Research Article

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FTIR-based comparative analysis of glucomannan contents in some tuberous orchids, and effects of pre-processing on glucomannan measurement

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Abstract

BACKGROUND: Glucomannan (GM) is a polysaccharide of the mannan family of compounds found in some plant species. The dried and powdered tubers of some orchid species, collectively known as 'salep powder,' are a commercially important crop for human consumption and are one of the primary sources of GM. GM content is the primary indicator for the yield and quality of salep powder. We hypothesized that it would be more practical and accurate to measure GM content within tuber powder directly, prior to any purification or pre-processing. The GM content of tubers of 14 different orchid species was evaluated and compared using Fourier transform infrared (FTIR) spectroscopy and an enzymatic colorimetric method.

RESULTS: Among the analyzed modes, the sum of the peak areas at 873 and 812 cm^{-1}, which represent the CH bending attributed to the β-pyranose form of D-glucose and D-mannose, respectively, gave the only confirmation using colorimetric methods. It was found that the tubers of *Himantoglossum caprinum* and *Serapias vomeracea* had the highest GM concentrations among the analyzed species. After conducting different pre-processing steps on *Serapias vomeracea* tubers, it was found that treating the tubers with milk, or high temperature resulted in an apparent increase in GM concentrations.

CONCLUSION: *Himantoglossum caprinum* and *Serapias vomeracea* give the highest yields of GM and should be used for commercial horticulture. GM estimation should be made prior to any pre-processing. FTIR spectroscopy is effective and reliable for directly comparing GM content of different orchid species, without the need for any purification or pre-processing.
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Keywords: FTIR; glucomannan; glucose; mannose; Orchidaceae; pre-processing

INTRODUCTION

As members of the mannan family of compounds, glucomannan (GM) polysaccharides are natural water-soluble fibers consisting of β-1,4-linked D-mannose, D-glucose and an acetyl group every 12–18 repeating units.[1,2] Mannose and glucose monomers form GMs with different molecular structures. GM from a common GM-source, the konjac plant (*Amorphophallus konjac*), has these monomers in a molar ratio of 1.6:1 (mannose/glucose) while GM from *Orchis morio* tubers has a ratio of 3.6:1.[3,4] Due to their hydrophilic nature, high viscosity and prebiotic properties, GMs are used as gelling agents, thickeners, stabilizers, film formers, emulsifiers, therapeutic agents and laxatives in different areas such as the food and pharmaceutical industries.[5] GMs have also gained attention as biosorbents, due to their biodegradability, renewability and low-cost.[6] In addition, they are reported to aid in normalizing blood sugar and relieve stress on the pancreas.[7]

The quantity of water-soluble, storage GMs in vegetative parts of plants such as tubers varies greatly.[8] Notably, some tuberous species of Orchidaceae family are the primary sources of these polysaccharides. Depending on the species, the tubers of these plants may contain variable concentrations of GMs (7–61%), starch (1–36%), moisture (6–12%), fat (~2%), protein (3.5–7%) and ash (0.2–7%).[9–11] Tuberous orchid species are mainly distributed across Mediterranean and Asian countries. In Turkey, Balkan and Middle Eastern countries, orchid tubers are dried and ground to obtain salep powder which is then mixed with boiling milk to produce salep which is a widely consumed traditional hot beverage. In addition, salep powder is used in the production process for Maraş ice-cream.[11]

In most countries, tuberous orchids are collected, often illegally, during the spring season and sold at high prices to be used mainly in the food industry. To protect the wild populations of GM-source orchids, it would be possible to culture these tuberous orchids in field conditions. However, orchids are slow growing in nature. Thus, it would be very time-consuming and costly to culture all tuberous orchids for industrial needs. To identify the most commercially important species, that is those with the

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Figure 1. Geographic locations of the sampling sites in Turkey. The stars show the sampling sites with the numbers on the stars indicating the number of sampled species.

highest concentrations of GM, a reliable GM productivity analysis should be conducted on tuberous orchids. This would result in the focused cultivation of only the highest yield tubers.

Those orchid species containing more GM are considered to be 'high quality,' especially in the food industry. Due to the commercial value and importance of orchid-sourced GM production, a practical method for determination of relative GM content in tubers of different orchid species is important. The method should be effective in the absence of any purification and/or pre-processing, typically heat drying, boiling in water/milk, peeling and/or hardening steps. However, conventional GM content estimation methods may be made after any pre-processing steps have been applied to the orchid tubers. These steps include boiling and high-temperature drying. These high-temperature processes may affect the mannosidic and/or glycosidic linkages in GM polymers, which in turn may make GM content estimation inconsistent. We hypothesize that Fourier transform infrared (FTIR) spectroscopy may offer a valuable confirmative technique, since it has high sensitivity in detecting changes in the functional groups belonging to plant tissue components, such as carbohydrates and proteins, simultaneously.[12–15] To test this hypothesis, we first collected tubers of various orchid species and analyzed their GM contents by using a well-known enzymatic colorimetric assay, before and after a pre-processing step. The results were then validated using FTIR spectroscopy. This is the first report of the effects of pre-processing steps on GM content estimation.

Table 1. The list of sampled orchid species and sampling sites

Species name	Sampling station (province/district)	Sample code
Anacamptis pyramidalis (L.) Rich.	Kocaeli/Kandıra	AN1
Himantoglossum caprinum (M.Bieb.) Spreng.	Kocaeli/Kandıra	HI1
Ophrys apifera Huds.	Kocaeli/Gebze	OP1
Orchis collina Banks et Sol.	Aydın/Söke	OR1
Orchis coriophora L.	Muğla/Fethiye	OR2
Orchis laxiflora Lam.	Aydın/Söke	OR3
Orchis palustris Jacq.	Aydın/Söke	OR4
Orchis papilionacea L.	Kocaeli/Kandıra	OR5
Orchis purpurea Huds.	Kocaeli/Kandıra	OR6
Orchis sancta L.	Muğla/Milas	OR7
Platanthera chlorantha (Custer) Rchb.	Kocaeli/Yuvacık	PL1
Serapias parviflora Parl.	Kocaeli/Gölcük	SE1
Serapias vomeracea (Burm. F.) Briq.	Kocaeli/Aslanbey	SE2
Steveniella satyrioides (Spreng.) Schltr.	Kocaeli/Kartepe	ST1

MATERIAL AND METHODS

Collection and identification of the specimens

The specimens collected from their natural habitats in Turkey (Fig. 1) are listed in Table 1. The identification of the orchid species was accomplished using Davis's Flora of Turkey.[16] In order not to harm natural populations of these species, sampling size was limited to a maximum of ten individuals having at least two tubers. All species were sampled during their flowering stages. None of the collected specimens are endemic to Turkey, and only *O. palustris* is listed in the Least Concern (LC) category of The International Union for Conservation of Nature (IUCN), the other species collected being more common. Therefore, the sample size for *O. palustris* was limited to five individuals.

Preparation of the tubers

Collected tubers were washed under tap water, to clean soil residues, before they were cut into thin slices. The sliced samples were dried overnight using a freeze dryer (Martin Christ Alpha 1–2 LD plus, Osterode, Germany). The dried materials were then ground into fine powder using an agate mortar and pestle which were cleaned between each sample grinding to avoid cross-contamination of the samples. The resulting tuber powders were assayed using the GM Assay Kit (catalog code: K-GLUM;

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Direct method	Pre-processed 1*	Pre-processed 2**
1) Tuber collection	1) Tuber collection	1) Tuber collection
2) Washing - Slicing	2) Boiling in water (10 min)	2) Boiling in milk (10-15 min)
3) Freeze-drying	3) Peeling skins of tubers	3) Peeling skins of tubers
4) Grinding	4) Heat drying 1 (10 min, 60-70 °C)	4) Natural drying 1 (7-10 d, RT)
5) Measurements	5) Heat drying 2 (15 min, 140-150 °C)	5) Grinding
	6) Hardening for a few days	6) Measurements
	7) Grinding	
	8) Measurements	

* Farhoosh and Riazi (2007) ** Tekinşen and Güner (2010) RT: Room temperature

Figure 2. Comparison of sample preparation methods for analysis of glucomannan content.

Megazyme™ International Limited, Bray, Ireland) which is specific for measuring GM content.

For the FTIR studies, the remaining powder from each sample was mixed with dried potassium bromide (KBr) at a ratio of 1:100 (*m/m*). The mixture was again dried using overnight freeze drying to remove all remaining traces of water. To produce a thin KBr disk for analysis, each sample was compressed at a pressure of ~100 kg cm^{-2} (1300 psi) using a hydraulic pellet press.

Colorimetric determination of GM content

Samples were prepared according to the manufacturer's instructions for the GM Assay Kit (K-GLUM 03/14). The GM contents of the samples were estimated by measurement of absorbance values of prepared blank and sample solutions (for GM A_1, A_2, A_3) in a UV-visible spectrophotometer (Shimadzu UV Mini 1240, Kyoto, Japan) at 340 nm. The GM contents of the samples were calculated using the following formulae:[17]

$$\Delta A_{\text{glucomannan}} = (A3 - A1)\,\text{sample} - (A3 - A1)\,\text{blank}$$

$$\text{Total glucomannan content } (\%) = \Delta A_{\text{glucomannan}} \times 36.8.$$

Relative comparison of GM contents by FTIR spectroscopy

The spectra of samples were acquired using a Perkin Elmer – Spectrum One spectrometer (Perkin Elmer Inc., Norwalk, CT, USA) equipped with a deuterated triglycine sulfate (DTGS) detector. The sample compartment in the FTIR spectrometer was continuously purged with dry air to prevent water vapor contamination. Pellets were scanned at 4 cm^{-1} resolution with 100 scans in the spectral range 4000 – 400 cm^{-1} (mid-IR region) at room temperature.

To minimize intra-sample variability and to eliminate any interference which may be caused by variations in experimental conditions, three independent pellets prepared by taking separate portions randomly from the same powder sample were used. After scans were taken, the resulting triplicate spectra were averaged to represent a spectrum of each sample. Subsequently, the average spectrum of each sample was used for data evaluation as described previously.[13,15] In order to evaluate the differences in GM content between the samples, the area values of the modes assigned to GM were measured. For the calculation of peak areas, the Perkin-Elmer Spectrum One FTIR software was used. First, baseline correction for each band was applied in order to identify the start and end points. The corrected area, which is the area between the spectrum and baseline points, was calculated.

Determination of the effect of pre-processing on GM contents

In order to investigate the effects of pre-processing steps on measured GM content of tubers, the tuber pre-processing methods reported by Tekinşen and Güner[11] were applied to *Serapias vomeracea* tubers, since the species is easily accessible in nature and has rich GM content (Fig. 2). After pre-processing the GM content of tubers was evaluated using both FTIR spectroscopy and GM Assay Kit.

Statistical analysis

The experiments were repeated in triplicate. The results were expressed as mean ± standard deviation (SD). All data were compared and statistically analyzed using analysis of variance (ANOVA) and Duncan's multiple range test using Statistical Package for Social Sciences (SPSS, version 22, IBM Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

The results of enzymatic colorimetric analysis of the GM content of sample powders are shown in Table 2. GM content varied considerably from species to species. The most GM-productive species was found to be *Himantoglossum caprinum* while the lowest amount of GM was obtained in *Steveniella satyrioides* tubers. The morphologically similar species, *Serapias vomeracea* and *Serapias parviflora* gave similar results. The GM contents varied greatly between *Orchis* species. For example, *O. sancta* contained almost seven times higher GM quantity than *O. collina*. This variation may be explained by the physiological age of the species[18] for the following reason. Although the specimens were sampled during their flowering stages, some of them start flowering in different months. A late flowering date means a physiologically older plant which has more time to produce primary metabolites and enrich its tubers' nutritional content. Also, micro/macro-climatic conditions at the different sampling sites could affect both flowering dates and tuber development.

Interpretation of IR spectra of complex samples follows the 'group frequency' approach in which absorption bands are attributed vibrational modes of particular functional groups based on comparison with model systems. Using the group frequency approach for spectral analysis, the absorptions can be assigned to vibrational modes of molecules present within biomolecules.[19] Accordingly, depending on the Beer – Lambert Law, relative quantitative information can be obtained by measuring the intensity of the absorption modes to provide relative concentration information on the corresponding functional groups.[20–22] Previously reported biochemical assays had confirmed this for analysis of

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Table 2. Estimated glucomannan (GM) content in the tubers of sampled orchid species

Rank	Code	GM content (%)
1	HI1	29.51 ± 0.81^{a}
2	SE2	29.15 ± 0.59^{a}
3	SE1	26.28 ± 1.16^{b}
4	AN1	24.62 ± 0.61^{c}
5	OR7	23.11 ± 0.19^{cd}
6	OR6	22.48 ± 0.35^{d}
7	OP1	18.43 ± 0.49^{e}
8	OR4	16.16 ± 0.21^{f}
9	OR5	13.06 ± 0.43^{g}
10	PL1	11.00 ± 0.18^{h}
11	OR3	10.57 ± 0.18^{h}
12	OR2	8.45 ± 0.27^{i}
13	OR1	7.41 ± 0.14^{i}
14	ST1	4.73 ± 0.63^{j}
F		767.777

Data represent mean ± SD. Means having the same letter in a column are not significantly different by Duncan's multiple range test ($P < 0.001$).

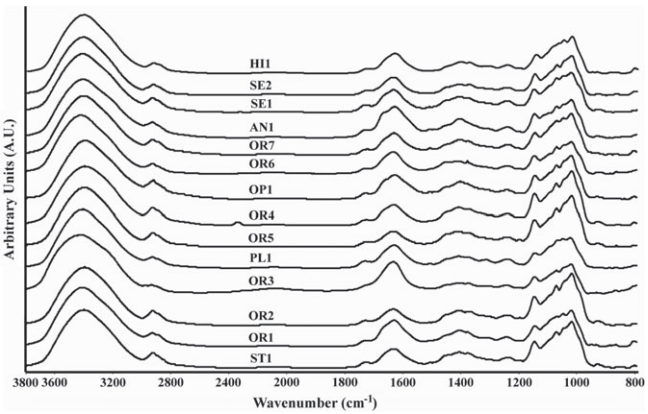


Figure 3. The representative FTIR spectra of tubers of 14 orchid species at wavelengths over the range 3800–800 cm^{-1}.

lipid peroxidation products[23–25] and proteins.[26] It must be remembered that since FTIR spectroscopy cannot distinguish specific molecules within a complex sample, it is restricted to provide information about all the biomolecules present and is unable to focus on any specific one. Since we did not apply any extraction method for GM to the samples, in our case, the tuber samples contain not just GM but also a wide range of other biomolecules. Therefore, several modes originating from all the biomolecules present in the samples, which included GM, were observed in the IR sample spectra as shown in Fig. 3. The main observed modes in the orchid tuber samples appeared at 3396, 2929, 1732, 1635, 1413, 1375, 1247, 1151, 1076, 1053, 1027, 873 and 812 cm^{-1}. However, it must be borne in mind that in addition to GM, tuber samples also contain other molecules such as proteins, water, starch and other nitrogenous substances like ash, all of which can also contribute to the modes associated with GM.[11] For example, carbohydrates, proteins, phospholipids and cell wall polysaccharides also give absorptions at around 3390, 1640, 1740, and 1240 and 1025 cm^{-1}, respectively.[13–15]

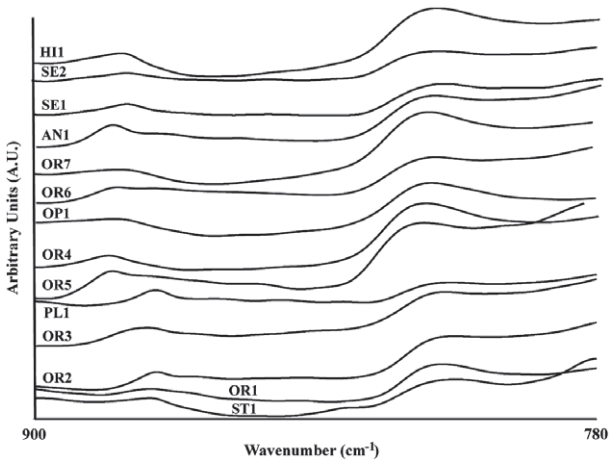


Figure 4. The representative FTIR spectra of evaluated tubers of orchid species at wavelengths over the range 900–780 cm^{-1}.

Seven absorption values, which GM has the potential to contribute, were analyzed.[27–29] The mode at 3396 cm^{-1} is the characteristic stretching vibration of the -OH groups coupled with hydrogen bonding. Additionally, the mode at 2929 cm^{-1} represents C-H stretching and arises from methyl units.[30] Ester carbonyl groups (C=O), mainly sourced from acetyl group of glucomannan and hemicellulose appear at 1732 cm^{-1}[31,32] while the C=O stretch vibration from GM with the contribution of protein amide groups may be found at 1635 cm^{-1}.[27] In addition, the C-O-C fragment was observed at 1154 cm^{-1}.[33] The existence of β-1,4 glucosidic and β-1,4 mannosidic linkages characteristic of GM are assigned to C-O-C stretching at 1027–1246 cm^{-1}.[27] CH bending appeared at 873 and 812 cm^{-1} and is attributed to the β-pyranose form of glucose and mannose, respectively.[34]

In order to evaluate GM contents of the samples, peak areas of the modes most strongly associated with GM, at 1732 1635, 1246, 1154, 1027, 873 and 812 cm^{-1}, were measured. The results are shown in Table 3. In order to compare the accuracy of the data obtained from the enzymatic colorimetric assay and FTIR spectroscopy, the data were further compared as mentioned earlier. There was no significant correlation between the measured individual FTIR peak areas and the results from GM Assay Kit as is evident by comparing the results shown in Tables 2 and 3. In order to obtain conformity between spectroscopic and colorimetric results the modes which have been widely used to identify GM content in different systems were thus taken into consideration. The peak area values of the GM specific vibrations at 873 and 812 cm^{-1} as reported by Hua *et al.*[34] were evaluated (see Fig. 4). As the measurement of GM content is based on both glucose and mannose units within the samples these two peak areas were summed (Table 3). This approach has been validated in the literature for various biomolecules.[25,35] Subsequently, accordance between IR and GM Assay Kit data was obtained by addition of the peak areas at 873 and 812 cm^{-1}. The corresponding findings revealed that the highest concentration of GM is present in *H. caprinum* while *Steveniella satyrioides* has the lowest, as shown in Tables 2 and 3.

A similar approach to the current study has also been previously described by Tekinşen and Güner.[11] However, in that study, GM contents in various orchid tuber samples were found to be higher than that of our samples. Tekinşen and Güner reported richer GM content in the species *Serapias vomeracea*, *O. coriophora* and *O. palustris* at 44.8%, 20.0% and 20.5%, respectively.[11] This

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Table 3. Peak area values of IR modes assigned to glucomannan in the tubers of 14 orchid species

Code	Peak areas 1732	1635	1246	1154	1027	873	812	873 + 812
HI1	3.98 ± 0.11^{bc}	35.69 ± 0.21^{a}	8.76 ± 0.80^{abc}	14.33 ± 1.80^{a}	31.38 ± 0.68^{ab}	0.47 ± 0.080^{cd}	2.82 ± 0.49^{a}	3.29 ± 0.71^{a}
SE2	3.70 ± 0.22^{bcd}	36.99 ± 2.17^{a}	7.53 ± 0.27^{bcd}	11.66 ± 2.07^{a}	22.91 ± 0.75^{bcd}	0.53 ± 0.011^{c}	2.36 ± 0.40^{a}	2.89 ± 0.60^{ab}
SE1	3.67 ± 0.19^{bcd}	28.39 ± 3.11^{b}	9.51 ± 0.77^{ab}	13.14 ± 4.01^{a}	30.37 ± 2.80^{abc}	0.74 ± 0.020^{a}	0.90 ± 0.07^{bc}	1.64 ± 0.99^{abc}
AN1	2.39 ± 0.34^{de}	24.88 ± 3.99^{bcd}	7.40 ± 0.61^{bcd}	14.14 ± 0.80^{a}	33.52 ± 3.61^{a}	0.64 ± 0.090^{b}	0.78 ± 0.092^{bc}	1.42 ± 0.91^{bc}
OR7	3.04 ± 0.39^{cde}	36.25 ± 2.15^{a}	9.33 ± 0.58^{ab}	12.84 ± 2.99^{a}	$27.74 \pm 3.12^{a-d}$	0.28 ± 0.004^{ef}	1.10 ± 0.088^{b}	1.39 ± 0.87^{bc}
OR6	2.02 ± 0.04^{e}	19.21 ± 1.05^{de}	5.03 ± 1.01^{d}	9.05 ± 0.70^{a}	29.72 ± 4.09^{abc}	0.33 ± 0.005^{ef}	1.02 ± 0.077^{bc}	1.35 ± 0.81^{bc}
OP1	2.80 ± 0.14^{cde}	13.82 ± 0.91^{e}	6.70 ± 0.39^{cd}	10.11 ± 1.22^{a}	$23.84 \pm 2.74^{b-d}$	0.30 ± 0.007^{ef}	0.94 ± 0.045^{bc}	1.24 ± 0.77^{bc}
OR4	2.54 ± 0.57^{cde}	26.10 ± 1.33^{bcd}	6.57 ± 0.84^{cd}	9.80 ± 0.74^{a}	23.16 ± 1.50^{bde}	0.10 ± 0.001^{g}	0.90 ± 0.056^{bc}	1.00 ± 0.158^{bc}
OR5	5.58 ± 0.31^{a}	26.59 ± 1.44^{bc}	10.69 ± 1.09^{a}	9.28 ± 0.64^{a}	21.84 ± 1.22^{cd}	0.26 ± 0.008^{f}	0.69 ± 0.067^{bc}	0.95 ± 0.172^{bc}
PL1	4.65 ± 0.61^{ab}	42.22 ± 3.55^{a}	8.89 ± 1.37^{abc}	11.41 ± 1.22^{a}	$23.47 \pm 2.87^{b-d}$	0.06 ± 0.001^{g}	0.85 ± 0.072^{bc}	0.91 ± 0.092^{c}
OR3	2.57 ± 0.18^{de}	25.79 ± 2.47^{bcd}	6.15 ± 0.90^{d}	8.58 ± 0.94^{a}	21.01 ± 3.09^{d}	0.38 ± 0.004^{de}	0.50 ± 0.011^{bc}	0.88 ± 0.022^{c}
OR2	2.24 ± 0.29^{de}	19.82 ± 1.39^{cde}	5.64 ± 0.56^{d}	9.51 ± 1.80^{a}	$23.54 \pm 1.21^{b-d}$	0.10 ± 0.005^{g}	0.70 ± 0.064^{bc}	0.80 ± 0.079^{c}
OR1	2.33 ± 0.94^{de}	17.47 ± 0.86^{e}	6.49 ± 0.74^{cd}	12.08 ± 0.96^{a}	30.35 ± 2.55^{abc}	0.25 ± 0.008^{f}	0.44 ± 0.057^{c}	0.69 ± 0.006^{c}
ST1	2.12 ± 0.75^{e}	24.75 ± 1.64^{bcd}	5.07 ± 0.28^{d}	8.32 ± 0.27^{a}	20.07 ± 2.07^{d}	0.13 ± 0.002^{g}	0.45 ± 0.079^{c}	0.58 ± 0.109^{c}
F	17.284	44.032	15.139	4.154	9.097	118.258	44.700	5.610

Data represent mean ± SD. Means having the same letter in a column are not significantly different by Duncan's multiple range test ($P < 0.001$).

might be due to a number of reasons including differences in sample preparation methods (Fig. 2), the sample size, physiological age of the plant samples and climatic factors.[18] In Tekinşen and Güner's sample preparation method,[11] the authors air-dried the tubers for 7 to 10 days after which they were boiled in milk for 10 to 15 min. As a result of the boiling process, milk sugar (lactose) might degrade into its subunits, glucose and galactose. These lactose-derived glucose units might contribute to signal strength, in addition to the glucose molecules in GM, when using colorimetric analysis, which in turn would lead to higher reported GM concentrations. Also, due to its hydrophilic nature, orchid tubers can easily absorb liquids such as milk. In our study, to investigate whether pre-processing treatments affect GM estimation, we examined tuber samples of *Serapias vomeracea* and compared the GM content of samples that had been boiled in milk with samples that had not been boiled, using both colorimetric assay and FTIR spectroscopy (Fig. 5). It was found that the same tuber sample yielded apparent higher GM concentration when it was boiled in milk during sample preparation. This result was validated using the GM Assay Kit. After the same pre-processing steps conducted by Tekinşen and Güner,[11] GM content in the *Serapias vomeracea* tubers was found to be 36.21% compared to our result for unprocessed sample of 29.15%, an increase of 24.22%. Although colorimetric method includes washing steps with ethanol to remove low-molecular weight sugars (LMWS), such as D-glucose and D-fructose, our results indicated that these washing steps may not be enough to eliminate all LMWS contribution coming from a possible pre-processing treatment. We did not investigate the pre-processing methodology of Farhoosh and Riazi[10] since they did not indicate the species names, used a mixture of unknown tuber samples and compared GM contents according to tuber shapes. However, the authors found that the pre-processing steps they conducted influenced the GM content of both palmate and round-shaped tubers (Fig. 2). The authors showed that boiling the tuber samples in water for 10 min and application of high temperatures (10 min at 60–70 °C and 15 min at 140–150 °C) to dry them out increased measured GM content. The increase was found to be 22.44% and 14.45% for palmate and round-shaped tubers, respectively.[10] Consequently, when analyzing the GM content of orchid tuber samples, any contribution that might come from different sources other than GM should be taken into consideration.

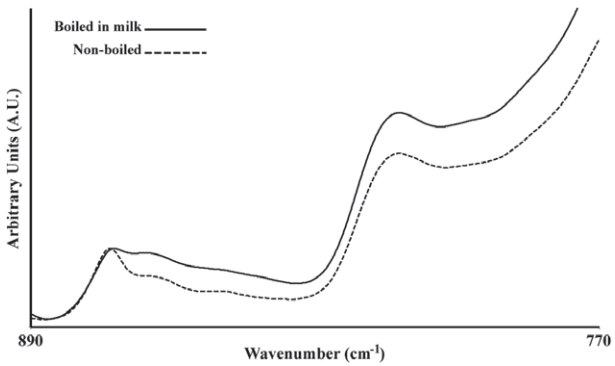


Figure 5. Comparison of the representative FTIR spectra of tubers of *Serapias vomeracea* boiled in milk with non-boiled tubers at wavelengths over the range 890–770 cm^{-1}.

CONCLUSION

We evaluated the potential of FTIR spectroscopy in estimating relative GM concentrations in various orchid species, without the need for any purification. We also show that pre-processing treatments, including heat application, cause an apparent significant increase in the GM concentration. Therefore, we advise that no pre-processing steps should be performed when an accurate measurement of GM content in orchid tubers is required. In addition, we have shown for the first time that this technique is suitable for comparison of GM content in orchid tuber samples and is easy, time-effective and reliable. By summing the peak areas of 873 and 812 cm^{-1} obtained using FTIR spectroscopy the orchid species with the highest GM yield can be identified. However, quantitative analysis of GM concentration requires other methods in addition to FTIR data. Additionally, in light of these results, two widespread

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and abundant species from the Mediterranean climate zone, *H. caprinum* and *Serapias vomeracea*, which both have rich GM content, are suggested as being suitable for commercial horticultural use by the GM industry in the future.

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