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Effect of roasting on tocopherols of gourd seeds *(Cucurbita pepo)*

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RESUMEN

Efecto del tostado sobre los tocoferoles de semillas de calabaza *(Cucurbita pepo)*

Se ha estudiado el efecto del tostado a alta temperatura sobre la vitamina E en semillas de calabaza *Cucurbita pepo*. El tostado a 140°C durante 5 min indujo una hinchazón de la semilla con un aumento en el volumen del 43 %, y una pérdida de peso del 6,5 %. La semilla adquirió la apariencia deseada después del tostado. La actividad del agua en la semilla cruda fue de 0,544 y disminuyó durante el tostado a 0,105. Las semillas de *Cucurbita pepo* contenían un 51,0 % de grasa. El contenido de tocoferol de las semillas tostadas fue de 68mg/100g y el de las semillas no tostadas de 107mg/100g de aceite. El γ-tocoferol representó el 96% de los tocoferoles totales. La pérdida de tocoferoles totales durante el tostado fue del 36% siendo la má alta la del β-tocoferol con un 50%; la del α-tocoferol fue del 41% y la del γ-tocoferol del 36%.

PALABRAS-CLAVE: Calabaza - Cucurbita pepo - Tocoferol - Tostado - Vitamina E.

SUMMARY

Effect of roasting on tocopherols of gourd seeds *(Cucurbita pepo)*

The effect of roasting at high temperatures on the Vitamin E in hulled gourd seeds of *Cucurbita pepo* was studied. Roasting at 140°C for 5 min induced a swelling of the seed with an increase in volume of 43 %, and a weight loss of 6.5 %. The seed acquired the desired puffed-up appearance. The water activity of raw seeds was 0.544 and decreased during roasting to 0.105.

Cucurbita pepo seeds contained 51.0 % fat. Tocopherol content of roasted seeds was 68mg/100g oil and of non roasted seeds was 107mg/100g oil. γ-tocopherol represented 96% of the total tocopherols. Total tocopherol loss during roasting was 36%. β-tocopherol loss was the highest at 50%; for α-tocopherol it was 41% and for γ-tocopherol it was 36%.

KEY-WORDS: Cucurbita pepo - Gourd - Roasting - Tocopherol - Vitamin E.

1. INTRODUCTION

The Cucurbitaceae family consists of 90 genera and approximately 700 species. The Cucurbitaceae are characterised by long flexible stems, a crawling or climbing growth habit and fruits that differ widely in colour and shape, having a thick and impermeable skin protecting a juicy fibrous pulp.

The majority of the Cucurbitaceae used as food are found in five genera: *Citrullus* (water melons and wild colocynths), *Cucumis* (cucumbers, gherkins and melons), *Lagenaria* (gourds), *Sechium* (chayotte) and *Cucurbita.* The last one is the most important genus economically and is represented by four species: the musky pumpkin *Cucurbita maxima*, the gourd *Cucurbita moschata*, the gourd of Siam *Cucurbita ficifolia* and the gourd *Cucurbita pepo*.

The Cucurbitacae have long been cultivated not only for food but also for their medicinal properties. Particular medical properties have been attributed to each part of the fruit and the plant. In the south of France, a slice of gourd pulp (*Lagenaria*) mixed in one liter of water was used as laxative to treat intestinal diseases and to satisfy thirst. The seeds are noted as an effective remedy against Taenia, a parasitic worm that is paralyzed by the action of cucurbitine (3-amino-3-carboxypyrrolidine) contained in the skin of the seed (Armougom, 1998).

Increasingly, consumers want foods that not only taste good but are also good for their health. This is reflected in the demand for food products that have optimal bioavaliblity of essential fatty acids, vitamins, and antioxidants.

Vitamin E is an essential liposoluble vitamin which was discovered by Evans and Bishop in 1922. The term vitamin E is a general descriptor of tocopherols and all their derivatives which present the biological activity of the RRR α-tocopherol (Machlin, 1984; Tomassi and Silano, 1986). Recommended dietary allowances for vitamin E are around 10 to 30mg for an adult in good health (Veris Inc., 1993).

The main tocopherols are: α tocopherol (5,7,8-trimethyl), β tocopherol (5,8-dimethyl), γ tocopherol (7,8-dimethyl) and δ tocopherol (8-methyl). The basic structure of tocopherols is made up of two cycles and a side chain. In tocopherols, the side chain is saturated, but in tocotrienols, it contains three double bounds.

Tocopherols are antioxidant molecules and their primary task is to prevent the damage caused by free radicals on tissues. This is achieved by donating a hydrogen atom to a peroxide radical which results from the degradation of unsaturated

lipids and as a consequence tocopherols are minor but omnipresent components in membranes (Kamal-Eldin and Appelquist, 1996).

Tocopherols are particularly sensitive to heating at high temperatures (Barrera-Arellano *et al*., 2002). As a result, most tocopherols are lost or destroyed during the refining of vegetable oils. Most commercial vitamin E is thus prepared by chemical synthesis. In order to increase the shelf-life of food, the addition of antioxidants during food processing is common. However because of the possible toxic effects of synthetic antioxidants, industry is increasingly turning to natural antioxidants. Many authors consider that natural vitamin E is more active than the synthetic analogue because the chemical synthesis produces many isomers with less biological activity (Horwitt, 1986). A mixture containing 60% in weight of natural a, g and d isomers is largely used as an additive (Shimada *et al*., 2000).

α-tocopherol is the most easily absorbed by the intestinal wall. A high plasma content of α-tocopherol decreases the natural absorption of γ and δ-tocopherol (Huang and Appel, 2003). α-tocopherol has the highest antioxidant activity (Chunhieng, 2003). This activity decreases from α to δ-tocopherol (Dziezak, 1986). The tocotrienols are more effective antioxidants because they are unsaturated.

In blood and animal cells, α-tocopherol is the most abundant and represents 87% of the tocopherols in the plasma (Gonzales, 1990). ß and γ-tocopherol represent 2 and 10 % of plasmatic vitamin E respectively (De Leenheer *et al*., 1988).

The α isoform has many functions similar to the γ isoform, such as, for example, increasing the activity of superoxide dismutase in arterial plasma and cells. γ-tocopherol has some specific biological properties which are different from those of α-tocopherol. People who have high plasmatic concentrations of γ-tocopherol tend to get less cancer of the prostate, while those who have low levels tend to have more cancer of the higher aero-digestive tract (Jiang *et al*., 2001).

Along with selenium, present in *Cucurbita pepo* seed (0.3 ppm) (Kreft *et al*., 2002), vitamin E can act on the ageing of cells. This can be proven from the fact that the product resulting from the metabolism of g tocopherol plays an important role in the prevention of cardiac diseases and in the regulation of blood pressure by controlling the drainage of water and of different metabolites in the body.

The liver selectively exports α-tocopherol, using lipoproteins as carriers, to other tissues of the body. The liver eliminates other forms of vitamin E that the chromane cycle has not totally methylated (Schmidt and Nikoleit, 1993). One hypothesis is that the antioxidant activity of tocopherols in the body is linked to their chiral structure and to the mechanism of membrane transfer (Meydani *et al*., 1986). The RRR α-tocopherol is more readily accepted by the lipoproteins of the membranes than other forms such as SRR α-tocopherol (Ingold *et al*., 1987).

However, γ-tocopherol may be a more potent cancer chemopreventive than α-tocopherol (Campbell *et al*., 2003). Thus, it is considered that the structure of γ-tocopherol confers on it a better stability against oxidation at high temperatures and consequently its loss is less and its effectiveness against oxidation is greater than α-tocopherol (Cheng *et al*., 1987).

This study is related to the effect of roasting at high temperature (140°C) on vitamin E present in the oil of hulled gourd seed *Cucurbita pepo* Lady Godiva variety.

2. EXPERIMENTAL

2.1. Samples

The hulled seed of *Cucurbita pepo* Lady Godiva variety is derived from a mutant closely related to the variety Styriaca, which is widely grown in North-East Europe, particularly in Austria. In France, our samples were produced by the company "Les fleurs de Jaussely" (81470 Aguts, France).

The hulled character of the gourd seed lends itself to efficient analysis as losses associated with process manipulation are reduced.

For each experiment, 75 g of seeds were roasted at 140°C for 5min, 24h before the extraction of oils. To protect tocopherols, the roasted seeds were hermetically sealed in plastic packaging.

2.2. Moisture content and water activity

Analysis of the moisture content was carried out by drying 4 lots of 10 g of seeds at 105 ± 2°C for 24h. The water activity was determined with an Aqualab A_w meter. The plastic cap of the Aqualab was filled with ground seeds and the analysis was done in two replicates at 24 +/-1°C. The water activity (A_w) is a good indicator of the preservation potential of the product and its microbiological stability with time. Below a threshold of A_w = 0.62, there is usually no notable fungal development (Guiraud, 1998).

2.3. Extraction of oil

The gourd seeds were crushed using a domestic crusher and dried in an oven at 105°C for 24 h. In order to obtain a sufficient quantity of oil, we used 20 g of dried seeds. The extraction was done by Soxhlet with petroleum ether for 6 hours.

2.4. Analysis and quantification of tocopherols by HPLC

Tocopherol quantification (Figure 1) was carried out using Thermo Separation Products (TSP) with a P1000XR pump, using 4 external tocopherol standards (Tocopherol set, Calbiotech™). The standard solutions were prepared every day and

were preserved in HPLC grade hexane in flasks without actinic activity. These tests were repeated 4 times on each oil before and after roasting to obtain the most representative possible equations calculated by linear regression (Table 1). Standard samples were made at concentrations of 1, 2, 2.5, and 5μg/mL, which is the maximum limit of the detection threshold of the fluorometer (TSP FL3000). 2mg of oil were dissolved in 25mL hexane for HPLC, then analyzed directly by HPLC.

A hypersil silica column (250mm x 4,6 x 5μm) coupled with a 100μL injection loop was used for separation. The mobile phase was a mixture of hexane and dioxane 97/3 v/v (flow: 1mL/min). The detection of tocopherols was done using a fluorometer, which made it possible to inject and proportion the oil directly in hexane. Excitation was set at 290nm, thus inducing fluorescence emission by tocopherols at 330nm.

Table 1
Correlations between peak areas and the concentrations of tocopherols

Tocopherols	Equations	R^2
α-tocopherol	$y = 28651\ x^2 + 1.10^7\ x$	0.9941
β-tocopherol	$y = -49636\ x^2 + 1.10^7\ x$	0.9995
γ-tocopherol	$y = 1.10^7\ x$	0.9898
δ-tocopherol	$y = 2.10^6\ x^2 + 2.10^7\ x$	0.9943

2.5. Specific volume

During heating, the pumpkins seeds swell. To measure this swelling, a graduated test-tube was used. The volume occupied by a measured quantity of 75g of seeds was measured in 3 replicates before and after treatment.

2.6. Seed roasting

Roasting was carried out in a coffee roaster (Probat™ Type BRZ 2) at 140°C for 5min. Seventy five grams of raw seeds were placed in a rotary metal drum (Diameter 11 cm, length 18 cm, 1.7 Kw) which was heated by electrical resistance. The seeds were simultaneously roasted by contact with the metal plate, and also by the hot air in the drum. Under these conditions, the seeds obtained are generally appreciated by a panel of tasters.

3. RESULTS

3.1. Characterisation of roasted seeds

The water activity A_w of raw seed was 0.544 +/- 0.002. A_w decreased considerably during roasting at 140°C for 5 min to only 0.105 +/- 0.002. Thus, in both cases, the seeds were stable with respect to potential growth of micro-organisms (Guiraud, 1998). The roasting of seeds will therefore increase sanitary quality by increasing microbiological stability with time.

Specific volume as well as the weight of the seeds were measured before and after roasting. We calculated the swelling and the loss in weight due to the drying of seeds at 140°C for 5 min. Roasting at 140°C for 5 min caused an increase in volume of almost 43.2 +/-4.4%, and a loss of weight of 6.4 +/- 0.24% which can be explained by the drying of seeds. The loss in density was - 34.6+/-1.8%. The seed thus acquired the desired puffed-up appearance**.**

3.2. Tocopherol analysis

- Calibration

The results were obtained from four repetitions. An average curve of peak areas relating to the four selected concentrations was then plotted using a linear regression calculation. The coefficients of correlation R^2 were close to 1 (Table 1).

- Analysis of *Cucurbita pepo* seeds

Cucurbita pepo seeds contained 51.0 +/- 1% of fat, which is comparable to the data given by Ucciani (1995).

Figure 1 shows the analysis of tocopherol standards and Figure 2 the analysis of the tocopherols from roasted seeds at 140°C for 5min. The seeds had a tocopherol content of 107.4 +/- 2.9 mg/100g oil, similar to other oleaginous seeds such as soybean (98mg/100g oil) and maize (81.6mg/100g oil). This content was higher than those of olive (11mg/100g oil), palm (38mg/100g oil) and sunflower (70mg/100g oil) (Kamal-Eldin and Andersson, 1997). Murkovic and Pfannhauser (2000) analysed 15 samples of pumpkin seed oil and found a vitamin E content of between 100 and 600 mg/100g oil.

γ-tocopherol, which represented 89.7% of the total amount of tocopherols detected in the

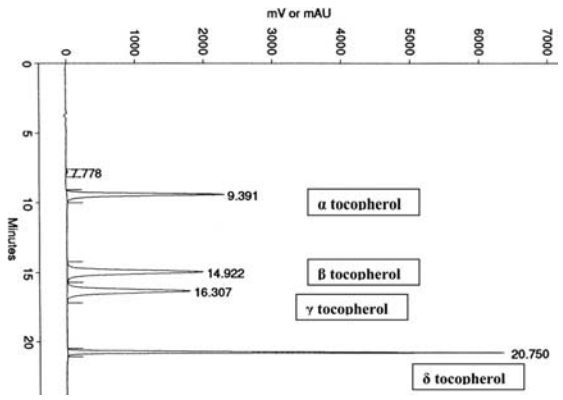

Figure 1
HPLC analysis of standard tocopherols

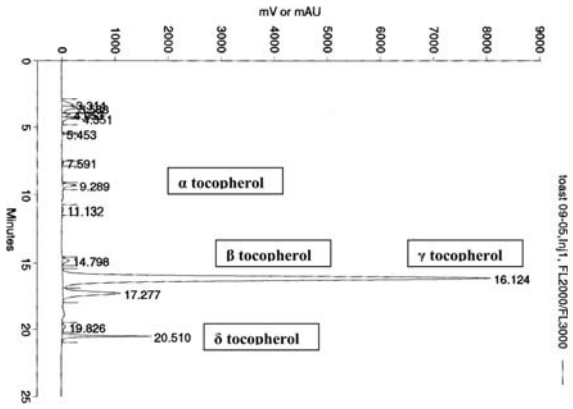

Figure 2
HPLC analysis of tocopherols from roasted *Curcubita pepo* seeds at 140°C for 5min

Cucurbita pepo seed, was the most significant, just as it is in many other vegetable oils (Table 2 and Figure 2).

The total tocopherol contents of roasted seeds (68.2 +/- 5.9 mg/100g oil) compared to raw seeds (107.4 +/- 2.9 mg/100g oil) indicates a loss (107.4 - 68.2)/107.4 of total tocopherol content of 36% (Table 3).

Roasting caused a loss in all tocopherols (Table 3). But, the loss was not the same for all the tocopherol molecules. The loss of β-tocopherol was the highest at 50%, for α-tocopherol it was 41%, while for γ-tocopherol it was 36%. For δ-tocopherol, the loss was less at 25%.

Antioxidant capacity decreases from α-tocopherol to γ-tocopherol (Dziezak, 1986). γ-tocopherol is thus more resistant to oxidation due to ambient air. In the roasted oil, its percentage increased and stayed very high at 90% (Tab. 3), whereas α-tocopherol decreased most notably from 7.1 to 6.7%.

4. CONCLUSIONS

Tocopherols have already been the subject of many studies describing their content in plant materials and some papers describe the effect of physical treatments like frying at 180°C (Barrera-Arellano *et al*., 2002) and subjecting them to microwave heating (Yoshida *et al*., 1991; 1999), but none on the effect of roasting.

Our results showed that roasting at 140°C for 5min affected the content of tocopherols by preferentially reducing (36%) those with the greatest antioxidant capacity (α-tocopherol: loss of 41%) thus concentrating δ (loss of 25%) and γ-tocopherols (loss of 36%), which have the least antioxidant capacity (Gottstein and Grosch, 1990). γ-tocopherol which is less active, is more stable at high temperatures than α-tocopherol. It was thus used to stabilize the formation of non volatile

Table 2
Composition (mg/100g oil) of tocopherols of *Curcubita pepo* compared with various vegetable oils (Kamal-Eldin and Andersson, 1997)

Oils	α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total
Curcubita pepo	7.69	1.35	96.4	1.99	107.4
Soybean	11.6	1.7	57.8	26.3	97.4
Maize	22.2	0.1	57.0	2.3	81.6
Sunflower	67.1	2.3	0.4	0	69.8
Olive	9.6	0.6	1.2	0	11.4

Table 3
Comparison of the concentrations (mg/100g of oil) of tocopherols present in the oil of raw and roasted *Curcubita pepo* seeds at 140°C for 5min

	Raw seeds mg/100g oil	Roasted seeds %	mg/100g	oil%
α-tocopherol	7.69 +/- 0.98	7.15	4.56 +/- 0.64	6.68
β-tocopherol	1.35 +/- 0.22	1.26	0.67 +/- 0.15	0.98
γ-tocopherol	96.4 +/- 10.62	89.74	61.53 +/- 22.81	90.16
δ-tocopherol	1.99 +/- 0.07	1.85	1.49 +/- 0.11	2.18
Total	107.4 +/- 2.97	100	68.25 +/- 5.93	100

compounds during the frying of potato chips (Neff *et al*., 2003). Barrera-Arellano *et al*. (2002) showed the same tendency with a quicker loss of α-tocopherol than the other tocopherols and a better preservation of δ-tocopherol at frying temperatures.

After treatment at 140°C for 5min, the amount of tocopherols in roasted seeds remained significant with 68mg per 100g of seeds (Table 3). Yoshida *et al.* (1992) showed that the stability of these compounds was connected to the influence of fatty acids present in the lipid fraction. When vegetable oils were heated, the levels of free fatty acids increased through hydrolysis of the triglycerides. They also showed that the accumulation of short chain fatty acids which are saturated was linked to the reduction of tocopherols in coconut and palm oils. The lipid fraction of *Cucurbita pepo* seed contains essentially long polyunsaturated fatty acids (62% of C18:2) (Younis *et al*., 2000) which protect the tocopherol fraction against oxidation by scavenging free oxygen.

In conclusion, the most sensitive tocopherols are those that are more susceptible to oxidation. However, the differences observed in the loss of tocopherols would be in favour of their oxidative role over their degradation at roasting temperature (140°C). Barrera-Arellano *et al*. (2002) showed the opposite tendency for the loss of tocopherols during frying at high temperatures and they estimated that the loss is due to the chemical degradation of tocopherol molecules. It could be explain by the difference in the experiment duration. In fact, Barrera-Arellano *et al*. (2002) studied frying for 2 to 10 hours, which could explain the degradation. In our case, roasting was done only for 5 min and the oxidation, more than the chemical degradation, is certainly the reason for the loss in tocopherols.

It would be interesting to conduct a further study on the combined effect of oxidation on fatty acids and tocopherols and in particular the pro-oxidative or co-oxidative effect of tocopherols when they are subject to roasting temperature as suggested by Kamal-Eldin and Andersson (1997) who proposed the hypothesis that α-tocopherol could be involved in pro-oxidation in oils rich in 18:3 fatty acid. Another instructing technology could be the roasting by microwave tested by Yoshida *et al.* (1999) who showed a good stability of all tocopherols for 6 to 8 min and a preservation of 80% of tocopherols after 20 min of roasting.

REFERENCES

Armougom PR. 1998**.** Etude de la fraction lipidique des graines de Cucurbitacées tropicales des genres *Lagenaria*, *Luffa, Momordica*. Thèse de docteur es Sciences, Université de la Réunion.

Barrera-Arellano D, Ruiz-Mendez V, Velasco J, Marquez-Ruiz G, Dobarganes C. 2002. Loss of tocopherols and formation of degradation compounds at frying temperatures in oils differing in degree of unsaturation and natural antioxidant content. *J. Sci. Food Agric.* **82**, 1696-1702.

Campbell S, Stone W, Whaley S, Krishnan K. 2003. Development of gamma-tocopherol as a colorectal cancer chemopreventive agent. *Crit. Rev. Oncol. Hematol*. **47** (3) 249-259.

Cheng SC, Burton GW, Ingold KU, Foster DO. 1987. Chiral discrimination in the exchange of alpha-tocopherol stereoisomers between plasma and red blood cells. *Lipids* **22**, 469-473.

Chunhieng T. 2003. Développement de nouveaux neutraceutiques à partir de graines et fruits d'origine tropicale : Application à la Noix du Brésil *Bertholletia excelsa* et au fruit de Cambodge *Morinda Citrifollia*. Thèse de Docteur es Sciences, Université de Nancy.

De Leenheer AP, Nelis HJ, Lambert WE, Bauwens RM. 1988. Chromatography of fat-soluble vitamins in clinical chemistry. *J. Chromatogr*. **429**, 3-58.

Dziezak JD. 1986. Preservatives, antioxidants. The ultimate answer to oxidation. *Food Technol*. **9**, 94-102.

Gonzales MJ. 1990. Serum concentrations and cellular uptake of vitamin E. *Med. Hypotheses* **32**, 107-110.

Gottstein T, Grosch W. 1990. Model study of different antioxidant properties of alpha- and gamma-tocopherols in fats. *Fett. Wiss. Technol*. **92***,* 139-144.

Guiraud J. 1998. In "Microbiologie alimentaire - Rappels de microbiologie générale" Ed. Dunod, France.

Horwitt MK. 1986. The promotion of vitamin E. *J. Nutr*. **116**, 1371-1377.

Huang HY, Appel LJ. 2003. Supplementation of diets with alpha-tocopherol reduces serum concentrations of gamma- and delta-tocopherol in humans. *J. Nutr*. **133***,* 3137-3140.

Ingold KU, Burton GW, Foster DO. 1987. Biokinetics of and discrimination between dietary RRR- and SRR-alpha-tocopherols in the male rat. *Lipids* **22***,* 163-172.

Jiang Q, Christen S, Shigenaga MK, Ames BN. 2001. Gamma tocopherol, the major food in the US diet, deserves more attention. *Am. J. Clin. Nutr*. **74**, 714-722.

Kamal-Eldin A, Andersson R. 1997. A multivariate study of the correlation between tocopherol content and fatty acid composition in vegetable oils. *J. Am. Oil Chem. Soc*. **74**, 375-376.

Kamal-Eldin A, Appelquist LA. 1996. The chemistry and antioxidant properties of tocopherols and tocotrienols. *Lipids* **31**, 671-701.

Kraft I, Stibilj V, Trkov Z. 2002. Iodine and selenium contents in pumpkin (*Cucurbita pepo* L.) oil and oil-cake. *Eur. Food Res. Technol*. **215**, 279-281.

Macklin LJ. 1984. "Vitamin E" En L.J. Machlin (Ed.) *Handbook of vitamins: nutritional biochemical and clinical aspects.* Marcel Dekker Inc, New York.

Meydani M, Macauley JB, Blumbergn JB. 1986. Influence of dietary vitamin E, selenium age on regional distribution of alpha-tocopherol in the rat brain. *Lipids* **21**, 786-791.

Murkovic M, Pfannhauser W. 2000. Stability of pumpkin seed oil. *Eur. J. Lipid Sci. Technol.* **102** (10) 607-611.

Neff WE, Warner K, Ellern F. 2003. Effect of gamma-tocopherol on formation of non volatile lipid degradation products during frying of potato chips in triolein. *JAOCS*. **80 (**8) 801-806.

Schmidt K, Nikoleit DA. 1993. Vitamine E naturelle, vitamine E synthétique : en quoi diffèrent-elles ? *STP Pharma pratiques* **3 (**4) 259-273.

Shimada Y, Nakai S, Suenaga M, Sugihara A, Kitano M, Tominaga Y. 2000. Facile purification of tocopherols from soybean oil deodorizer distillate in high yield using lipase. *JAOCS*. **77**, 1009-1013.

Tomassi G, Silano V. 1986. An assessment of the safety of tocopherols as food additives. *Food Chem. Toxicol*. **24**, 1051-1061.

Ucciani E. 1995. In "Nouveau dictionnaire des huiles végétales". *Tec et Doc Lavoisier*, Paris, France.

Veris, Anonymous. 1993. "Vitamin E Research Summary". *Veris Inc.*, June.

Yoshida H, Hirooka N, Kajimoto G. 1991. Microwave heating effects on relative stabilities of tocopherols in oils. *JAOCS.* **68**, 1042-1046.

Yoshida H, Tatsumi M, Kajimoto G. 1992. Influence of fatty acids on tocopherol stability in vegetable oils during microwave heating. *JAOCS.* **69**, 119-125.

Yoshida H, Takagi S, Mitsuhashi S. 1999. Tocopherol distribution and oxidative stability of oils prepared from the hypocotyl of soybeans roasted in a microwave oven. JAOCS. **76**, 915-920.

Younis Y, Ghirmay S, Al-Shihry S. 2000. African *Cucurbita pepo*; properties of seed and variability in fatty acid composition of seed oil. *Phytochemistry* **54**, 71-75.

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