Quantitative assessment of the main antioxidant compounds, antioxidant activities and FTIR spectra from commonly consumed fruits, compared to standard kiwi fruit

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1 This article was written in memory of Dr. Zeev Tashma, who encouraged our research group and participated in our research.
2 Prof. Simon Trakhtenberg died in November 2011.

ABSTRACT

Bioactive compounds (polyphenols and ascorbic acid) and dietary fibers, and related antioxidant activities of commonly consumed apples, bananas, peaches, pears, blond and red grapefruits, pomelos, oranges, lemons, red plums, white grapes, mango, persimmon and strawberries grown in the same geographical and climatic conditions were compared with standard kiwi fruit. The presence of polyphenols was studied by Fourier transform infrared (FT-IR) spectroscopy. The contribution of dietary fibers to antioxidant activity of the fruits was minimal (R² from 0.3078 to 0.3626), of the ascorbic acid moderate (R² from 0.6402 to 0.6734) and of total polyphenols - decisive (R² from 0.9792 to 0.9827). It was found that strawberries and kiwi fruits have the highest phenolic contents and their antioxidant activities following by red plum > mango > white grapes > persimmon > apples > pears > red grapefruit > lemons = oranges = blond grapefruits > pomelos > bananas > peaches. FTIR spectroscopy and radical scavenging assays are suitable for bioactivity determination of these fruits. In order to receive best results for human consumption, a combination of these fruits has to be included in the everyday diet. The methods used are applicable for bioactivity determination in food analysis in general.

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1. Introduction

Epidemiological studies and experiments on laboratory animals show that consumption of fruits and vegetables is associated with a low risk of cardiovascular diseases, cancer, and inversely related to coronary atherosclerosis (Dauchet, Hercberg, & Dallongeville, 2006; Gorinstein et al., 2006; Perez-Jimenez et al., 2011; Serafini, Bellocco, Wolk, & Ekstrom, 2002). This information is essential to characterize the health effects of individual phenolic compounds, which differ in their bioavailability, physiological and antioxidant properties (Perez-Jimenez et al., 2011). The health protective effect of the mentioned natural products is mostly related to their antioxidants: phenolic compounds, which prevent oxidation of low-density lipoprotein cholesterol (Chun et al., 2005; Sun, Chu, Wu, & Liu, 2002) and to a less extent - dietary fibers (Lairon et al., 2005; Mahattanatawee et al., 2006). Phenolics and antioxidant activities were determined by various methods in dry and fresh fruits at different phases of simulated gastrointestinal digestion (Kamiloglu, Pasli, Ozcelik, & Capanoglu, 2014). Digestion enhances total polyphenol content and antioxidant activity also in...
2. Materials and methods

2.1. Chemicals

Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid); 2,2′-azobis-2-methyl-propanimidamide; 1,1-diphenyl-2-picrylhydrazyl (DPPH), β-carotene, α-amylase, protease, amylglucosidase, Folin–Ciocalteu reagent (FCR); Griess reagent; Tris, tris(hydroxymethyl)aminomethane; potassium persulfate, gallic acid, and catechin were obtained from Sigma Chemical Co., St. Louis, MO, USA. All reagents were of analytical grade.

2.2. Fruits

Israeli apples (Malus sylvestris), bananas (Musa acuminate), and red and green grapefruits (Citrus paradisi), lemons (Citrus limon), mangoes (Mangifera indica L.), oranges (Citrus sinensis), peaches (Prunus persica), pears (Pyrus communis), persimmons (Diospyros kaki L. Triumph), pomelos (Citrus maxima), red plums (Prunus domestica L.), strawberries (Flagara ananassa), white grapes (Vitis riparia) and kiwi fruits (Actinidia delicosa) were used. These fruits and berries were of the same ripeness, grown in the same geographical region and climatic conditions and picked up at the peak of their respective harvest time in 2011. One kilogram of each fruit was used for the analyses.

The fruits were randomly selected and washed in distilled water. Except for white grapes and strawberries all fruits were peeled. Five replications of five extracts from each cultivar were done.

2.3. Determination of dietary fibers, total polyphenols and ascorbic acid

The contents of dietary fibers were determined according to Prosky, Asp, Schweizer, De Vries, and Furda (1992). Samples were treated with heat-stable α-amylase, protease, and amyloglucosidase, followed by centrifugation (15 min, 3000 g) in order to separate the soluble and insoluble fractions, and dialysis against water. Defatted samples were extracted from a 50-mg aliquot with 5 mL of 50% methanol/water with heating at 90 °C for 3 h.

The second extract was obtained with 5 mL of 1.2 mol/L HCl in 50 mL/100 mL methanol/water and treated as above with heating (Vinson et al., 2001). The clear supernatants obtained from two different extractions were used for determination of total polyphenols by Folin–Ciocalteu method and measured at 675 nm (Singleton, Orthofer, & Lamuela-Raventos, 1999). Ascorbic acid was determined by HPLC at 254 nm (Perez, Olías, Espada, Olías, & Sanz, 1997).

2.4. Fourier transform infrared (FT-IR) spectra of polyphenols

Total phenols in the investigated fruit extracts were studied by IR spectroscopy. A Nicolet iS 10 FT-IR Spectrometer (Thermo Scientific Instruments LLC, Madison, WI, USA), with the smart iTRTM ATR (attenuated total reflectance) accessory was used to record IR spectra (Cui et al., 2012; Derenne et al., 2013; Maoela et al., 2009).

2.5. Determination of antioxidant activity (AA)

2.5.1. Trolox equivalent antioxidant capacity test (TEAC)

This test was done using the relative ability of antioxidant substances to scavenge the 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonate) radical cation (ABTS+*). The relative ability was measured at 734 nm (Rice-Evans & Miller, 1994).

2.5.2. Radical scavenging activity using 1, 1-diphenyl-2-picrylhydrazyl (DPPH)

Five milliliters of a 0.1 mmol/L methanolic solution of DPPH were added to 100 μL of studied samples. Changes in the absorbance of the samples and standards were measured at 517 nm (Brand-Williams, Cuvelier, & Berset, 1995).

gooseberries (Chiang, Kadouh, & Zhou, 2013). Some published articles gave different values of antioxidant activity of the same regularly consumed fruits and vegetables. So, one group of authors (Proteggente et al., 2002) has shown that their antioxidant activities are in the following order: strawberry > red plum > grapefruit = orange > green grape > apple > pear > peach. The second group (Sun et al., 2002) claims that apples have the highest total phenolic content and the highest antioxidant activity, followed by red grapes, strawberries, peaches, lemons, oranges, pears and grapefruits. Therefore, the first group of authors found that among studied fruits the antioxidant activity of grapefruits and oranges is higher than the antioxidant activity of apples. On contrary, the second group reported that apples have the highest total phenolic content and the highest antioxidant activity. As was already mentioned, some investigators recommended to include in the diseases preventive diets fruits and vegetables only with high antioxidant activity (Haruenkit et al., 2007; Paganga, Miller, & Rice-Evans, 1999; Saxena, Venkaiah, Anitha, Venu, & Raghunath, 2007; Vinson, Su, Zubic, & Bose, 2001). We suppose that the discrepancy in the published results may be connected to the fact that the studied fruits and berries were from different geographical regions and were grown in different climatic conditions. While many research gaps remain, kiwi fruits with their multiple health benefits have the potential to become part of our “daily prescription for health” (Stonehouse et al., 2013). Then, we had a unique opportunity to determine the real contents of the essential antioxidant compounds and related their antioxidant activities of regularly consumed fruits and berries from the same geographical and climatic conditions in comparison with standard kiwi fruit. Therefore, we have decided to investigate 14 regularly consumed fruits and berries: apples, bananas, blond grapefruits, lemons, mangos, oranges, peaches, pears, persimmons, pomelos, red grapefruits, red plums, strawberries and white grapes and to compare with standard kiwi fruit. There are many assays for total antioxidant activity determination and everyone has its limitations (Yu et al., 2002). Some of these antioxidant assays give different antioxidant activity trends (Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002). In order to find out the source of the discrepancy in the published results of different groups of investigators (Proteggente et al., 2002; Sun et al., 2002) it was decided to use fruits and berries of the same ripeness and to determine their antioxidant activity (AA) by four different radical scavenging assays. Fourier transform infrared (FTIR) spectroscopy is used in the characterization of polyphenols, even in analysis of cancer cells exposed in vitro to polyphenols (curcumin, epigallocatechin gallate and quercetin) (Cui et al., 2012; Derenne, Van Hemelryck, Lamoral-Theyes, Kiss, & Goormaghtigh, 2013; Maelope et al., 2009). The polyphenol content was determined by FTIR and UV spectroscopy. The role of ascorbic acid in the AA of fruits is controversial. Some authors claim that the AA of fruits might be attributed mainly to the content of phenols (Rapisarda et al., 1999; Wang, Cao, & Prior, 1996), and the contribution of ascorbic acid to the AA is less than 15%. On the other hand, there are investigators, who claim that ascorbic acid plays a major role in the AA (Vinson et al., 2001). Therefore, we also decided to determine the contents of ascorbic acid in the studied samples and its contribution to AA.
2.5.3. Antioxidant assay using β-carotene linoleate model system (β-carotene)

To emulsion [β-carotene (0.2 mg) in 0.2 mL of chloroform, linoleic acid (20 mg), and Tween-40 (polyoxyethylene sorbitan monopalmitate) (200 mg)] were added 40 mL of oxygenated water which initiated this assay as an oxidant. Four milliliter aliquots of this emulsion were added to test samples. AA of the extracts was evaluated in terms of bleaching β-carotene, measuring the absorbance at 470 nm, during $t = 180$ min at an interval of 15 min (Jayaprakash & Rao, 2000).

2.5.4. Scavenging activity against nitric oxide (NO)

A portion of 0.5 mL of a mixture (0.4 mL of each samples extract and 0.1 mL of sodium nitroprusside solution) was diluted with 0.3 mL of Griess reagent. The absorbance of the chromophore formed during the diazotination of nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was immediately read at 570 nm (Marcocci, Packer, Droy-Lefaix, Sekaki, & Gardès-Albert, 1994).

2.6. Statistical analysis

The results are means ± SD of five measurements. To compare several groups analysis of variance (ANOVA) was applied. In the assessment of the AA Spearman correlation coefficient (R) was used. P values of <0.05 were adopted as statistically significant.

3. Results

3.1. Dietary fibers

The contents of dietary fibers in pears, peaches, plums and strawberries were comparable. These values were significantly higher in the above mentioned fruits than in apples, lemons, oranges, grapefruits and white grapes (Table 1, P < 0.05).

Slightly higher content of dietary fibers was shown in kiwi fruit (P < 0.05), following by red plums, pears, strawberries and peaches.

3.2. Ascorbic acid

The significantly highest content of ascorbic acid was in kiwi fruit and strawberries, following by citrus fruits and the lowest contents were found in pears, white grapes, apples and red plums (Fig. 1, P < 0.05).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total ($\mu$g/g FW)</th>
<th>Insoluble ($\mu$g/g FW)</th>
<th>Soluble ($\mu$g/g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple</td>
<td>12.2 ± 1.1$^b$</td>
<td>8.6 ± 0.7$^b$</td>
<td>3.6 ± 0.3$^b$</td>
</tr>
<tr>
<td>Banana</td>
<td>15.5 ± 1.4$^a$</td>
<td>9.6 ± 0.8$^a$</td>
<td>5.9 ± 0.5$^a$</td>
</tr>
<tr>
<td>Blond grapefruit</td>
<td>11.2 ± 1.1$^b$</td>
<td>8.9 ± 0.7$^b$</td>
<td>4.3 ± 0.3$^b$</td>
</tr>
<tr>
<td>Kiwi fruit</td>
<td>24.7 ± 2.2$^a$</td>
<td>18.3 ± 1.7$^a$</td>
<td>6.4 ± 0.5$^a$</td>
</tr>
<tr>
<td>Lemon</td>
<td>13.1 ± 1.1$^b$</td>
<td>8.7 ± 0.7$^b$</td>
<td>4.4 ± 0.3$^b$</td>
</tr>
<tr>
<td>Mango</td>
<td>12.9 ± 1.0$^a$</td>
<td>8.4 ± 0.6$^a$</td>
<td>4.5 ± 0.3$^a$</td>
</tr>
<tr>
<td>Orange</td>
<td>13.0 ± 1.1$^b$</td>
<td>8.6 ± 0.7$^b$</td>
<td>4.4 ± 0.3$^b$</td>
</tr>
<tr>
<td>Peach</td>
<td>20.7 ± 2.1$^a$</td>
<td>12.7 ± 1.1$^a$</td>
<td>8.0 ± 1.1$^a$</td>
</tr>
<tr>
<td>Pear</td>
<td>21.3 ± 2.1$^a$</td>
<td>12.9 ± 1.2$^a$</td>
<td>8.4 ± 0.6$^a$</td>
</tr>
<tr>
<td>Persimmon</td>
<td>13.9 ± 1.1$^b$</td>
<td>8.6 ± 0.7$^b$</td>
<td>4.3 ± 0.3$^b$</td>
</tr>
<tr>
<td>Pomelo</td>
<td>13.5 ± 1.1$^b$</td>
<td>9.3 ± 0.7$^b$</td>
<td>4.2 ± 0.3$^b$</td>
</tr>
<tr>
<td>Red grapefruit</td>
<td>13.2 ± 1.1$^b$</td>
<td>8.9 ± 0.7$^b$</td>
<td>4.3 ± 0.3$^b$</td>
</tr>
<tr>
<td>Red plums</td>
<td>22.2 ± 2.1$^a$</td>
<td>13.3 ± 1.2$^a$</td>
<td>8.9 ± 0.7$^a$</td>
</tr>
<tr>
<td>Strawberries</td>
<td>20.7 ± 2.1$^a$</td>
<td>12.7 ± 1.1$^a$</td>
<td>8.0 ± 1.1$^a$</td>
</tr>
<tr>
<td>White grape</td>
<td>13.2 ± 1.1$^b$</td>
<td>8.9 ± 1.1$^b$</td>
<td>4.3 ± 1.1$^b$</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 5 samples per fruit, each sub-sampled and analyzed 5 times. Means in columns without letters in common differ significantly (P < 0.05).

3.3. FT-IR spectra

The wavelength numbers of FTIR spectra for catechin (Fig. 2b) at 831, 1040, 1113, 1142, 1283, 1474, 1513 and 1608 cm$^{-1}$ were assigned to −C−H alkenes, −C−O alcohols, −C−O−H alcohols, −OH aromatic, −C−O alcohols, C−H alkenes, C−C aromatic ring and C−C alkenes, respectively. The comparison of all investigated fruits showed slightly more intensive bands in 2927, 1380, 1086 and 880 cm$^{-1}$ in kiwi fruit (Fig. 2a, upper line) than in the other samples. The main bands in all fruit samples were from 3400 to 3300 cm$^{-1}$ (O−H, broad, normal polymeric OH, stretch, hydroxyl group, H-bonded OH stretch) and from 1400 to 700 cm$^{-1}$ (aromatic ring stretch). A shift in the difference between the standards and the investigated samples can be explained by the method of extraction of the main polyphenols. The obtained spectra were similar, therefore we present only 4 spectra and their comparison with catechin.

3.4. Total and free polyphenols

As can be seen the contents of the total polyphenols differ significantly (Table 2). The significantly highest contents of total (7.8 ± 0.7 and 7.7 ± 0.7 g/kg FW) and free polyphenols (1.2 ± 0.10 g/kg FW) were in strawberries and kiwi fruit, following by red plums (7.4 ± 0.7 g/kg FW) and (1.1 ± 0.10 g/kg FW), respectively (Table 2, P < 0.05).

3.5. AA values

The results of the determination of the antioxidant activities of all studied fruits by all four used tests were summarized in Table 2. AA values by TEAC test varied in the studied samples and ranged from 1.83 ± 0.1 to 21.29 ± 1.9 μmol TE/g FW, with the lowest numbers for peaches and the highest ones for strawberries, following by kiwi fruit and red plums. The AAs for these fruits were significantly higher than for other studied samples (Table 2, P < 0.05). The DPPH values (% DPPH inhibition) varied from 11.9 ± 1.1 to 94.1 ± 8.9, with the lowest numbers in peaches and with the highest ones in strawberries, following by kiwi fruit and...
red plums (Table 2, P < 0.05). The β-carotene values (% β-carotene bleaching) ranged from 10.1 ± 1.1 to 89.8 ± 7.7, with the lowest results for peaches and the highest ones for strawberries, following by kiwi fruit ad red plums (Table 2, P < 0.05).

It was also found that also the NO values (% NO-scavenging activity) varied in the studied samples and ranged from 11.6 ± 1.1 to 87.8 ± 8.2 with the lowest value for peaches and the highest ones for strawberries, following by kiwi fruit and red plums and were significantly higher than of other studied samples (P < 0.05). As can be seen (Table 2, P < 0.05), strawberries and kiwi fruit possess the highest AA, following by red plums. Among the four used antioxidant tests the β-carotene and the DPPH were the most sensitive. Therefore, for the assessment of the contribution of total dietary fibers, ascorbic acid and total polyphenols to the total AA of the studied fruits and berries the β-carotene and the DPPH tests were used.

The correlations between the total dietary fibers and AAs were from 0.3078 to 0.3626 (Fig. 3A and B); between ascorbic acid and AAs were from 0.6402 to 0.6734 (Fig. 3C and D) and between total polyphenols and AAs were from 0.9792 to 0.9827 (Fig. 3E and F). Therefore, the contribution of total dietary fibers to the AA was minimal, of the ascorbic acid - moderate and of total polyphenols - decisive.

Fig. 2. FTIR spectra of extracted polyphenols. a, lines from the top kiwi fruit, red plum, persimmon, peach. b, standard catechin.
4. Discussion

As was already mentioned, different groups of investigators have given different results of the AA values of the same fruits and vegetables (Proteggente et al., 2002; Sun et al., 2002; Wang et al., 1996). In order to find out the sources of this discrepancy and to receive reliable results, fruits and berries of the same ripeness and from the same geographical region grown in the same climatic conditions were used. The results of our investigation showed that the studied fruits contained significantly different amounts of total and free phenols and ascorbic acid (P < 0.05). So, the contents of total phenols varied from 0.9 to 7.8 and of free phenols – from 0.1 to 1.2 g/kg FW and ascorbic acid – from 5.2 to 64.2 mg/100 g FW (P < 0.05).

These results correspond with the results of others (Kähkönen et al., 1999; Proteggente et al., 2002; Sun et al., 2002), who also found that the contents of these bioactive compounds in fruits and berries differ significantly. Also Wang et al. (1996) found that the content of ascorbic acid varied considerably from one kind of fruit to another: from 2.9 to less than 0.49 μmol/g FW for strawberries and pears, respectively. Determination of AA by all four used assays has shown the same tendency: significantly different AA values of different fruits. We have found that the AA of the studied samples is in the following order: strawberry = kiwifruit > red plum > apples > white grapes > pears > lemons = grapefruits = oranges > peaches. Also these results correspond with the results of others, who have shown that AA of fresh strawberries was twice the capacity measured in oranges or red grapes, 7 times - in apples, 11 times - in pears, and 16 times - in honeydew melon (Wang et al., 1996). This study shows why the authors of the cited investigations of regularly consumed fruits and vegetables gave different estimations of the content of bioactive compounds and AA (Proteggente et al., 2002; Sun et al., 2002). Our results can be compared with Kaulmann, Jonville, Schneider, Hoffmann, and Bohn (2014), where the polyphenols of plum were about 185 ± 14 mg/100 g. Vitamin C and polyphenols were found to be the best predictors of antioxidant capacity in plums (R² = 0.853, and R² = 0.711). Our results are in line with other recent reports, where the polyphenols of mostly consumed fruits ranged from 22.78 to 1423.74 mg GAE/100 g FW with the highest amounts in chokeberry. The lowest levels (less than 30 mg of GAE/100 g) were found in plum Renkloda, pears and peach (Podsędek, Majewska, Redzyńska, Sosnowska, & Kosiolkiewicz, 2014). In some reports was shown that between the total polyphenols and AAs there is no correlation (Kähkönen et al., 1999; Yu et al., 2002). Our results did not support our claims. We have found a very high correlation between the total polyphenols content and AA (R² from 0.9792 to 0.9827). These data are in accordance with others, who have reported that high polyphenol content increases AA (Eberhardt, Lee, & Liu, 2000; Rinaldi et al., 2013; Toaldo et al., 2013; Vinson et al., 2001). Linear correlation between phenolic content and AA was estimated (Paganga et al., 1999; Rapisarda et al., 1999).

The ATR FT-MIR spectroscopy has allowed to investigate extracted polyphenols. The spectra observed bands between 1800 and 600 cm⁻¹ allow to identify specific band profiles, which are typical for the different hydroxyl-aromatic molecules that constitute the tannin extracts (Cui et al., 2012; Fernandez & Agosín, 2007; Jensen, Egebo, & Meyer, 2008). The tannins which belong to the “hydrolysable” family, present intense absorptions in the region of the C=O at around 1700 cm⁻¹ and also two major “single” bands at around 1315 and 1180 cm⁻¹ to be assigned to aromatic C–O–O. The tannins of the family of “condensed” tannins present an overall structured profile especially in the region between 1400 and 1100 cm⁻¹, where the differences were marked between the samples (Fig. 2). The detailed observation of the peaks in the zone of C–O can give also interesting suggestions related to the classification of different condensed tannin: The hydroxyl distribution in the A-ring is observed in the region between 1200 and 1100 cm⁻¹, while the one of the B-ring can be identified checking the region between 1300 and 1200 cm⁻¹. Our results are in correspondence with others, showing that the FT-IR absorption spectra of catechin, gallic acid and quercetin confirm (Maela et al., 2009) the presence of these substances in the fruit extracts. The spectra of catechin show the characteristic absorption regions for O–H group (3400–3100 cm⁻¹), C–C group around 1600 cm⁻¹, as well as C–O group (1150–1010 cm⁻¹), which are found as well in all extracts (Fig. 2b). The absorption peaks of active substances moved to the smaller wave number direction, and the intensity of characteristic peaks represents the content of corresponding compound. In general, the present findings are consistent with literature data (Podsędek et al., 2014), wherein phenolic content in fruits may be affected by a large number of factors such as climatic conditions, agrotechnological processes, cultivars, harvest time, and storage conditions. No doubt, that the samples used by the above-mentioned authors were not from the same geographical region, have grown not in the same climatic conditions and most probably were not of the same ripeness. So, for one of the above-cited investigations,
fruits were purchased on three separate occasions from different local supermarkets (Wang et al., 1996). The conditions of our investigation were unique: the fruits and strawberries were from the same geographical region, grown in the same climatic conditions and were of the same ripeness. Therefore, we suppose, that the results of our investigation are reliable.

There is also a controversy concerning contribution of ascorbic acid to AA: some authors reported that its contribution to AA of fruits is minimal (Rapisarda et al., 1999; Wang et al., 1996) and others claim that the AA is well-correlated with the content of vitamin C (Proteggente et al., 2002). The results of our investigation show that the contribution of ascorbic acid to AA of studied fruits is moderate ($R^2$ from 0.6402 to 0.6734). On the contrary, the correlation between the contents of the dietary fibers and AA was low: ($R^2$ from 0.3078 to 0.3626).

In summary, the contents of the studied bioactive compounds and the AA are varied significantly. The total polyphenols are the main contributor to the AA of fruits and berries, the contribution of the ascorbic acid is moderate and of dietary fibers is only minimal. The most possible reasons of the discrepancy in the results of the investigations of the cited authors is the use of samples from different geographic regions, which were grown in different climatic conditions.

5. Conclusion

Strawberries and kiwi fruit have the highest total phenolic content and antioxidant activity following by red plum > mango > white grapes > persimmon > apples > pears > red grapefruit > lemons = oranges = blond grapefruits > pomelos >
bananas > peaches. FT-IR spectroscopy was used as a rapid method and as an additional indicator for comparison of methanol extracts from the studied fruits. Therefore, methanol extracts were investigated by FTIR spectroscopy, and the similarity and differences between these fruits were based on the peaks and bands in the polyphenol region.

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References