-18 % DM [14]. Thus, the wild fruit that has the highest amount of pectins, is the hawthorns obtained from Nabha.

Regarding the state of maturity, we shall not neglect that pears that were harvested from Arsal were more mature than those harvested from Nabha. This condition would have resulted in a decrease in the amount of pectin (6.83 % DM for Nabha and 1.411 % DM for Arsal).

The characterization of wild fruit pectin was based on DE, DA, DM, and on the galacturonic acid content forming each pectin. Concerning the quality of pectin, all pectins extracted were highly methylated. Pears from Nabha, having a DE of 80 % (DM 80 % and DA zero), yielded a highly methylated pectin which would have a significant gelling power. However pears from Arsal, having a DE of 58.33 % (DM 49.87 % and DA of 0.12 %), yielded a moderately methylated pectin. The gel strength increases with the degree of esterification, and

especially the degree of methylation. This phenomenon was confirmed on the beet pectin and that of citrus, where the gel strength of pectins of citrus (DE 67.80 %) is higher than that of beet (DE 58.92 %) [14].

Vitamin C

The results presented in Table 2 show that the ascorbic acid varies from one fruit to another. These differences are due to several factors: the type of soil, climate, and the varieties of the same species or a different species [15,16].

Plums were the richest type of fruit in vitamin C, having the highest content (0.66 g/100 g of pulp). Its concentration was even greater than that of domestic pears (0.005 g/100g of pulp) [17].

Pears grown at Arsal and Nabha had very low levels of vitamin C (0.052 g/100g pulp and 0.046 g/100g pulp respectively), but were greater than those of domestic pears (0.004 g/100g of pulp) [17].

On the other hand, hawthorns of Arsal contain an amount of vitamin C (0.183 g/100g pulp) lower than those from Nabha (0.40 g/100g pulp) and those from Ham (0.7 g/100g pulp), but greater than that of Berberis (0.12 g/100g pulp).

The anthocyanins

The sensory qualities of fruits, such as color and flavor, are the main factors that influence the consumer's acceptance [18]. Anthocyanins are responsible for the color of the fruit (orange, red and blue), and play a role in attracting insects for pollination [19]. Among the studied fruits, it was found that only two contained anthocyanins: Hawthorns from Arsal and Berberis (7.36 g/100g pulp and 2.14 g/100 g pulp respectively). The amount of anthocyanin contained in the hawthorn of Arsal was higher than that found by Sherger et al. [20] on the other varieties of hawthorn (4.06 g/100 g of pulp). This difference may be due to the type of fruit (species and variety), and also to the culture's conditions (environment and culture techniques) [16].

The minerals

The results obtained for the mineral elements are gathered in Table 3 and Table 4. The calcium content was the highest despite the great variability of the contents of this element: this variability may be due to several factors: sampling, mineralization method, and dosage. Hawthorns from Arsal, Nabha and Ham, are rich in minerals and were the most abundant in pink fruits [5], but their levels are low compared to those obtained by Ozcan [3]. Plums from Nabha contain fewer minerals than the wild plums studied by Cahsir et al. [4]. Also, pears from Arsal were rich in Ca, Cu, K, and Zn, but poor in Fe, Mn and Na. Therefore, those from Nabha were rich in Ca and K.

Lipid content

Domestic fruits contain lipid content below 0.5 %**.** The results obtained in Table 5 indicate that wild fruits are richer in lipids than the domestic fruits with content greater than 1%.

The studied wild fruits contain equal amounts of lipids (between 1.5 and 2 % of the fresh material) which are identical to those of wild plums (1.58 %) analyzed by Cahsir et al. [4] and to flowers (1.5 %) analyzed by Demir and Ozcan [5]. The studied hawthorns contain quantities greater than the hawthorns studied by Ozcan et al. [3]. On the other hand, berberis were the richest fruit in lipids (3.82 %) as seen in table 5.

Table 1
Characterization of pectin

Fruits	Quantity of pectin (g/100g pulp)	DE (%)	ACG (%)	DM (%)	DA (%)
Hawthorn Arsal	11	66.68 ± 1.05	4 ± 0.53	66.32 ± 1.36	0.04 ± 0.008
Hawthorn Ham	5.7	66.66	4.81	66.4935	0.16
Hawthorn Nabha	11.69	69.66 ± 0.91	11.39 ± 1.42	69.62 ± 0.89	0.03 ± 0.01
Pear Nabha	6.83	80	2.30 ± 0.46	80	0
Pear Arsal	1.41	58.33 ± 8.33	2.17	49.87	0.12
Prunus Nabha	10.37	71.4 ± 0.82	8.46 ± 0.8	71.4 ± 0.82	0
Apple Standard		0.36	7.6 ± 0.15	36.52 ± 0.03	0.07 ± 0.03

Table 2
Composition of vitamin C per 100g of fresh material

Fruit	Pear Arsal	Plums Nabha	Pear Nabha	Hawthorn Nabha	Hawthorn Ham	Berberis	Hawthorn Arsal
% Vit C	0.05 ± 0.003	0.66 ± 0.08	0.04 ± 0.001	0.4 ± 0.03	0.7 ± 0.21	0.12 ± 0.003	0.18 ± 0.03

Table 3
The minerals content per 100 g of dry matter

Sample	Ca (ppm)	Mg (ppm)	Fe (ppm)	Cu (ppm)
Hawthorn Ham	449.5 ±125.5	34.65 ±5.225	3.53 ±0.465	92.05 ±84.95
Hawthorn Nabha	421.5 ±178.5	31.22 ±3.395	5.24 ±0.485	115.2 ±112.2
Berberis	344 ±131	21.17 ±9.47	1.04 ±0.035	20.95 ±15.85
Plums Nabha	246.5 ±59.5	14.13 ±2.03	1.17 ±0.215	310.85 ±12.35
Pear Nabha	397.5 ±127.5	16 ±3.39	0.89 ±0.03	1.85 ±0.55
Pear Arsal	463.5 ±92.5	24.75 ±4.57	1.05 ±0.045	257.55 ±67.65
Almond Nabha	168 ±20	50.04 ±1.07	1.12 ±0.045	74.8 ±7.4
Hawthorn Arsal	265.67 ±18.5	17.99 ±1.015	2.15 ±0.015	315.7 ±60.25

Table 4
The minerals content per 100 g of dry matter

Sample	Mn (ppb)	Zn (ppb)	K (ppm)	Na (ppm)
Hawthorn Ham	115.55 ±5.85	278.1 ±0.7	215 ±15	13.65 ±4.35
Hawthorn Nabha	40.95 ±7.35	321.6 ±42.8	195 ±5	6.9 ±0.4
Berberis	231.8	230.4	130	6.55 ±0.55
Plums Nabha	17.9 ±15	77.9 ±18.1	220 ±10	17
Pear Nabha	34.35 ±3.65	26.2 ±23.5	235 ±15	7.15 ±0.15
Pear Arsal	8.05 ±3.65	125.65 ±20.55	210 ±10	6
Almond Nabha	163.1 ±20.5	668.35 ±14.65	160 ±50	6.6 ±0.4
Hawthorn Arsal	22.43 ±6.2	106.23 ±26.25	186.67 ±35	13.47 ±0.8

Table 5
Lipid content per 100 g of fresh material

Fruit	Plums Nabha	Pear Arsal	Hawthorn Nabha	Hawthorn Ham	Hawthorn Arsal	Pear Nabha	Berberis
% of lipids	1.58 ± 0.05	1.9 ± 0.26	2.07 ± 0.23	1.65 ± 0.03	2.23 ± 0.16	1.22 ± 0.18	3.82 ± 0.07

Table 6
Composition of wild fruits in protein per fresh material

Fruits	Hawthorn Arsal	Pear Arsal	Hawthorn Nabha	Pear Nabha	Plum	Hawthorn Ham	Berberis
% CP= HCl (ml)*0.14*6,25/m (Sample)	0.89 ± 0.09	0.64 ± 0.05	0.8 ± 0.01	0.62 ± 0.04	0.67 ± 0.04	0.64 ± 0.05	2.91 ± 0.13

Table 7
Fibers content per 100 g of dry matter

Fruit	Pear Arsal	Pear Nabha	Hawthorn Arsal	Hawthorn Nabha	Hawthorn Ham	Plum	Berberis
Fibers (%)	29.79 ± 0.79	29.24±3.38	12.56±0.1	18.65±0.2	13.04±0.33	21.74 ± 0.7	7.56 ± 0.07

Table 8
Chemical composition of wild fruit in 100g fresh material:

Fruit	Proteins	Lipids	Fibers	Ash	Humidity	Vitamin C	Brix
Hawthorn Arsal	0.89 ± 0.09	2.23 ± 0.16	4.25 ± 0.16	32.38 ± 0.05	66.15	0.18 ± 0.03	20.4
Hawthorn Nabha	0.80 ± 0.01	2.07 ± 0.23	7.45 ± 0.08	38.32 ± 0.006	60.04	0.4 ± 0.03	21.3
Hawthorn Ham	0.64 ± 0.05	1.65 ± 0.03	5.33 ± 0.13	39.47 ± 0.006	59.12	0.69 ± 0.21	19.2
Plum Nabha	0.67 ± 0.04	1.58 ± 0.05	6.93 ± 0.22	30.94 ± 0.04	68.09	0.66 ± 0.08	18.6
Pear Nabha	0.62 ± 0.04	1.22 ± 0.18	11.45 ± 1.32	37.67 ± 0.04	60.79	0.04 ± 0.001	17.7
Pear Arsal	0.64 ± 0.05	1.90 ± 0.26	11.84 ± 0.31	38.17 ± 0.04	60.24	0.05 ± 0.003	17.1
Berberis	2.91 ± 0.13	3.82 ± 0.07	2.77 ± 0.16	35.93 ± 0.06	63.23	0.12 ± 0.003	19.3

Protein content

The protein content is less than 5% in the wild fruits [21], and 0.1% in domestic fruits. The obtained results presented in the Table 6 of wild fruits were consistent with those obtained by Marakoglu et al. [21].

All wild fruits contain protein levels between 0.6-0.8 percent. Domestic plums and pears contain a respective protein content of 0.5-0.8% and 0.7% fresh materials [22]. Therefore, hawthorn varieties studied contain amounts (2.48%) similar to those studied by Ozcan et al. [3].

Fibres

Results in Table 7 show a difference in fibers content between varieties of the same species or different species. Pears from Arsal and Nabha, had the highest fibers content with respective levels of 29.7% and 29.24% DM but less rich than domestic fisheries (31-36% DM) [23] and domestic pears (36.1% DM). Wild plum from Nabha were higher in fibers content (21.74%) than the domestic plums (3.003%).

Hawthorns from Ham and those from Arsal contain, respectively, a fiber content of 12.56% and 13.04% which is less than those from Nabha with 18.65% of fibers content, but higher than those of blackthorn with 4.6% [21].

CONCLUSION

The main objective of this study was to determine the nutritional and technological values in wild fruits. After viewing their chemical composition, wild fruits were compared together. Arsal's and Nabha's pears were rich in fibers, low in vitamin C and minerals, along with a moderate amount of proteins and lipids. Oon the other hand, Nabha's plums were rich in vitamin C, with an average content in proteins and lipids. Moreover, the berberis was rich in proteins and fats, and contains reduced amounts of vitamin C. Regarding hawthorns, they were rich in minerals. Hawthorns from Nabha contain levels of fiber and vitamin C higher than those from Arsal and Ham.

Taking into account the degree of methylation and acetylation, all wild fruits contain highly methylated pectin and may have a high gel strength, with the exception of pears grown in Arsal, that had a low methylated pectin content and a high degree of acetylation.

On the other hand, regarding biodiversity, it can be rescued from being reduced, by encouraging the Lebanese people to take care of this food source in their own villages, and work on providing the market with new types of jams that interests the customers. It will be a very important, and a promising step, commercially.

In the future research, it will be interesting to characterize better the pectin's content through determining its gelling power and to identify the flavonoids present in wild fruits.

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