Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

Enzymatic Synthesis and Characterization of PolyQuercetin

Ferdinando F. Bruno[a]; Adam Trotta[b]; Stephen Fossey[a]; Subhalakshmi Nagarajan[b]; Ramaswamy Nagarajan[c]; Lynne A. Samuelson[a]; Jayant Kumar[d]
[a] U.S. Army Natick Soldier Research, Development and Engineering Center RDECOM, Natick, MA [b] Department of Chemistry, University of Massachusetts, Lowell, MA [c] Plastic Engineering, University of Massachusetts, Lowell, MA [d] Physics, Center for Advanced Materials, University of Massachusetts, Lowell, MA

Online publication date: 12 October 2010

To cite this Article Bruno, Ferdinando F. , Trotta, Adam , Fossey, Stephen , Nagarajan, Subhalakshmi , Nagarajan, Ramaswamy , Samuelson, Lynne A. and Kumar, Jayant(2010) 'Enzymatic Synthesis and Characterization of PolyQuercetin', Journal of Macromolecular Science, Part A, 47: 12, 1191 — 1196
To link to this Article: DOI: 10.1080/10601325.2010.518839
URL: http://dx.doi.org/10.1080/10601325.2010.518839

Journal of Macromolecular Science, Part A: Pure and Applied Chemistry (2010) **47**, 1191–1196

ISSN: 1060-1325 print / 1520-5738 online
DOI: 10.1080/10601325.2010.518839

Enzymatic Synthesis and Characterization of PolyQuercetin

FERDINANDO F. BRUNO[1,*], ADAM TROTTA[2], STEPHEN FOSSEY[1], SUBHALAKSHMI NAGARAJAN[2], RAMASWAMY NAGARAJAN[3], LYNNE A. SAMUELSON[1] and JAYANT KUMAR[4]

[1]*U.S. Army Natick Soldier Research, Development and Engineering Center RDECOM, Natick, MA*
[2]*Department of Chemistry, University of Massachusetts, Lowell, MA*
[3]*Plastic Engineering, University of Massachusetts, Lowell, MA*
[4]*Physics, Center for Advanced Materials, University of Massachusetts, Lowell, MA*

Quercetin is a flavonol found in fruits, onions and wine. Recently, quercetin has been extensively investigated for its antioxidant behavior in food such as poultry and fish. While quercetin has been shown to be a good stabilizer for several oils, the thermal stability and solubility at neutral and slightly alkaline conditions continue to be an area of huge concern. However, increasing the stability and the antioxidant potency of this flavonol would be beneficial to the food industry. We utilized horseradish peroxidase, a phytochemical enzyme, to polymerize quercetin in biocompatible water/ethanol mixtures. This unique, one pot procedure has provided a method to synthesize polyquercetin under mild conditions. The final polymer is soluble in a water/ethanol mixture. Preliminary data were presented in the Material Research Society fall meeting in Boston on December 3, 2008. However, in this article, enzymatically synthesized polyquercetin has also been characterized by a variety of techniques. Using UV-Vis FTIR, GPC, NMR and TGA we have deduced certain aspects of the structure. Structural elucidation was further refined by results from molecular modeling. Density functional theory calculations predict that the UV-Vis spectra of polyquercetin can show high conjugation relative to those of quercetin monomer. This was confirmed by the experimental results. This novel thermally stable polymer, synthesized using Green Chemistry principles, can be used as a potent antioxidant in the highly regulated food industry.

Keywords: Quercetin, Green Chemistry, enzymatic polymerization, horseradish peroxidase

1 Introduction

The phenomenon known as the 'French Paradox' is the correlation of a high-fat diet with a low incidence of coronary heart disease (1). This phenomenon is often found in Mediterranean cultures. This occurrence is associated with a major dietary difference: the moderate consumption of red wine by the Mediterranean population (2). Investigations on the components of red wine led researchers to conclude that flavonoids including quercetin have been shown to exhibit numerous therapeutic properties (3).

Flavonoids are known to be regular constituents in the diet (4). They are divided according to chemical structure into different sub-classes. Among the various subclasses of flavonoids, flavonols are a distinct class of compounds with the 3-hydroxyflavone backbone. Quercetin is the most common flavonol found in the human diet, and has been the subject of much discussion. Recent reports suggest that the estimated average daily intake of quercetin by an individual in the United States is 25 mg. (5)

Quercetin has been shown to possess numerous therapeutic properties. Quercetin is believed to be an active anti-inflammatory agent (6), anti-aggregator agent (7), and vasodilating agent (8). The anti-cancer activity of quercetin has been demonstrated in both in-vitro and in-vivo studies. Quercetin completely inhibits reverse transcriptase of both Raucher murine leukemia virus (concentration of 1 μg/mL) and human immunodeficiency virus (concentration 2 μg/mL), as well (9). In-vivo studies have shown that quercetin blocks the formation of skin tumors (10) and that it enhances the lifespan of mice with P-388 leukemia (11). Moreover, recently, it has been reported that quercetin inhibits carcinogenesis in laboratory animals (12).There are numerous other reports on quercetin exhibiting anticarcinogenic properties (13–15).

The anti-oxidant activity of quercetin and other flavonoids has been studied extensively (16–18). Studies have revealed that quercetin is a superior anti-oxidant when compared to butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (19). Preliminary antioxidant studies indicate that quercetin is a promising antioxidant.

*Address correspondence to: Ferdinando F. Bruno, U.S Army Natick Soldier Research, Development and Engineering Center RDECOM, Natick, MA 01854. E-mail: Ferdinando_Bruno@uml.edu

However, it is quite well-known the content of flavonoids and other polyphenols in food decline significantly on thermal processing. Common processes such as boiling or frying decreases the content of flavonols and other compounds.

However limited stability of quercetin under oxidative conditions and heating are a major issue. In order to address these limitations, we have modified quercetin using benign conditions using naturally occurring enzymes. Horseradish peroxidase (HRP), a phytochemical enzyme, was used as a catalyst to polymerize quercetin. HRP has previously been used to catalyze the formation of polyphenols and polyanilines (20–31). The reaction with HRP involves an initial two-electron oxidation of the ferric enzyme to form an oxidized intermediate (HRP-I) by the addition of hydrogen peroxide. The quercetin monomer then undergoes an oxidation by the intermediate (HRP-I) and this creates a monomeric radical. The radical species couple to first form dimers, and then to form trimers, and this repeats until an oligomer of considerable size is obtained. These oligomers are different from the well known natural anthocyanin.

The enzymatically synthesized polyquercetin was characterized using both experimental techniques and molecular modeling.

2 Experimental

2.1 Instrumental Methods

All UV—Vis spectra were obtained using a Perkin-Elmer Lambda 9 UV—Vis near-IR spectrophotometer. The concentration of the monomer and polymer was of 0.2 mmol. The Thermogravimetric Analysis was performed under nitrogen using a TA Instruments Q50 Thermogravimetric Analyzer. The TGA measurements were carried out under nitrogen at a rate of 10°C/min. A Thermo Nicolet Avatar 370 FT-IR spectrometer was used to obtain the FTIR spectra in the form of KBr pellets or by casting a film on a ZnSe substrate. GPC analysis was carried out on an Agilent GPC with RI detector. GPC analysis was done on a DMF column calibrated with polystyrene standards. 2 mg/ml of the poly(quercetin)was dissolved in DMF and a flow rate 1 ml/min was used. 0.01% lithium bromide was used in all the measurements. The HNMR, C13NMR and the COSY was collected using a Bruker (Billerica, MA) 400 MHZ NMR. The solvent used was d^6-DMSO (Aldrich Chemical Co., St. Louis, MO).

2.2 Materials

Quercetin Bihydrate (≥95%) was purchased from Aldrich Chemical Co. (St. Louis, MO) and was used as received. Peroxidase (Type II: from Horseradish) was purchased from Sigma Chemical Co. (St. Louis. MO) and was used as received. Ethanol (absolute, 200 proof, 99.5%, A.C.S. Reagent) hydrogen peroxide were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) and were used as received. Hydrogen Peroxide (30%) was diluted with water to obtain 0.03% solution and used for polymerization reactions. For dialysis, Spectra/Por® Membranes (MWCO: 8000 and MWCO: 1000) were obtained from Spectrum Laboratories Inc. (Rancho Dominguez, CA) and were used after a thorough wash with distilled water.

2.3 Polymerization Reaction

The polymerization of quercetin was carried out under 1 atmosphere pressure and a temperature of approximately 25°C. First, 6 mL of pH 6.8 phosphate buffer (10 mM) was taken in a 20ml vial. Next, 4 mL of pure ethanol was added to the vial. 10mg of quercetin hydrate was then added to the solution. The pH of the solution is then raised to 11, and then slowly brought down to 7.8 and held there. This was done to increase the solubility of the quercetin. Next, 4 mg of Horseradish Peroxidase (Type: II) was added to the solution. To commence the polymerization 1.5 mL of Hydrogen Peroxide solution (0.03%) was added in small increments to the solution while stirring. To complete the reaction, the solution was left to sit for an hour. The final products were dialyzed using Spectra/Por® Membranes. The samples were dried under vacuum at 50°C and stored until further analysis. The percent yield was typically 90% or higher. Control samples, using denatured enzyme, were prepared following the same procedure. The enzyme was denatured by placing the same in buffered water at 100°C for 30 min. The denatured HRP was then tested using purpurogallin, and was found to be inactive. The denatured HRP did not polymerize the quercetin monomer.

2.4 Modeling Calculations

Calculations were carried out using the Gaussian 03 (32) program. Visualization was with the GaussView 03W program on a personal computer. Density Functional Theory (DFT) calculations were at the B3LYP/6-31G(d) level. UV-Vis absorption was calculated using Time Domain DFT also at B3LYP/6-31G(d).

3 Results and Discussion

The enzymatic polymerization of quercetin in the presence of HRP was monitored using UV-Vis spectroscopy (Fig. 1). The polyquercetin, compared to the quercetin control, showed a blue shifted absorption peak for quercetin monomer residue, and a long wavelength tail (390–600 nm) indicating the presence of extended conjugation. These results are different from those previously observed in the enzymatic polymerization of phenol using, e.g., the LB technique (34) where only an extended conjugation was

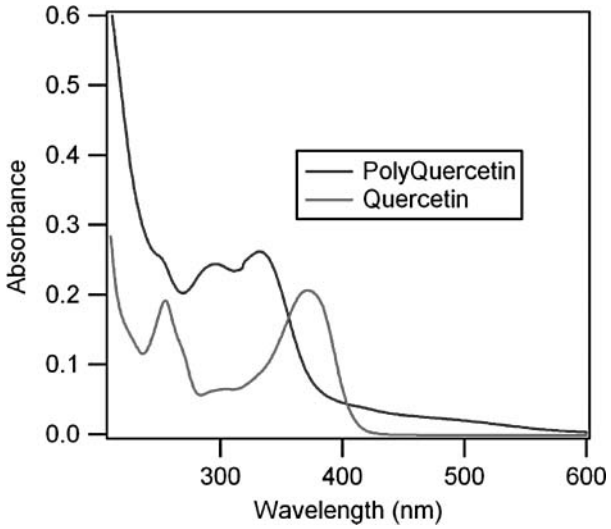


Fig. 1. UV-Vis of the quercetin monomer and of polyquercetin.

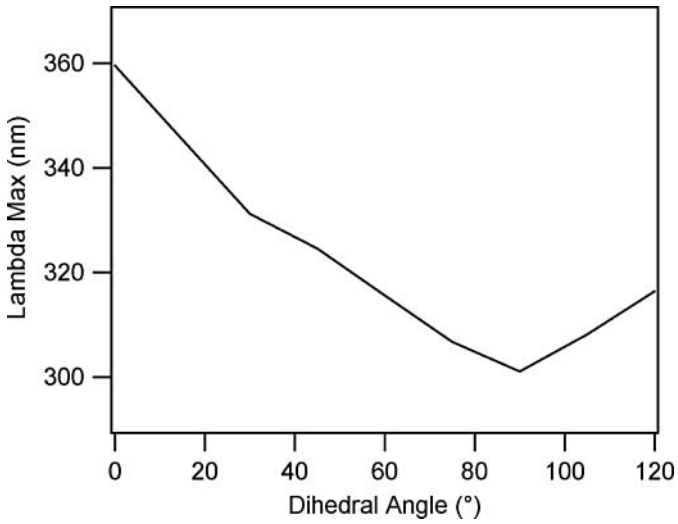


Fig. 3. Calculated lambda max for quercetin at different dihedral angles.

observed. The blue shifted absorption can be explained from electronic structure modeling.

There is only one rotatable carbon-carbon bond in quercetin. Steric interactions in quercetin dimers result in a loss of planarity between the catechol and the remaining moieties in the quercetin molecule (rotation of 2-1′ bond as shown in Figure 2). Based on DFT calculations this bond can have substantial double bond character before polymerization. The calculated bond length is 0.146 nm. The calculated dihedral from DFT, 0.0 degrees, is not substantially different from the 8 degrees seen in the dehydrate crystal (35). For quercetin, TD-DFT calculations predict a lambda max of 358 nm (transition electric dipole moment 2.318 A.U., oscillator strength 0.4562) with a weaker absorption at 266 nm and another significantly weaker at 289 nm for quercetin. When quercetin is polymerized the 2-1′ bond rotates out of plane losing the double bond character (Fig. 2). The loss of the planar conformation for polymerized quercetin causes the absorption wavelength to blue shift. To investigate the effect of this loss of planarity on the UV-Vis absorption, the rotatable carbon-carbon bond in the dimer was set to a torsion angle of 90 degrees (the two ring systems are orthogonal). In this case, the lambda max is approximately 300 nm (Fig. 3). There is a strong absorption at 301 nm (transition electric dipole moment 1.175 A.U., oscillator strength 0.1321) with a weak shoulder at 303 nm. There is also a series of weak absorptions between 287 nm and 250 nm. Figure 3 shows the calculated lambda max as a function of the 2-1′ torsion angle. The presence of two strong peaks between 300 nm and 360 nm suggests there may be two distinct populations of rotamers for the quercetin monomer residues. For quercetin the highest occupied molecular orbital (HOMO) is concentrated on the catechol group. Therefore we expect the reaction to take place at the two more negative carbons of that group (2′ and 5′, see Figure 6 in the supplementary information). If the 2-1′ bond is rotated to 90 degrees then the HOMO shifts to the resorcinol group. Given the partial double bond character of this bond in quercetin we would not expect to see this. Moreover, the tail between 390-600 nm in the UV-Vis is indicative of conjugation created by the polymerization, via catechol, of quercetin. This result is not surprising and was already observed in previous works (33, 34). An average molecular weight Mn of 14000 g/mol (Polydispersity = 3.64) was found for the polymer. Fourier transform infrared (FTIR) spectroscopy of the polyquercetin is shown in Figure 4. Two important absorptions are observed in the

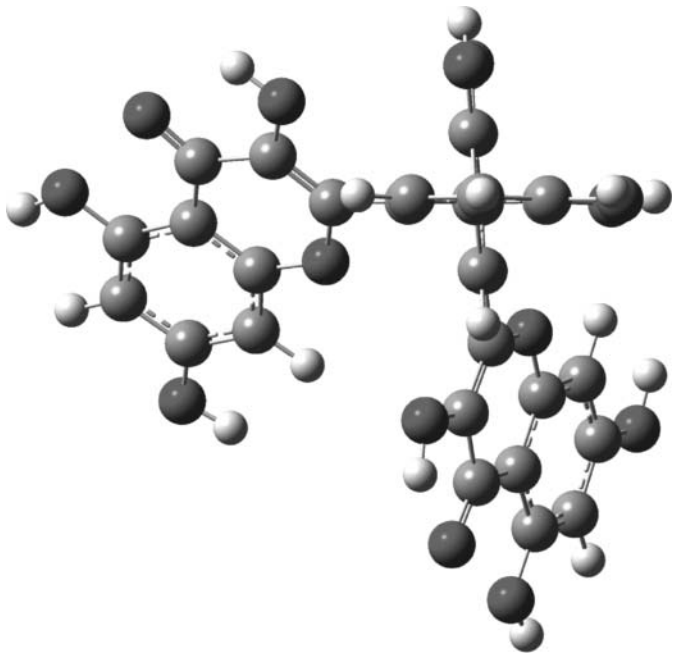


Fig. 2. Quercetin dimer. The bond 2-1′ (carbons number 46–49 and 8–18) rotates out of plane losing the double bond character.

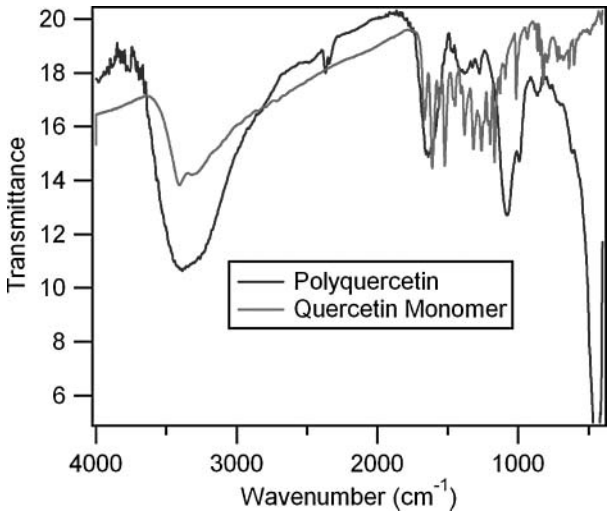


Fig. 4. FTIR spectra of the monomer (quercetin) and of the polyquercetin.

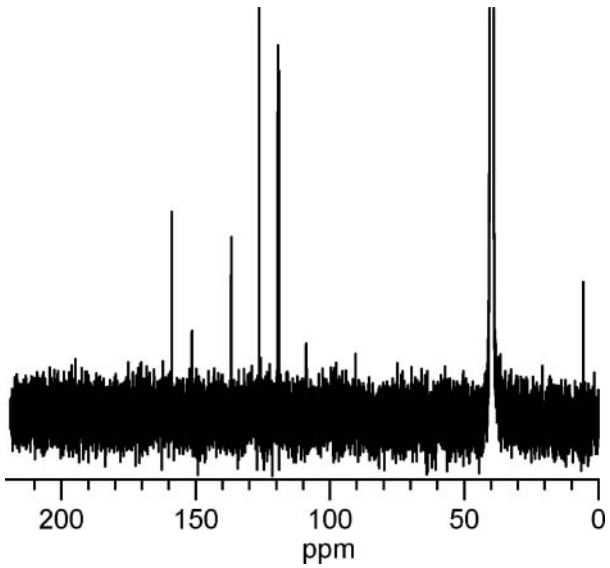


Fig. 6. C^{13}-NMR of the polyquercetin collected in d^6-DMSO.

spectra that support the formation of polyphenol in this enzymatic reaction. The first is a significant OH stretch band (3000–3500 cm^{-1}) suggesting the presence of H-bonded OH groups. The large presence of OH groups accounts for the solubility of the polymer in mix solvents such as ethanol/water (ratio 40/60). The second notable peak is in the 1550–1650 cm^{-1} region and is assigned to o-quinone molecules already reported in previously published work. Similar spectral features were found for polyquercetin polymerized with laccase (33).

H-NMR analysis of the polyquercetin (Fig. 5) showed a presence of the two predominant aromatic peaks (H-NMR ppm 7.948 and 7.274). Quercetin has a catechol and resorcinol subunits in its chemical structure. To assign the aromatic sub-structure following enzymatic polymerization, a C^{13}-NMR (Fig. 6) and a correlation spectroscopy (COSY-H1-C13)) two dimensional NMR (Fig. 7) were performed. From COSY, it was observed that the peaks assigned for C6 and C8 carbons in the resorcinol ring for the monomer, (C6 at 99.5 ppm and C8 at 94.5 ppm) shifts downfield of 19.89 ppm (C6) and of 14.48 ppm (C8) in the polymer indicating a change in the structure of the resorcinol subunit. However also the C5′ and C2′ assigned to the catechol substructure is downshifted from the monomer (monomer assignments 116.5 ppm C5′ and 116.0 ppm C2′) of 9.2 ppm for each carbon. This indicates that the polymerization occur on both sub-phenolic structure. The

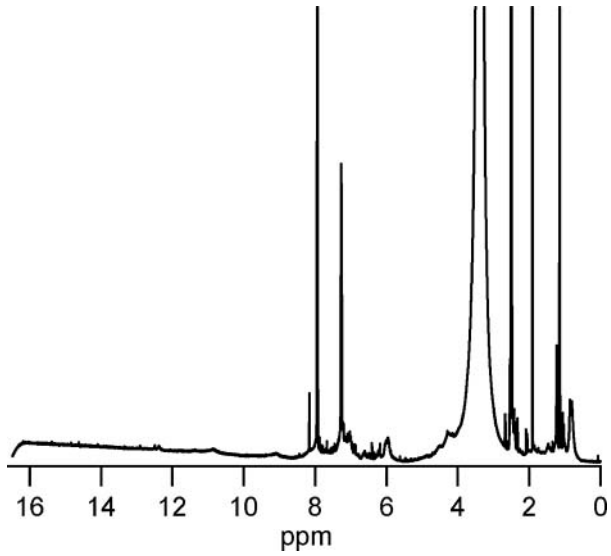


Fig. 5. H-NMR of the polyquercetin collected in d^6-DMSO.

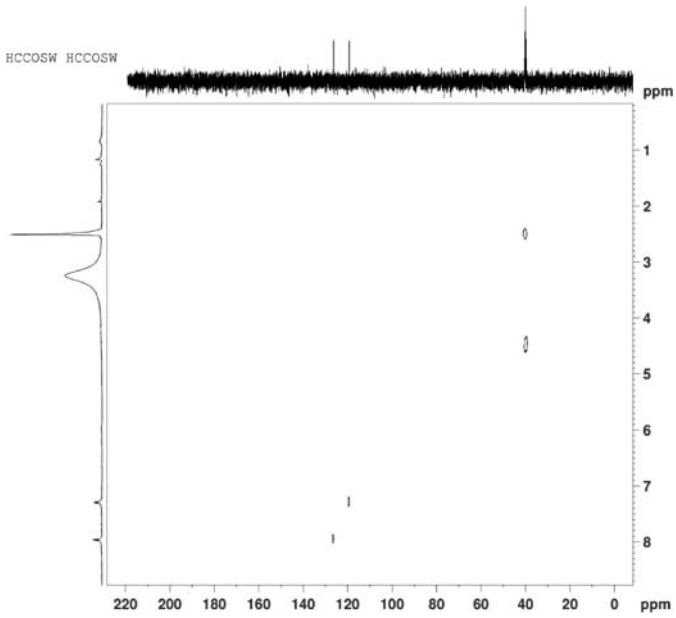
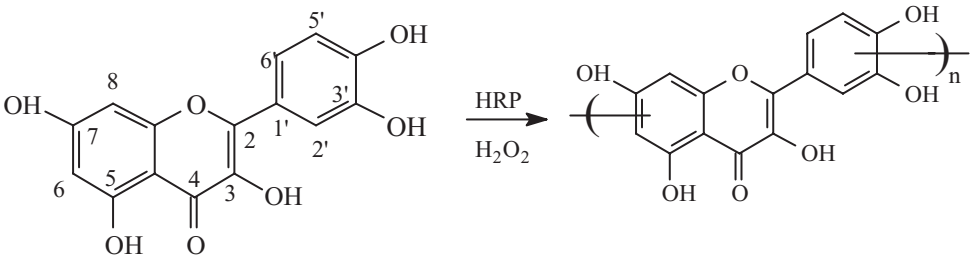


Fig. 7. COSY-NMR of the polyquercetin collected in d^6-DMSO.



Sch. 1. Polymerization reaction with a proposed structure of the polymer.

polymerization reaction and the proposed structure of the polymer are illustrated in Scheme 1. The TGA in Figure 8 shows the thermal stability of polyquercetin vs. the monomer.Polyquercetin proved to be highly hygroscopic, as confirmed by the FTIR, and a first decrease in weight is due to the presence of bonded water. However, it was found that a significant amount of complex (75%) remains after heating the polymer to 600°C. This is attributed to the formation of new catechol-catechol bonds in the backbone of the polymer. A first degradation is observed at 320°C. Then gradually the degradation occurs for the higher molecular weight compounds. These results were similar to that found for insoluble polyphenols synthesized from organic media (36). The final residue was not analyzed.

4 Conclusions

A biocompatible route for the direct synthesis of water soluble, polyquercetin, using HRP as enzyme is described. The approach employed here involves the use of benign solvents and the products can be isolated using simple purification techniques such as dialysis. Instrumental analysis and modeling seem to suggest that the polymer does not contain substantial amounts of conjugated quercetin moieties. Rotation of the bond 2-1′, after polymerization, breaks the conjugation of the quercetin. This is in contrast with previous polyphenolic compounds synthesized enzymatically. However, the formation of new catechol-catechol bonds in the polymeric backbone creates a thermally stable polymer. A possible structure of the polymer is also proposed. The final polymers, however, are expected to be very complex and further purification and characterization are still in progress. Polyquercetin is envisioned to be a more thermally stable antioxidant suitable for the highly regulated food industry and/or drug industry since the starting materials, solvents and the catalyst used are biocompatible. Furthermore, it is anticipated that suitable immobilization of the enzymes used with this approach could potentially lead to a facile, cost effective and environmentally friendly route for large scale production of processable phenolic resins based on natural monomers.

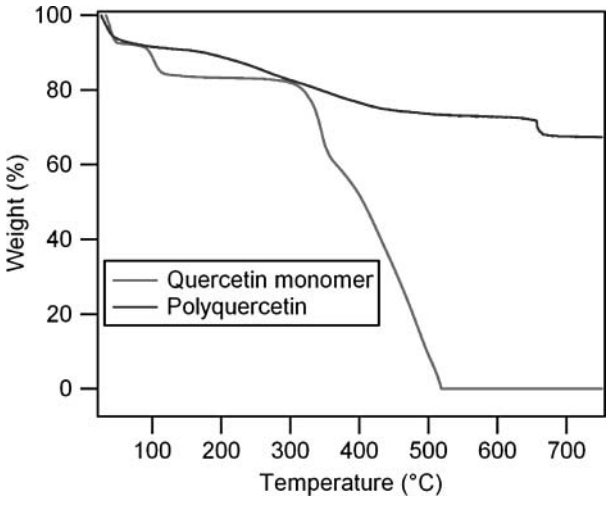


Fig. 8. TGA of quercetin and polyquercetin.

Acknowledgements

The authors thank the US Army Natick Soldier Research, Development and Engineering Center RDECOM for financial support (contract #06-08749).

This paper is dedicated in memory of Dr. William (Bill) Porter.

References

1. Richard, J.L. (1987) *Archives des maladies du coeur et des vaisseaux*, 80 Spec No., 17–21.
2. Renaud, S. and de Lorgeril, M. (1992) *Lancet*, 339(8808), 1523–6.
3. Frankel, E.N., Kanner, J., German, J, B., Parks, E. and Kinsella, J.E. (1993) *Lancet*, 341(8843), 454–7.
4. Pierpoint, (1986) *Progress in Clinical and Biological Research, 213 (Plant Flavonoids Biol. Med.)*, 125–40.
5. US Department of Health and Human Service. *National Toxicology Program Technical Report on the Toxicology and Carcinogenesis Studies of Quercetin in F344/N rats*. NIH publication no 91-3140. US Department of Health and Human Service, Research Triangle Park, NC, 1992.
6. Ferry, D., Smith, A., Malkhandi, J., Fyfe, D.W., de Takats, P.G., Anderson, D., Baker, J. and Kerr, D.J. (1996) Clin. *Cancer Res.*, 2, 659–68.
7. Pignatelli, P., Pulcinelli, F.M., Celestini, A., Lenti, L., Ghiselli, A., Gazzaniga, P.P. and Violi, F. (2000) Am. J. Clin. Nutr.; 72, 1150–5.
8. Perez-Vizcaino, F., Ibarra, M., Cogolludo, A.L., Duarte, J., Zaragoza-Amaez, F., Moreno, L. and Gustavo López-López, Juan Tamargo (2002) *J. Pharmacol. Exp. Ther.*, 302, 66–72.

9. Ono, K., Nakane, H., Fukushima, M., Chermann, J.C. and Barre-Sinoussi, F. (1990) *Eur. Jour. of Bioch.*, 190(3), 469–76.
10. Fujiki, H., Horiuchi, T., Yamashita, K., Hakii, H., Suganuma, M., Nishino, H., Iwashima, A., Hirata,Y. and Sugimura, T. (1986) *Prog. in Clin. and Biol. Res.*, 213, 429–40.
11. Armand, J., De Forni, M., Recondo, G., Cals, L., Cvitkovic, E. and Munck, J. (1988) *Prog. in Clin. and Biol. Res., 280(Plant Flavonoids Biol. Med. 2: Biochem., Cell., Med. Prop.)* 235–41.
12. Stavric, B. (1994) *Clin. Biochem.* 27, 245–8.
13. Verma, A.K., Johnson, J.A., Gould, M.N. and Tanner, M.A. (1988) *Cancer Res.*, 48, 5754–88.
14. Deschner, E.E., Ruperto, J., Wong, G. and Newmark, H.L. (1991) *Carcinogenesis*, 12, 1193–6.
15. Pereira, M.A., Grubbs, C.J., Barnes, L.H., Li, H. and Olson, G.R. (1996) *Carcinogeneisis*, 17, 1305–11.
16. Hayek, T., Fuhrman, B., Vaya, J., Rosenblat, M., Belinky, P., Coleman, R. and Elis, A. (1997) *Arter. Thromb Vasc. Biol.*, 17, 2744–52.
17. Chopra, M., Fitzsimons, P.E., Strain, J.J., Thurnham, D.I. and Howard, A.N. (2000) *Clin. Chem.*, 46, 1162–70.
18. Nixon, J.E., Hendricks, J.D., Pawlowski, N.E., Pereira, C.B., Sinnhuber, R.O. and Bailey, G.S. (1984) *Carcinogenesis*, 5(5), 615–9.
19. Udaya, N.W. and Shahidi, F. (1994) *Food Chemistry*, 50(40), 393–396.
20. Dordic, J.S. (1989) *Enzyme Microb. Technol.*, 11, 194–211.
21. Akkara, J.A., Senecal, K.J. and Kaplan, D.L. (1991) *J. Polym. Sci., Polym. Chem.*, 29, 1561–1574.
22. Kim, Y-J., Shibata, K., Uyama, H. and Kobayashi, S. (2008) *Polymer*, 49(22), 4791–4795.
23. Uyama, H. (2006) *Kagaku Seibutsu*, 44(12), 814–822 (and references in the paper).
24. Uyama, H. and Kobayashi, S. (2006) *Advances in Polymer Science, 194 (Enzyme-Catalyzed Synthesis of Polymers)*, 51–67.
25. Kurisawa, M., Chung, J.E., Uyama, H. and Kobayashi, S. (2003) *Macromolecular Bioscience*, 3(12), 758–764 (and references in the paper).
26. Uyama, H. and Kobayashi, S. (2003) *Current Organic Chemistry*, 7(13), 1387–1397.
27. Ghan, R., Shutava, T., Patel, A., John, V.T. and Lvov, Y. (2004) *Macromolecules*, 37(12), 4519–4524.
28. Kim, Y-J., Uyama, H. and Kobayashi, S. (2004) *Polymer Journal* (Tokyo, Japan) 36(12), 992–998.
29. Mita, N., Tawaki, S-H., Uyama, H. and Kobayashi, S. (2003) Macromolecular Bioscience, 3(5), 253–257.
30. Eker, B., Zagorevski, D., Zhu, G., Linhardt, R.J. and Dordick, J.S. (2009) *Journal of Molecular Catalysis B: Enzymatic*, 59(1–3), 177–184.
31. Irvin, G., Banerjee, S., Premachandran, R., Simmons, B.A., Li, S., John, V.T. McPherson, G., Akkara, J., Kaplan, D. and Zhou, W. (2001) *Surfactant Science Series, 100 (Reactions and Synthesis in Surfactant Systems)*, 515–524.
32. Gaussian 03, Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Montgomery, J.A., Jr., Vreven, T., Kudin, K.N., Burant, J. C., Millam, J.M., Iyengar, S.S., Tomasi, J., Barone, V., Mennucci, B., Cossi, M., Scalmani, G., Rega, N., Petersson, G.A., Nakatsuji, H., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Klene, M., Li, X., Knox, J.E., Hratchian, H.P., Cross, J.B., Bakken, V., Adamo, C., Jaramillo, J., Gomperts, R., Stratmann, R.E., Yazyev, O., Austin, A.J., Cammi, R., Pomelli, C., Ochterski, J.W., Ayala, P.Y., Morokuma, K., Voth, G.A., Salvador, P., Dannenberg, J.J., Zakrzewski, V. G., Dapprich, S., Daniels, A.D., Strain, M.C., Farkas, O., Malick, D.K., Rabuck, A.D., Raghavachari, K., Foresman, J.B., Ortiz, J.V., Cui, Q., Baboul, A.G., Clifford, S., Cioslowski, J., Stefanov, B.B., Liu, G., Liashenko, A., Piskorz, P., Komaromi, I.; Martin, R.L.; Fox, D.J.; Keith, T., Al-Laham, M.A., Peng, C. Y., Nanayakkara, A., Challacombe, M., Gill, P.M. W., Johnson, B., Chen, W., Wong, M.W., Gonzalez, C., and Pople, J.A., Gaussian, Inc., Wallingford, CT, 2004.
33. Desentis-Mendoza, R.M., Hernandez-Sanchez, H., Moreno, A., Rojas del, C.E., Chel-Guerrero, L., Tamariz, J. and Jaramillo-Flores, M.E. (2006) *Biomacromolecules*, 7(6), 1845–54.
34. Bruno, F.F., Akkara, J.A., Samuelson, L.A., Kaplan, D.L., Mandal, B.K., Marx, K.A., Kumar, J. and Tripathy, S. (1995) *Langmuir*, 11, 889.
35. Jin, G.Z., Yamagata, Y. and Tomita, K. (1990) *Acta Cryst.*, C46(10), 310–313.
36. Bruno, F.F., Nagarajan, R., Sidhartha, J.S., Yang, K., Kumar, J., Tripathy, S. and Samuelson, L. (2000) *Mater. Res. Soc. Symp. Proc.*, 600, 255.