Effect of Gamma Radiation on Anthocyanin and Flavonol Pigments in Cranberries (Vaccinium macrocarpon Ait.)

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Abstract. The effectiveness of gamma radiation as an enhancer of anthocyanin and flavonol pigment synthesis in cranberries was determined. Three different maturities of cranberries, based on their degree of coloration, and radiation levels of 150 and 300 krad were employed. The changes in the anthocyanin and flavonol pigments were measured quantitatively at regular intervals during storage. Radiation had a beneficial effect on the pigmentation of full-red cranberries and resulted in a significant increase in the anthocyanin and flavonol pigment contents. Effects on the less colored berries were not as great and in some cases flavonoid synthesis was reduced. The radiation induced changes were strictly quantitative in nature and there were no qualitative changes in the anthocyanins and flavonols. The visual effects of radiation on cranberries were minor softening and a stimulation of pigment production in the endocarp area of the fruit, resulting in internal coloration of the fruit. It was concluded that gamma radiation has an effect on the biosynthesis of the pigments involved and that the maturity stage of the cranberries was the controlling factor in determining the degree of response to radiation treatment. A possible mode of action of radiation on flavonoid synthesis was postulated.

The most important attribute of the cranberry is its bright red attractive coloration. This is due primarily to the 4 major red anthocyanin pigments as identified by Sakamura and Francis (29) and Zapsalis and Francis (35), and to a minor contribution by the yellow flavonol pigments identified by Puski and Francis (25).

The intensity of color in the cranberry is of prime importance in determining the ultimate use of the crop and the rapid increase in the production of cranberry cocktail has resulted in the need for a larger supply of well colored berries. When the fruit is left on the vines for a longer time, more pigment is produced resulting in a better color. However, due to the inconvenience and sometimes the impracticability of a late harvest, often the fruit must be harvested before optimum color maturity is reached. Any means of increasing the rate of anthocyanin and flavonol synthesis and concn of these pigments prior, or subsequent, to harvest would be of economic benefit to the grower and the processor.

The use of preharvest sprays of various chemicals showed some promise as a means of promoting early red color development in cranberries. Shawa and Ingalsbe (30) found malathion (as an emulsifiable concentrate) caused a significant increase in anthocyanin formation in cranberries and similar results have been reported by Eck (7). The color enhancement occurred within 4 days of treatment and the difference between the treated and untreated berries remained throughout the harvest period. Eck (8) reported that malathion and ethephon increased pigment content while SADH delayed anthocyanin development in cranberries. Devin et al. (5) also increased pigment content with malathion treatment, while reporting indole-3-acetic acid to be ineffective as an enhancer of cranberry anthocyanin. Eaton et al. (6) reported an increased anthocyanin content resulting from sprays of malathion for cranberries in 7 commercial growing regions of North America.

In recent years, there has been a growing alarm over the extensive use of chemicals on our food materials and efforts are being made to reduce the use of these chemicals. Therefore, if an alternative non-chemical method of anthocyanin (and flavonol) pigment enhancement in cranberries was available it could be of potential value. The use of gamma radiation for increased pigmentation and coloration in fruits and vegetables has received considerable attention in the last few years.

Maxie et al. (21) first noted the increase in red color development in nectarines and peaches subjected to 200 krad of radiation. They showed that the unirradiated fruit never developed as much anthocyanin as the irradiated fruit and attributed the higher pigmentation to a radiation induced response. Dennison (4) reported increased anthocyanin content in peaches and nectarines, following an immediate loss after treatment. Bramlage and Couey (2), also working with peaches and nectarines, found yellow and red colors developing sooner and more fully after irradiation doses of 200 krad. However, these authors reported a reduced and delayed coloration in plums, pears, and tomatoes.

In strawberries, some authors reported that radiation decreased anthocyanin content (18) while others (15,23,17) found no change or an increase. More recently Belli-Donini and Stornaiuolo (1) stated that radiation at 200 krad slightly increased the anthocyanin content in “less ripe” fruit, but slowed down pigment synthesis in “riper fruit.”

Sparrow et al. (39) found an increase in the concn of anthocyanin pigments in leaves of Rumex and other plant genera, with the increase being as great as 20 times that of the untreated plants. They concluded that enhanced pigment formation is a common response of higher plants to ionizing radiation.

We determined the effects of gamma radiation from Cobalt-60 on the anthocyanin and flavonol pigments of cranberries. Post-harvest radiation was employed to try to increase pigmentation of the fruit. Also the relationship of the individual anthocyanins and flavonols was investigated to determine if the changes produced were quantitative or qualitative in nature.

Materials and Methods

Cranberries. The cranberries we used were obtained from Ocean Spray Cranberries, Inc., Middleboro, Massachusetts. All fruit was of first quality obtained within 24 hr of harvest.

Three maturities of cranberries, based on their degree of pigmentation, were used, white-pink, half-red, and full-red. The berries were hand sorted into these categories and put into 5-lb. polyethylene bags for radiation treatment. The fruit was put into small polyethylene bags (perforated with 56 holes for gas.

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2 Present address: Research Division, Atomic Energy of Canada Limited, P.O. Box 6300, Ottawa, K2A 3W3, Ontario. The senior author wishes to express his gratitude to Atomic Energy of Canada Limited whose educational leave policy enabled him to pursue this investigation at the University of Massachusetts. Appreciation is also expressed to Ocean Spray Cranberries, Hanson, Mass. for financial support of this work.

The cranberries were irradiated and radiation treatment included a preliminary 35.7\,36.3 \,35.9 \,33.4 \,33.4 \,62.5a \,61.9 \,34.2 \,33.4 \,57.7 \,57.7 \,57.7 Flavonol (mg/100 g) 150 300
and radiation treatment, and subdivided into various temp groups. Samples for the initial readings were immediately put into the freezer at -20°F. At regular intervals, samples were removed from the various storage temp and placed in the freezer for subsequent pigment analyses. All material in the freezer was stored in double walled polyethylene bags to prevent desiccation of the samples and possible errors due to wt change.

Three different trials covering 3 crop years were conducted with storage periods of 35, 60, and 56 days, respectively. The storage temp used were 34°C, 38°C, 40°C, and 50°F depending on the particular trial involved.

‘Early Black’ cranberries were used in Trials I and III, while ‘Howes’ was used in Trial II.

**Radiation treatment.** The cranberries were irradiated immediately after color sorting. In Trial I the radiation treatment was done at the Marine Products Development Irradiator, Gloucester, Massachusetts, and in Trials II and III at the U.S. Army Natick Laboratories, Natick, Massachusetts. In all cases the radiation source was Cobalt-60.

The conditions for radiation treatment were as follows: In Trial I the dose rate was 82 krad/min, and the total dose was 150 and 300 krad minimum with a ratio of 1.04/1.00 rad. Temperature during irradiation was 75°F. In Trials II and III the dose rate was 60 and 50 krad/min, respectively, with the total doses being 150 and 300 krad min plus up to 12% and 12.5%, respectively. The cranberries were contained in punctured polyethylene bags for treatment and irradiation was carried out at room temp.

**Quantitative analyses of anthocyanins.** The total anthocyanin content of the cranberries was determined using the quantitative techniques of Fuleki and Francis (12) using photodensitometry. However, the improved solvent system of Fuleki (10) of butanol: formic acid: water (100:25:60) was used in place of the recommended in the earlier work.

**Quantitative analyses of flavonols.** The total flavonol content of the cranberries was determined as mg quercetin/100g using the quantitative spectrophotometric technique of Lees and Francis (16). The flavonol and anthocyanin contents were measured on the same cranberry extract.

The individual flavonol pigments were determined quantitatively using the TLC technique of Lees and Francis (16) and the samples employed in the densitometric measurement of the individual anthocyanins after these studies were completed.

The method whereby the qualitative TLC technique was made quantitative was as follows. Cellulose TLC plates were prepared and developed as before (16). The flavonol extract of the cranberry extract was prepared by solvent-solvent extraction and taken up in 500 ml of 95% ethanol:1.5N HCl (85:15) The extract was spotted at the rate of 75 lambda per TLC plate. Five plates were spotted and developed 2-dimensionally. The separated flavonols were detected under long-wave UV with ammonia vapors and the pigments collected by quantitatively scraping off the cellulose containing the pigments. The flavonols were eluted off the cellulose with 10 ml of methanol through a fine porosity sintered glass funnel, with a millipore filter added (Millipore Filter Corp., Bedford, Mass., Cat. No. BCWP02500), into a test tube. The methanol was evaporated off using a stream of air and then 1.5 ml of 95% ethanol:1.5N HCl were added to dissolve the pigment.

The optical density (O.D.) was measured at 374 nm, with distilled water as a blank, in quartz cuvettes with 1 cm pathlength and vol of 1.5 ml. The ratios of the flavonol pigments to each other in cranberries was calculated by the following formula:

\[
\% \text{ No. 1 Flavonol} = \frac{\text{O.D. Flavonol No. 1 \times 100}}{\text{Total O.D.}}
\]

and so on for each of the flavonols. A blank sample was obtained by scraping cellulose off the TLC plate in an area which had been exposed to both solvents but which contained no flavonols.

**Results and Discussion**

**Effect of gamma radiation on total anthocyanin and flavonol pigment content.** The radiation trials included a preliminary trial to determine if radiation had any effect on cranberry pigmentation and a 2nd trial to elucidate the optimum conditions for these effects. Three maturities of berries were chosen to determine if the effects varied with maturity. The storage temp included a temp at which pigment synthesis is known to be favored (i.e., 50°F) and a lower temp at which pigment production occurs at a much slower rate (34°F).

The results of Trials I and II indicated that radiation treatment of cranberries did have an effect on cranberry pigmentation with this effect varying with fruit maturity. Radiation had no beneficial effect on white-pink cranberries and resulted in a decreased flavonoid synthesis as compared to the control. For the half-red category of fruit the irradiation had no beneficial effect on pigmentation with the pigment content of treated and untreated fruit being essentially the same at all times. Radiation increased pigmentation of fully-mature, full-red fruit. At storage temp of 34°F and 40°F, the irradiated samples showed a more rapid rate of flavonoid synthesis during the early part of the storage period than at later storage periods. At the end of the studies the pigment contents of the control and treated fruit were approximately the same. The rate of increase was greater for 300 krad than 150 krad and for 40°F then 34°F. No advantage due to radiation treatment was found at 50°F.

There was no change in the ratio of the anthocyanin and flavonol pigments in the cranberries (all maturities), and no shift from the flavonols to the anthocyanins or vice-versa occurred as a result of radiation. This indicates that radiation exerts its effect at a metabolic point prior to that of the synthesis of these pigments and thus affects each pigment the same.

On the basis of the above, only full-red cranberries were employed in Trial III with storage temp of 34°F and 40°F. Triplicate samples were analyzed at each storage interval to allow a statistical evaluation of the effects of radiation by means of the “t” test.

At 34°F, the irradiated samples exhibited a greater anthocyanin and flavonol content at all sampling intervals except for the final one of 56 days (Table 1). After 14 days, the anthocyanin content of the 150 krad fruit was significantly higher (5% level) than the control, while the 300 krad sample achieved this level of significance after 28 days, for both anthocyanin and flavonol content. In comparison with the initial anthocyanin content of 57.7 mg/100g, the controls

<table>
<thead>
<tr>
<th>Storage time in days</th>
<th>Dose level (krad)</th>
<th>Anthocyanin (mg/100 g)²</th>
<th>Dose Level (krad)</th>
<th>Flavonol (mg/100 g)²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>150</td>
<td>300</td>
<td>0</td>
</tr>
<tr>
<td>Initial</td>
<td>57.7</td>
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<td>57.7</td>
<td>33.4</td>
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<td>61.9</td>
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<td>61.2b</td>
<td>62.1</td>
<td>64.9b</td>
<td>34.6c</td>
</tr>
<tr>
<td>42</td>
<td>60.5</td>
<td>61.9</td>
<td>62.6</td>
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</tr>
<tr>
<td>56</td>
<td>62.9</td>
<td>62.0</td>
<td>62.6</td>
<td>36.9</td>
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</table>

²The figures for pigment content represent the means of 3 analyses.

YMeans not followed by a common letter are significantly different at the 5% level.
The effect of gamma radiation on the pigment content of full-red cranberries at 40°F.

Table 2.

<table>
<thead>
<tr>
<th>Storage time in days</th>
<th>Dose level (krad)</th>
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<td>0</td>
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</tr>
<tr>
<td></td>
<td>300</td>
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<tr>
<td>42</td>
<td>66.6d</td>
<td>63.4de</td>
</tr>
<tr>
<td>56</td>
<td>69.4f</td>
<td>64.8f</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Anthocyanin (mg/100 g)</th>
<th>Flavonol (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>57.7</td>
<td>57.7</td>
</tr>
<tr>
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</table>

2The figures for pigment content represent the means of 3 analyses.

The results obtained in the studies of the individual anthocyanin pigments. These findings are in agreement with those of Maxie et al. (21) on peaches and Sparrow et al. (32) on leaves, who found no change in the absorption spectra of the anthocyanin pigments after irradiation, only a greater amount of those normally present. Rogachev (28) reported that cyanin synthesis was inhibited in strawberries by irradiation while pelargonin synthesis was unaffected. However, the latter study used a dose level of 600 krad while the previous mentioned studies were at dose levels of 200 krad, and 24 krad, respectively. It is thought that the higher dose used (28) accounts for the partial destruction of anthocyanin synthesis.

The effect of gamma radiation on the individual anthocyanin pigment content of cranberries.

Table 3.

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Peak area^2</th>
<th>% Individual anthocyanin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peonidin-3-arabinoside</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>Cyanidin-3-arabinoside</td>
<td>66</td>
<td>77</td>
</tr>
<tr>
<td>Peonidin-3-galactoside</td>
<td>112</td>
<td>131</td>
</tr>
<tr>
<td>Cyanidin-3-galactoside</td>
<td>124</td>
<td>140</td>
</tr>
</tbody>
</table>

The results with myricetin-3-digalactoside were not obtained since not enough of this material was available from the small number of TLC plates and sample size used. However, since values for each of the other flavonols were determined it may be assumed that this flavonol behaves in a similar manner. The aglycones were grouped for measurement and their combined O.D. was not included in the calculation of the individual flavonols since they are believed to be artifacts of the method used (16).

The results indicate that radiation had no effect on the ratios of the flavonol pigments in cranberries.

The results obtained in the studies of the individual anthocyanin and flavonol pigments ratios suggest that the action of radiation on the biosynthesis of these pigments occurs at an earlier metabolic point than the formation of these pigments and also lends credence to the theory mentioned earlier that
radiation causes an increased rate of flavonoid biosynthesis rather than a total greater biosynthesis of the flavonoids.

Visual evaluation of radiation induced changes in cranberries.

The visual changes observed were the same for all experiments. The white-pink fruit did not exhibit any increase in internal or external coloration for the irradiated berries, while the control berries did acquire more skin color as a result of anthocyanin synthesis. Radiation resulted in a softening of the fruit with the degree increasing as the dose level and storage temp increased, but by the conclusion of the trials there was very little difference between the control and irradiated fruit. Half-red cranberries underwent some visual changes as a result of radiation treatment and these were similar to those outlined below for the full-red category of fruit but of a smaller degree.

Full-red cranberries showed very little external differences between the control and irradiated samples after long storage periods, regardless of temp. Some softening occurred, as outlined above, however this is not anticipated to be a problem since the radiation treatment is recommended for full-red cranberries for an increased pigment production for use in processed cranberry products where a high pigment content is required and the integrity of the berries is of little importance.

The internal changes in the full-red cranberries involves the coloration of the fleshy tissues. Initially, no internal coloration was present in the fruit and only the exocarp was red. As the storage time progressed, the irradiated fruit developed a strong red coloration in the endocarp area (after approx 14 days) giving evidence that anthocyanin synthesis was occurring in this area. A section perpendicular to the first was made through the carpel and stem ends of the berry and revealed that the mesocarp was still white around the red colored endocarp indicating the absence of diffusion. Internal coloration became more intense with time and was greater as the radiation dose and storage temp increased. Eventually diffusion of the pigment from the endocarp and exocarp to the mesocarp did occur and resulted in a bright red coloration throughout the berry. The appearance of the red endocarp coincided with the increased anthocyanin pigment content in Trial III. The results indicate that the radiation treatment stimulated the earlier development of the anthocyanins (and presumably the flavonols) in the endocarp area of the fruit leading to an increased pigment content as compared to the controls. Some synthesis normally occurs in this area but only after much longer storage periods, (i.e., in this research it required 42 days).

Although radiation treatment of full-red cranberries resulted in statistically significant increases in the anthocyanin and flavonol pigments, it is necessary to examine the actual magnitude of the changes and to compare this to the anthocyanin enhancement resulting from chemical treatments. Eck (8) reported a significant increase in cranberry anthocyanins as a result of ethephon treatment, but the actual pigment increase was only 1.8 mg/100 g of cranberries over a total anthocyanin content of 7.7 mg/100 g for the control. Devlin et al. (5) reported a 2.5-3.5% increase with indole-3-acetic acid after 2 weeks storage at ambient temp when applied up to 3 weeks before harvest, with an actual pigment increase of 1.6 mg/100 g.

Malathion is the main chemical recommended for pigment enhancement and Eck (7) found an increase of 6, 8 and 6 mg/100 g respectively (over a control of 32 mg/100 g at the end of the study) when applied at a rate of 2.5 lb./acre and 1 1/2, 2 and 3 weeks before harvest. Eck (8) later reported that malathion, applied at the same rate, 2 weeks before harvest gave an increase of 12.3% in pigment, which was not significant at the 5% level. This is an actual increase of 1.0 mg/100 g over a control value of 7.7 mg/100 g. At the rate of 1600 ppm applied 2 weeks before harvest, as recommended by Devlin et al. (5), malathion gave an increase of 9.3 mg/100 g of cranberries over the control fruit with an anthocyanin content of 42 mg/100 g. Also, they found no enhancement of color development for stored cranberries with malathion and the closer the application of the chemical to the time of harvest, the less beneficial was the effect on anthocyanin production. Eaton et al. (6) found a variable effect of malathion on different varieties of cranberries with the results obtained for application 3 and 2 weeks prior to harvest, respectively, being: 'Early Black' 8.6 and 7.7 mg/100 g over a control of 38.6 mg/100 g; 'Montmorency' 5.1 and 3.9 mg/100 g over a control of 44.0 mg/100 g; and 'Searles' 0.4 and 2.4 mg/100 g over a control of 22.7 mg/100 g. (The negative result being attributed to rain at the 3 week period).

Treatment of full-red cranberries with gamma radiation at 300 krad and subsequent storage at 40°F for 14 days gave an increase in anthocyanin of 5.9 mg/100 g over a control value of 60.7 mg/100 g. This increase compares favorably with those reported for the chemicals and it can be said that radiation is effective as a post-harvest anthocyanin enhancer. In evaluating the effectiveness of radiation and chemicals in promoting anthocyanin development, there are factors which must be taken into consideration. In all of the chemical studies the greatest effects were obtained with the earliest application of the chemical; i.e., with the most immature fruit, and the effectiveness dropped off with maturation to a point where the increases were less than those obtained with the chemicals. Also, in no case, had the cranberries used in a chemical study achieved the maturity of those used in the radiation trials, as shown by the low anthocyanin content of the controls at the end of the experiments; i.e., 7.7 to 44.0 mg/100 g. At the same time the effectiveness of radiation dropped off as the maturity of the fruit decreased. Thus it would appear that radiation and chemicals are effective at the opposite ends of the maturity scale for cranberry pigmentation.

Possible mode of action of radiation on cranberry pigment synthesis. A possible mode of action of radiation on flavonoid synthesis to account for the stimulation of a greater rate of flavonoid synthesis was postulated. Phenylalanine is generally accepted as a precursor in flavonoid biosynthesis and as one of the main sources of flavonoids (13,14). However, recent work by Swain and Williams (33) has indicated that L-phenylalanine may not be an obligate precursor in the biosynthesis of flavonoids and they suggest that it may act as a reserve substance which is called into play under conditions where a rapid synthesis of flavonoids is induced. This is supported by the earlier work of Zapolmetov and Bukhleeva (34) who proposed that plants had a flavonoid synthetic pathway which was not coupled with the amination of intermediate products. Also, the enzyme phenylalanine-ammonia lyase (PAL) which acts in flavonoid synthesis by catalyzing the irreversible deamination of L-phenylalanine into trans-cinnamic acid and thus into flavonoid synthesis (27), has been shown to require an increased activity to permit high rates of flavonoid synthesis (3). Thus if the activity of PAL were stimulated an increase in the production of flavonoids would be the expected result. This was shown to be the case by Creasy (3) in strawberry leaf disks where he found that as the activity of PAL increased, the rate of synthesis of flavonoids increased and that a correlation existed between the 2. On the basis of the foregoing it is postulated that rapid increases in the flavonoid content of plant materials are due to an increased PAL activity utilizing the reserve phenylalanine of the tissue.

Two possible means of increasing PAL activity and consequently flavonoid biosynthesis have been shown to be exposure of plant tissues to ethylene (26) and gamma radiation at dose levels of 90 to 200 krad (27, 24). With the latter stimulating de novo synthesis of the enzyme resulting in increased activity.

In considering the action of radiation of flavonoid biosynthesis it is probable that the increased PAL activity is due to ethylene produced by the treated tissue since this is a common response of plant materials to radiation exposure. This has been reported by several workers in different fruits: peaches and nectarines (21), lemons (20); pears (22); apples (19). Maxie et al. (22) and Sommer and Maxie (31) have indicated that the effect of radiation on ethylene production appears to be related to the stage of the fruit in the climacteric sequence of the fruit. They propose that if fruits are pre-climacteric, the effect of radiation will more likely be unfavorable than if the fruit was beyond the onset of the climacteric rise in respiration.

Cranberries have recently been shown to be climacteric by Bramlage3 and furthermore he indicated that there appears to be a relationship between the degree of coloration of the fruit and the stage of the climacteric sequence. Poorly colored fruit would be pre-climacteric and fully-colored fruit post-climacteric. Contrary to the findings of Fudge (9), it has recently been shown that ethylene stimulates anthocyanin production in cranberries3. In terms of these findings on the relationship between climacteric stage and radiation effect on ethylene production, the effect of radiation on flavonoid synthesis can be hypothesized.

It seems reasonable to consider that gamma radiation produces its effect on the biosynthesis of flavonoids in cranberries by causing an upsurge of ethylene which acts as a causal agent for increased PAL activity resulting in the increased biosynthesis of the flavonoid pigments involved. The climacteric stage of the cranberries is the controlling factor in determining the degree of response to the radiation treatment and explains why the radiation induced flavonoid synthesis increases as the maturity of the fruit increases. It must be noted, however, that the ethylene production in response to irradiation was not measured in this study, so that the above is only an untested theory of mode of action. The proposed mode of action of radiation explains the favorable results obtained with full-red (post-climacteric) fruit and also the increased response as the dose level and storage temp increased, and the unfavorable effect on the white-pink (pre-climacteric) category of fruit. The variable results obtained with the half-red cranberries (approximating the climacteric) are explained by the fact that some of the fruit may actually have been in the climacteric phase, while others were still pre-climacteric and thus the effects of radiation would be intermediate between those obtained with 2 categories of fruit.

The mode of action of chemicals has not been postulated. Great differences in the mode of action of chemicals and radiation must exist since they act at different ends of the climacteric scale.

**Literature Cited**


