

NutriHarmony

Multi-Vitamin Soy

Calcium Iron

Antioxidant

Enzymes Greens

Ion-Exchange Whey



NutriHarmony

Guide to Research Summaries

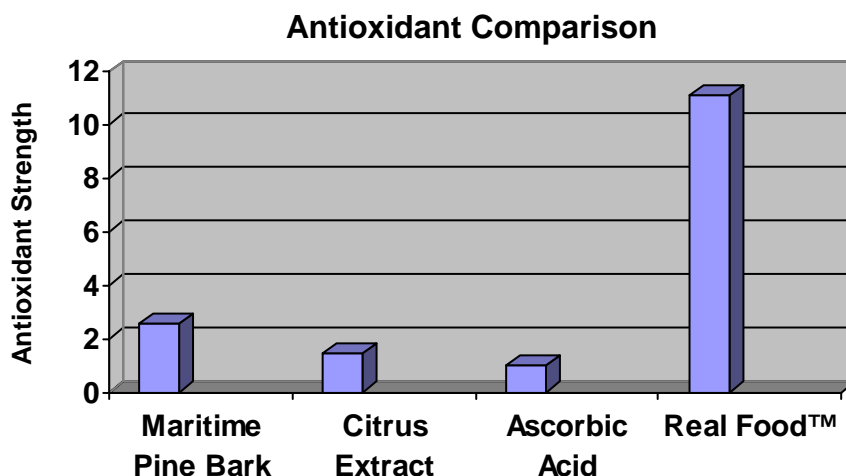
<u>Report Title</u>	<u>Page</u>
Phenol Analysis/Antioxidant Comparison	3
SEM/EDS/XPS Testing Explanation	4
Comparative IR Analysis of Beta-Carotene, Niacinamide and Vitamin C	5-6
Food Vitamins: The Newest, Oldest Idea	7
The Benefit of Active Yeast	8
Vitamin Analysis by Infra Red (IR)	9-10
Moderate Doses vs Mega Doses	11
Streamlined Test to Determine Identity and Potency	12-13
Introduction to Soy	14
Synergism of Citrus Extract and Vitamin C in the Real Food™ Formulation	15-16
Comparative NMR and IR Analyses of Bio-Interactive Vitamins	17-19

Report: Phenol Analysis/Antioxidant Comparison
Performed by: Joe A. Vinson, PhD, Professor Chemistry
Location: University of Scranton, Department of Chemistry
Date: October 1994

Enclosed is our report based on phenol analysis with the Folin reagent using catechin as the standard. The total phenols in the products, with the exception of ascorbic acid, were measured by A Retro-Hydrosis with methanolic hydrochloric acid. Total phenols represent all the free and sugar conjugated phenols in the products. After ingestion, free phenols would be produced in the digestive tract by enzymes and the acidic conditions.

The IC50 is the concentration of antioxidant phenols in the products or concentration of ascorbic acid to inhibit the oxidation of low density lipoproteins with cupric ion for 6 hours. The oxidation products are measured as thiobarbituric acid reactive substances. We have calculated I/IC5D to allow for comparison; the higher the I/IC, the more powerful the antioxidant. Thus, Real Food™ contains 10.1 times stronger antioxidants than ascorbic acid alone, 7.6 times stronger antioxidants than Citrus Extract which also contains vitamin C, and 4.3 times stronger than Pyncolgenol, pine tree extract.

Product	Total Phenols w/HCLl	I/C50 of Total Phenols	Antioxidant Strength Relative to Ascorbic Acid
Maritime Pine Bark	1367	1.36	2.56
Citrus Extract	745	0.77	1.45
Ascorbic Acid	0	0.53	1
Real Food™ Vitamin C (Antioxidant)	1255	5.88	11.1



Explanation:

The number 1 (one) is the smallest digit available for charting and represents "ZERO".

Report: SEM/EDS/XPS Testing Explanation

Performed by: Joe A. Vinson, PhD, Professor Chemistry

Location: University of Scranton, Department of Chemistry

Date: September 1994

TO WHOM IT MAY CONCERN:

Re: Simple explanation of what SEM/EDS/XPS testing indicates.

1. Scanning Electron Microscopy (SEM): Looking at morphological characteristics (size, shape, surface characteristics, etc. of the particles) of both the starting active yeast and inorganic mineral salt, and comparing those characteristics to those of the finished—Bio-Interactive mineral yeast, shows no apparent morphological relationship. This helps support a statement that these products are not identical to standard free-state USP.

Imagine cooking butter and rice in a pot. The morphological characteristics of the original butter patties and dry rice grains would no longer be detectable.

2. Energy Dispersive X-Ray Spectroscopy (EDS): Mapping the energy given off by a specific mineral (each one is different) would show isolated “hot spots” (discrete concentrations) rather than an even dispersion, if inorganic mineral crystals were simply dry-mixed into the yeast. In fact, there were numerous fine points of energy detected from all parts of the Bio-Interactive mineral yeast samples. The photos showing this did not make good copies and so are not sent. This helps support a statement that these products are not merely a simply mixture of USP with food.

Imagine just dropping butter patties into dry rice grains without any heat or water. The butter “hot spots” amongst the rice grains would readily be seen. After cooking, the butter is completely dispersed.

3. X-Ray Photoelectron Spectroscopy (XPS): It was hoped that determining shifts in energy due to changes in chemical bonding of the minerals would help support the statement that our products are bound or associated to, or in complex formation with, constituents of the food matrix. The results were inconclusive because the binding energies of the elements of interest were masked by the overwhelming strengths of the carbon and oxygen bonds associated with the organic nature of the matrix. For this particular test it would have been better if the element was present on the surface of the yeast in relatively high concentrations, which it was not. However, this test provided strong confirmation of the EDS observation that the products are not merely mixtures of minerals and yeast, but rather that the elements are somehow incorporated into the yeast structures.

Report: Comparative IR Analysis of Beta-Carotene, Niacinamide and Vitamin C
Performed by: Joe A. Vinson, PhD; John Proch, BS
Location: University of Scranton, Department of Chemistry
Date: June 1995

Experimental:

FTIR analysis of Bio-Interactive Beta-Carotene, Bio-Interactive Niacinamide and Bio-Interactive Vitamin C were performed using a Galaxy Series 5000 FTIR Spectrometer. A sample of the IR measurements were obtained in duplicate in order to verify the precision of the data obtained. The Bio-Interactive Beta-Carotene, Bio-Interactive Niacinamide, Bio-Interactive Vitamin C and the Food Matrix for each Bio-Interactive mixture were supplied by contractor. The Potassium Bromide used for preparing the sample pellets was obtained from Fisher Chemical Company. All sample pellets used for the analyses were prepared by compressing 120 milligrams of the powdered sample mixture at 20,000 pounds of pressure for 60 seconds.

Bio-Interactive Beta-Carotene Analysis:

A mixture containing 0.9 mgs of 30% Beta-Carotene in Vegetable Oil (Roche Vitamins and Fine Chemicals) and 310 mgs of Potassium Bromide; a mixture containing 7.4 mgs of Food Matrix for Beta-Carotene and 310 mgs of Potassium Bromide; a mixture containing 0.9 mgs, 30% Beta-Carotene in Vegetable Oil, 7.4 mgs of Food Matrix for Beta-Carotene and 31 mgs Potassium Bromide; and a mixture containing 7.7 mgs of Bio-Interactive Beta-Carotene and 310 mgs of Potassium Bromide were individually analyzed to obtain their respective IR spectra. Peak numbers were assigned to each peak which was to be analyzed. The mean value and the standard deviation for each peak were determined by comparing the peak values obtained from the two spectra plots for each sample. The values obtained for the individual peaks were compared between the four samples in order to determine variations in the peak positions.

A comparison of the three spectra containing Beta-Carotene peaks revealed several minor variations in peak positions which can be explained by experimental error. However, a comparison of the three spectra containing peaks for the Food Matrix for Beta-Carotene show three peaks which have a significant shift. The Bio-Interactive Beta-Carotene spectrum contain greater peak shifts than the spectrum obtained from the Pure Beta-Carotene + Food Matrix for Beta-Carotene sample.

Bio-Interactive Niacinamide:

A mixture containing 2.0 mgs of Niacinamide (Sigma Chemical Company) and 200 mgs of Potassium Bromide; a mixture containing 1.0 mgs of Niacinamide and 200 mgs of Potassium Bromide; a mixture containing 6.0 mgs of Food Matrix for Niacinamide and 200 mgs of Potassium Bromide; a mixture containing 2.0 mgs of Niacinamide, 6.0 mgs of Food Matrix for Niacinamide and 200 mgs of Potassium Bromide; a mixture containing 8.0 mgs of Bio-Interactive Niacinamide and 200 mgs of Potassium Bromide; and a mixture containing 4.0 mgs of Bio-Interactive Niacinamide and 200 mgs of Potassium Bromide were individually analyzed in order to determine their IR spectra.

Peak numbers were assigned to each peak to be studied. A mean value and a standard deviation were determined for each peak by comparing the peak values obtained from the two spectra plots for each sample. The values obtained for the individual peaks were compared between the samples in order to determine variations in the peak positions.

A comparison of the spectra containing Niacinamide peaks shows both major and minor variations in peak positions. In general, the variations are larger in the samples containing the Niacinamide + The Food Matrix For Niacinamide than in the samples containing the Bio-Interactive Niacinamide. A comparison of the spectra containing peaks for the Food Matrix for Niacinamide shows some variations in the peak positions. The variations are larger in the Bio-Interactive Niacinamide spectrum than in the spectrum obtained from the Niacinamide + The Food Matrix For Niacinamide sample. In general, the spectrum for the Bio-Interactive Niacinamide contains broader peaks with slightly higher absorbances than the spectrum obtained from the Niacinamide + The Food Matrix For Niacinamide.

Bio-Interactive Vitamin C:

A mixture containing 2.0 mgs of L-Ascorbic Acid (Mallinck-rodt, Inc.) and 200 mgs of Potassium Bromide; a mixture containing—6.0 mgs of Food Matrix For Vitamin C and 200 mgs of Potassium Bromide; a mixture containing 2.0 mgs of L-Ascorbic Acid, 6.0 mgs of Food Matrix for Vitamin C and 200 mgs of Potassium Bromide; and a mixture containing 8.0 mgs of Bio-Interactive Vitamin C and a mixture containing 8.0 mgs of Bio-Interactive Vitamin C and 200 mgs of Potassium Bromide were individually analyzed in order to obtain their IR spectra. Peak numbers were assigned to each peak to be studied. A mean value and a standard deviation were determined for each peak by comparing the peak values obtained from the two spectra plots of each sample. The spectra plots of the four samples were compared in order to determine variations in peak positions among the samples.

Major and minor variations were observed in the peak positions in the spectra obtained from the three samples containing L-Ascorbic Acid. In general, the variations in the peak positions were greater in the Bio-Interactive Vitamin C spectrum than in the L-Ascorbic Acid—The Food Matrix For Vitamin C spectrum. A comparison of the three spectra containing peak for the Food Matrix For Vitamin C show variations in the peak positions. In general, the variations are greater in the Bio-Interactive Vitamin C spectrum than in the spectrum for L-Ascorbic Acid + The Food Matrix for Vitamin C. A comparison of the spectrum for Bio-Interactive Vitamin C with the spectrum for L-Ascorbic Acid + The Food Matrix For Vitamin C reveals the absence of several peaks in the Bio-Interactive Vitamin C spectrum which are present in the spectrum for L-Ascorbic Acid + The Food Matrix For Vitamin C.

Conclusion:

The IR analysis of Vitamin C indicates the presence of interactions between L-Ascorbic Acid and the Food Matrix For Vitamin C in the Bio-Interactive Vitamin C. The IR analysis of Niacinamide indicates the presence of interactions between Niacinamide and the Food Matrix For Niacinamide by the shifting of only one Niacinamide peak in the Bio-Interactive Niacinamide spectrum. Stronger support for the presence of interactions in the Bio-Interactive Niacinamide can be shown by shifts which belong to peaks from the Food Matrix For Niacinamide. Support for the presence of interactions between Beta-Carotene and the Food Matrix For Beta-Carotene in the Bio-Interactive Beta-Carotene is shown by greater shifts in Food Matrix peaks in the Bio-Interactive Beta-Carotene spectrum than in the spectrum obtained from the Pure Beta-Carotene + Food Matrix.

The FTIR analyses shows that Food Beta-Carotene, Niacinamide and Vitamin C are:

- A. Not identical to free state USP.
- B. Not just a simple mixture of USP vitamins with food.
- C. Bound in complex formation with constituents of the food matrix

JOHN PROCH, B.S.
JOE VINSON, PhD.

Report: Food Vitamins: The Newest, Oldest Idea
Performed by: Joe A. Vinson, PhD, Professor Chemistry
Location: University of Scranton, Department of Chemistry
Date: October 1997

Real Food™ vitamins and minerals are produced with the help of Nature itself, in the form of living single-cell yeast's of the strain *Saccharomyces cerevisiae*. This strain is humankind's oldest microbial associate, helping to produce bread, beer and wine for over five thousand years.

The model for Real Food™ unique living-cell processes for transformation of “chemical” vitamins and minerals into foods for optimal human nutrition, is Nature's similar use of living microbial cells in the soil for optimization of plant nutrition. In soil, microorganism contribute to plant nutrition by transforming inorganic micronutrients into organic forms that are more available to plants.

The promotion of this soil micro-life through “feeding” with organic matter is a foundation stone of organic farming. Just as organic composts provide a “media” for soil micronutrient proliferation, Real Food™ whole plant-source media provides nutrients required for yeast growth and proliferation. Just as soil contains rocks and clays for the microbes to “digest” and transform, commercial vitamins and minerals are Real Food™ added to media for the yeast to “digest” and transform.

Still sounds like a new idea? How about the use of microbes for transformation of milk into yogurt or the use of yeast in Japan to transform soy beans into natto, or similarly into miso. Perhaps not coincidentally, these living cell products are considered by many as being amongst the most wholesome, nutritious, and health promoting foods on earth.

Report: The Benefit of Active Yeast

Performed by: Joe A. Vinson, PhD, Professor Chemistry

Location: University of Scranton, Department of Chemistry

Date: June 1997

RE: The Benefit of Active Yeast

RE: The Benefit of Active Yeast Fermentation

Dear Valued Customer or Interested Friend,

Enclosed is a test report on our interactive Vitamin C Yeast from Dr. J.A. Vinson, Professor of Chemistry at the University of Scranton in Scranton, Pennsylvania.

His tests investigated both the quantity and **quality** of anti-oxidant activity in our products. As you know, our nutritional media for Vitamin C Yeast contains a bioflavonoid extract in addition to USP ascorbic acid. By measuring the antioxidant activity of the ascorbic acid and bioflavonoid extract containing media separately, and then comparing their calculated combined activity with the obtained activity of the finished product, Dr. Vinson was able to measure any synergy resulting from submitting those ingredients to the proprietary active yeast fermentation process.

The results are wonderful. Dr. Vinson reports a 610% synergy! That's like saying the whole is six times better than the sum of the parts. As he points out, most investigations into synergy merely find a simple additivity (the whole is usually equal to the sum of the parts). This is a compelling response to competitors who express doubt that yeast growth has value and who think simply mixing vitamins with food is good enough.

In addition, please recall that our interactive Vitamin C Yeast was found to be 1.87 times more bio-available in humans than USP ascorbic acid. In a similar human study performed on a competitors product, reactor-vessel produced vitamin C (attempting protein reaction instead of yeast growth) a result of 1.35 was found. The superior bio-availability in our study also suggests that benefits are improved with yeast growth.

Report: Vitamin Analysis by Infra Red (IR)

Performed by: Bipin Patel, PhD, Organic Laboratory Manager

Location: Princeton Testing Laboratory, Inc., Sigma Chemical Company, Inc.

Date: May 8, 1992

The 3 Vitamin Packages: USP Vitamin, Blank Food Matrix (Placebo Vitamin), and Real Food™ Vitamin in Food Matrix

The 3 vitamin packages, USP Vitamin, Blank Food Matrix (Placebo Vitamin), and Real Food™ Vitamin in Food Matrix, were analyzed by Infra Red (IR) to determine the presence of complex formation of vitamin with the food matrix.

The IR spectra of all three USP Vitamins obtained by PTL from Sigma Chemical Company, Inc., distinctly differ from the spectra obtained from two sets of vitamin samples, blank food matrix and the respective vitamins in the food matrix, submitted as Real Food™.

Infra Red Spectra:

Following IR spectra are submitted with this report:

Figure 1a - USP Vitamin B1

Figure 1b - Comparison of Blank Food Matrix for Vitamin B1 and Real Food™ Vitamin B1 in food matrix.

Figure 1c - Subtraction of Blank Food Matrix for Vitamin B1 spectrum from the Real Food™ Vitamin B1 food matrix spectrum.

Figure 2a - USP Vitamin B6

Figure 2b - Comparison of Blank Food Matrix for Vitamin B6 and Real Food™ Vitamin B6 in food matrix.

Figure 2c - Subtraction of Blank Food Matrix for Vitamin B6 spectrum from the Real Food™ Vitamin B6 in food matrix spectrum.

Figure 3a - USP Vitamin C

Figure 3b - Comparison of Blank Food Matrix for Vitamin C and Real Food™ Vitamin C in food matrix.

Figure 3c - Subtraction of Blank Food Matrix for Vitamin C spectrum from the submitted Vitamin C in food matrix spectrum.

VITAMIN B1:

USP Vitamin B1 spectrum completely differs with the blank food matrix for Vitamin B1 and Real Food™ Vitamin B1 in food matrix spectra in the 2000-3000 cm^{-1} , and 850-1300 cm^{-1} wave-numbers. The USP Vitamin B1 spectrum (Figure 1a) has very sharp bands in the 500-2000 cm^{-1} wave-numbers while all bands in the other two spectra (Figure 1b) are very broad absorption band indicat-

ing presence of complex formation or hydrogen bonding in the sample. Blank matrix for Vitamin B1 and Vitamin B1 in food matrix spectra have similarity but band intensity and ratios differ between 2000-3000 cm⁻¹ wave-numbers and between 500-800 cm⁻¹ wave-numbers.

VITAMIN B6:

USP Vitamin B6 spectrum completely differs with the blank food matrix for Vitamin B6 and Vitamin B6 in food matrix spectra in the 700-1600 cm⁻¹ wave-numbers. The USP Vitamin B6 spectrum (Figure 2a) has very sharp bands in the 500-2000 cm⁻¹ wave-numbers while all bands in the other two spectra (Figure 2b) are very broad bands indicating presence of complex formation or hydrogen bonding in the sample. USP Vitamin B6 and Real Food™ Vitamin B6 in food matrix spectra have similarity but band intensity and ratios differ between 2000-3000 cm⁻¹ wave-numbers and between 1000-1700 cm⁻¹ wave-numbers.

VITAMIN C:

USP Vitamin C spectrum completely differs with the blank food matrix and Real Food™ Vitamin C food matrix spectra in the 700-1600 cm⁻¹ wave-numbers. The USP Vitamin C spectrum (Figure 3a) has very sharp bands in the 500-2000 cm⁻¹ wave-numbers. The USP Vitamin C and Real Food™ Vitamin C in food matrix spectra have similarity but band intensity and ratios differ between 2800-3000 cm⁻¹ wave-numbers and between 1400-1650 cm⁻¹ wave-numbers.

Conclusion: Infrared spectra of the Real Food™ vitamins:

- A. are not identical to that of infra red spectra of standard free-state USP vitamins.
- B. does not exhibit simple mixture of USP vitamin with food.
- C. differ from the USP vitamin and food matrix. This indicates complex formation between the vitamins and food matrix.

Report: Moderate Doses vs. Mega Doses

Performed by: Joe A. Vinson, PhD, Professor Chemistry

Location: University of Scranton, Department of Chemistry

Date: June 1997

Moderate Doses of Natural Form Nutrients as an Alternative to Mega Doses of Free-State Vitamins and Minerals.

Foods provide nutrients in complex forms. Plants for example, will take insoluble inorganic minerals from the soil and build them into complex soluble organic formations within their cellular structures. When we eat those foods, those complex formations are passed on to us and may be utilized by our bodies with relatively little changes.

In addition, certain foods may have constituents with a propensity to protect or work synergistically with particular vitamins or minerals. Dr. Szent Gyorgy, who won the Nobel prize for isolating vitamin C from green peppers, stated that crude extracts containing the substance (vitamin C) were biologically more effective than the isolated vitamin by itself. The reason is it's relationship to the food complex.

Proteins, in the form of enzymes, are the working substances in an organism. Proteins are usually closely associated with lipids and carbohydrates. The compounds are referred to as glycol-proteins, lipo-proteins, phosphor-proteins, etc. In most cases, enzymes need minerals and/or vitamins to be attached to them in order to function properly. These substances, found as they are in the complex matrices of foods, may take part in the absorption and transportation of nutrients as well as in their retention and storage.

Since we evolved on foods and not on free-state chemicals, it seems reasonable to conclude that it is the large more complex formations of nutrients that the body prefers.

Standard free-state USP (United States Pharmacopea) or BP (British Pharmacopea) and FCC (Food Chemical Codex) vitamins and minerals, which are purified to exacting standards and are not in complex formation with any constituent of food are, for the most part, tableted by manufacturers "as-is". Can we be sure that mega dosing with these free-state chemicals is the answer for optimum health? Examples to consider: The Linus Pauling Institute has reported that "'Rebound Scurvy' can be experienced when the body is removed from mega doses to which it has become accustomed. Calcium carbonate (an insoluble form of calcium not found in foods) can cause an "acid surge" in the stomach after two hours when taken in high doses.

Perhaps the answer to optimum health-giving potential lies in a nutrient's form rather than in its quantity. We believes it does. Therefore, to us a nutrient's similarity to food-state carries more importance than how many milligrams per penny a chemical isolate can provide.

That is why we put so much emphasis on utilizing living yeast cultures in the production of our Real Food™ nutrients. We want to provide the most natural, wholesome vitamins and minerals possible, for daily consumption. What could be a better model to emulate than food?

Report: Streamlined Test to Determine Identity and Potency , Explained

USP and FCC Vitamins and Minerals Have Relatively Streamlined Tests to Determine Identity and Potency

First Stage: Prepare sample for analysis.

Put sample directly into the solvent indicated in the USP or FCC* monograph for that particular nutrient.

Second Stage: Run analysis to determine identity and potency.

Run determinations on prepared sample by instrument (such as W Spectrophotometer, Fluorometer, Spectrophotometer, etc.), titration procedures, gravimetric procedures, etc., following the monograph in the wave range for that particular nutrient.

Third Stage: Record obtained results.

*The United States Pharmacopeia and Food Chemical Codex are standard reference texts containing standard analytical methods for identification and quantification of ingredients for which the molecular structure and composition are fully known.

Vitamins and Minerals in Natural Foods and Yeast-Grown Nutrients Require Additional Extraction Procedures Before Determining Identity and Potency

First Stage: Prepare sample for analysis.

1. As opposed to testing a synthesized chemical, when analyzing a nutrient in a natural food (vitamin C in a standard example) or in a yeast-grown food (yeast-grown vitamin B1 for example), effective "extraction" procedures were performed as a preliminary to sample preparation.

The purpose of extraction is to remove the numerous natural substances found in foods (proteins, carbohydrates, etc.) which may interfere with the analytical determinations. Only the subject nutrient must be left, undiminished after extraction.

The AOAC* suggests basic "starting point" extraction procedures which must be modified by the analytical procedure refining the complexity and composition of the matrix containing the nutrient (the presence of yeast, other vitamin ingredients, herbs, foods, etc.), and what type and which model instrument is intended to be used. Different procedures have different limitations and/or require particular modifications of the extraction procedures in order to analyze samples. Other factors, such as the ionic profile of the water to be used for dilution, may affect the modification.

Here are three examples from THE VITAMINS, second edition, volume II, published by Academic Press.

A look at pages 58 and 59 shows that the methods of assay for thiamin (vitamin B1) differ when testing milk and when testing red blood cells.

Reading the section about Extraction of Riboflavin (vitamin B2) on page 102, one finds “Since free riboflavin is found rarely in nature, it is usually necessary to treat natural products with acid or enzymes to liberate the riboflavin in some cases the protein or starch content of the sample may disturb the extraction.

Reading the section for Determination of Niacin on page 144, one finds “It is often necessary to use complicated procedures, purifications, and blank determinations.

Typically, the problems caused by interfering natural substance result in incorrectly low potencies, NOT Incorrect potencies. That is because the matrix tends to “hide” the nutrient so that it can’t be found.

2. Once an effective extraction method which will confidently yield re-producible results is devised** and utilized the sample preparation (diluting, measuring, etc.) can proceed.

Often, after the interfering substances have been removed by extraction with properly modified AOAC procedures then determinations on the resulting sample can then be run following analytical methods described in the USP or analytical methods are called “combination procedures” and are perfectly acceptable.

Second Stage: Run analysis to determine identity and potency.

Run determinations by instrument (such as W Spectrophotometer, Fluorometer, Atomic Absorption Spectrophotometry, titration procedures, gravimetric procedures, etc., following the monograph in the AOAC, USP, or FCC particular nutrient.

Third Stage: Record obtained results.

*Association of Official Agricultural Chemists is a standard reference text containing accepted analytical methods of extraction, identification, and quantification of ingredients from foods and for which the molecular composition and chemical nature of the environment (matrix) in which they are found is not fully known.

**Please note that development by a credible independent laboratory, of suitably modified extraction methodology of products, can be a time-consuming and very expensive - as much as \$3000.00 for a single product.

Report: Introduction to Soy

Performed by: Joe A. Vinson, PhD, Professor of Chemistry

Location: University of Scranton, Department of Chemistry

Date: May 1995

A Review of Soybeans, Soybean Products (Especially Fermented) and Eiealtei Benefits

Soybean Products and Composition

Soyfoods

For more than 1000 years, people throughout Asia have been consuming soybeans in a variety of traditional soyfood products. Only for the past 15 years have soyfoods begun to make a significant inroad into Western cultures and diets. These changes in Western diets have been primarily made for health and economic reasons.

There are many types of soyfoods available throughout the world today. Some are produced by modern processing techniques in large soybean-processing plants, whereas others are produced in more traditional ways, owing their history to Oriental processing techniques. These are the foods that are usually referred to as traditional soyfoods. The contractor has combined the natural fermentation process of traditional Japan with modern industrial manufacturing methods.

These soyfoods are typically divided into two categories: nonfermented and fermented. Traditional nonfermented soyfoods include fresh green soybeans, whole dry soybeans, soy nuts, soy sprouts, whole-fat soy flour, soymilk and soymilk products, tofu, okra and yuba. Traditional fermented soyfoods include tempeh, miso, soy sources, natto and fermented tofu and soymilk products.

Westerners have adopted some of these foods wholeheartedly, whereas others will undoubtedly take more time to accept. The most popular soyfoods in the US now are tofu, soymilk, soy sauce, miso and tempeh. Americans, known for the ability to adapt foreign foods to their own tastes, have developed a new class of "second generation" soyfoods, which include such products as tofu hot dogs, tofu ice cream, veggie burgers, tempeh burgers, soymilk yogurt, soymilk cheeses, soy flour pancake mix.

Largely because of the great entrepreneurial spirit of many small American companies, sales of soyfoods in the US have been growing steadily since 1980 and are projected to reach one billion dollars by the year 200 (1).

Report: Synergism of Citrus Extract and Vitamin C in the Real Food™ Formulation
Performed by: Joe A. Vinson, PhD, Professor of Chemistry
Location: University of Scranton, Department of Chemistry
Date: May 13, 1997

Synergism is an overused term in advertising and even in science. In fact, synergism is seldom shown experimentally even though we believe there are synergisms in living organisms. To define synergism we need to measure the effect of component of a mixture alone on a system. Then we need to calculate what the effect of the mixture would be, i.e. add the effect of the components. For example, suppose we have 2 antioxidant components A and B. Component A decreased the oxidation 20% and component B 15%. The accumulative total would be 35%. If the actual mixture of A and B at the same concentrations inhibited 55% then that would be an example of synergism to the magnitude of $55 - 35/35$ or 57% synergism. Most experimental systems demonstrate simple additivity.

Antioxidants were tested using a published in vitro model [Vinson, J. Agric. Food Chem 43: 401-3 (1995)] which mimics atherogenesis by oxidation of low-density lipoproteins in a test tube. Cupric ion was used as the oxidant of human low density and very low-density lipoproteins (LDL + VLDL) under standardized conditions. The lag time of the oxidation was determined by monitoring the production of lipid oxidation products known as conjugated dienes by their W absorption at 234 nanometers. A control was done with no antioxidants present. Antioxidants vitamin C at 0.84 μM and Citrus Extract (CE) (with phenols at 0.025 μM as measured by the Folin assay with catechin as the standard) were tested singly then in Real Food™ Vitamin C Formulation (CEC) at the same concentrations. The % increase of the lag time relative to the control was then calculated.

Discussion

The results are shown in Graph 1. Lag time is a measure of the quality and quantity of antioxidants present. The citrus extract polyphenols had no effect on the lag time at the low concentration of 0.025 μM. The Vitamin C increased the lag time relative to the control 21.4% at the much higher (33.6 times) concentration of 0.84. If you add the individual contributions of the two antioxidants you get a calculated value of 21.4% when they were actually present together in the Real Food™ Vitamin C Formulation was synergistic by almost a factor of 4 times the calculated value (i.e. 400%).

Protocol for Fixed Time Antioxidant Synergism

In this procedure we oxidized the lipoproteins under the same conditions as the lag time experiments except that we stopped the reaction after 6 hours and measured the amount of lipid peroxidation products by means of the production of thiobarbituric acid reactive substances using fluorometry. A control was done with no antioxidants present. In a dose-response study we examined the antioxidant effect of pure Real Food™ vitamin C alone, Citrus Extract without vitamin C and the Real Food™ Vitamin C Formulation. The concentrations are expressed in μM units. The

Sample	IC ₅₀	1/IC ₅₀	Sum C+CE	Synergism CEC-CE	Synergism %
Real Food™ Vitamin C Citrus Extract w/o C(CE)	1.90 μM	0.526			
Real Food™ Extract w/o C(CE) Real Food™ Vitamin C	3.30 μM	0.303			
Formulation (CEC)	0.17 μM	5.882	0.829	5.053	610%

Discussion

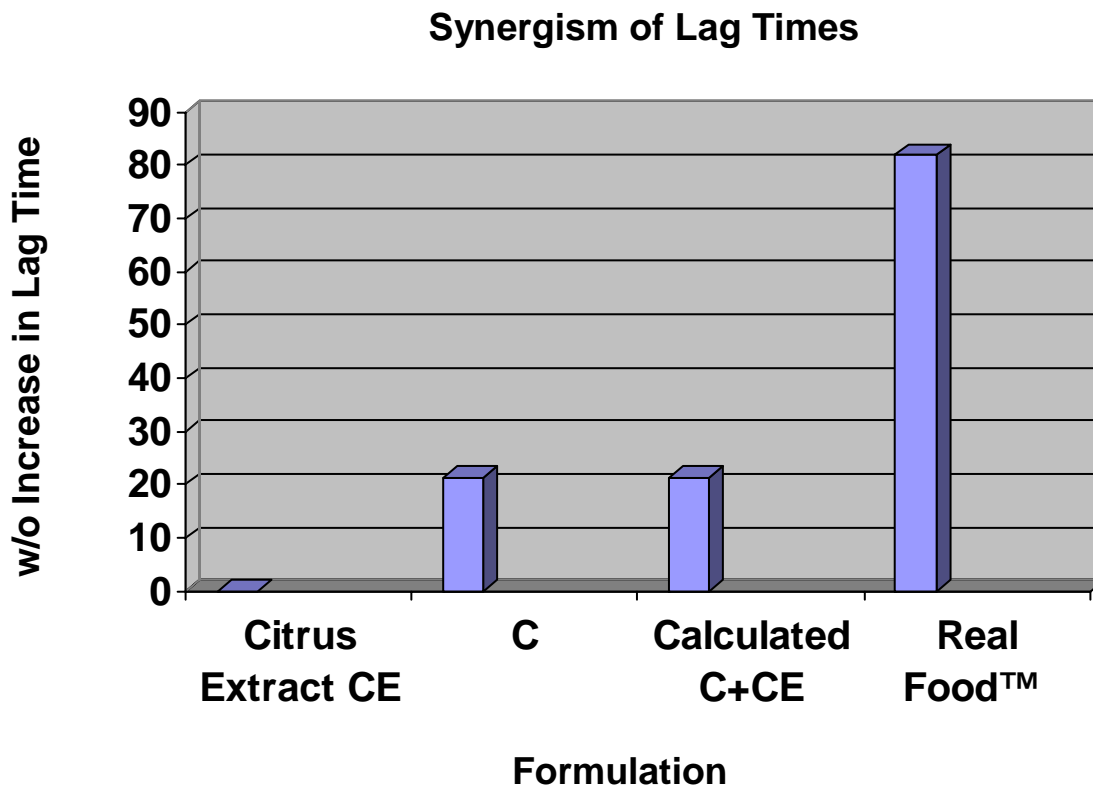
It is quite evident that there is a very large synergism when the flavinoids and Vitamin C are manufactured together in the Real Food™ Vitamin C Formulation.

Protocol for Different Ratios of Antioxidants on IC₅₀ Synergism

The same procedure was used as in the protocol above. Real Food™ CE and Real Food™ Vitamin C Formulation contain a mixture of flavinoids whose ideal concentration is 840 μmoles/g. We mixed vitamin C in various mole ratios with Real Food™ CE and measured the IC₅₀. Then we calculated the o/o synergism as in the table above. We also measured the synergism of the Real Food™ Vitamin C Formulation (CEC). The results are shown in the graph below.

Discussion

As can be seen there is a dose-response effect of the synergism when the ratio of vitamin C to Citrus-Extract is raised. However, at a ratio of 0.47:1 when the moles of flavinoids of the Citrus Extract were in excess of the Vitamin C there was a negative synergism, i.e. a pro-oxidant situation. As the ratio of vitamin C to flavinoids went above 1, then there was a distinct antioxidant effect. It is evident that synergism exists in the Real Food™ Vitamin C Formulation compared to just mixing the vitamin C and the flavinoids in the Real Food™ Extract without C since the ratio of 1:14.8 gives a greater synergism than the mixture of 1:17.7. In fact, the calculated % increase for a ratio of 1;14.8 is 409%. The actual increase is 610% and thus a 50% synergism exists in the Real Food™ Vitamin C Formulation above and beyond synergism from simple mixing of vitamin C and flavinoids in Real Food™ Citrus Extract without C.



Report: Comparative NMR and IR Analyses of Real Food™ Vitamins
Performed by: Joe A. Vinson, PhD, Professor of Chemistry; John Proch, B.S.
Location: University of Scranton, Department of Chemistry
Date: February 20, 1995

Comparative NMR and IR Analyses of Real Food™ Vitamin B1 and Real Food™ Vitamin B2 and Real Food™ Vitamin B6

Experimental:

NMR analyses of Real Food™ Vitamin B1, Real Food™ Vitamin B2 and Real Food™ Vitamin B6 were performed using a Gemini-300 Spectrometer. The NMR solvents used for the analyses were obtained from Aldrich Chemical Company. The NMR tubes used for the analyses were a Grade 6U –P obtained from Rontes Scientific Glassware.

IR analyses of Real Food™ Vitamin B1, Real Food™ Vitamin B2 and Real Food™ Vitamin B6 were performed using a Mattson FTIR (Galaxy Series 5000) Spectrometer. The IR—Grade Potassium Bromide used for the analyses was obtained from Fisher Chemical Company.

The Real Food™ Vitamin B1, Real Food™ Vitamin B2, Real Food™ Vitamin B6 and the Food Matrix for each Real Food™ Vitamin mixture were supplied by contractor. All of the NMR and IR measurements were obtained in duplicated in order to verify the precision of the data obtained.

Real Food™ Vitamin B1 Analysis:

A solution containing 10.0 mgs of Thiamine Hydrochloride (obtained from Eastman Kodak Company) and 1.0 ml of Deuterium Oxide; a solution obtained from filtering a mixture containing 60.0 mgs of Food Matrix for Vitamin B1 and 2.0 mls of Deuterium Oxide; a solution obtained from filtering a mixture containing 20.0 mgs of Hydrochloride, 60.0 mgs of Food Matrix for Vitamin B1 and 2.0 mls of Deuterium Oxide; and a solution obtained from filtering a mixture containing 80.0 mgs of Bio-Interactive Vitamin B1 and 2.0 mls of Deuterium Oxide were individually analyzed to obtain their respective NMR spectra. Peak numbers were assigned to each peak which was to be analyzed. The mean value and the standard deviation for each peak were determined by comparing the peak values obtained from the two spectra plotted for each solution. The values obtained for the individual peaks in each solution were compared to the values obtained for the same individual peaks in the other solutions.

A mixture containing 2.0 mgs of Thiamine Hydrochloride and 200.00 mgs of Potassium Bromide; a mixture containing 6.0 mgs of Food Matrix for Vitamin B1 and 200.0 mgs of Potassium Bromide; a mixture containing 2.0 mgs of Thiamine Hydrochloride, 6.0 mgs of Food Matrix for Vitamin B1 and 200.0 mgs of Potassium Bromide; and a mixture containing 8.0 mgs of Bio-Interactive Vitamin B1 and 200.0 mgs of Potassium Bromide were individually analyzed to obtain their respective IR spectra. Peak numbers were assigned to each peak which was to be analyzed. The mean value and the standard deviation for each peak were determined by comparing the peak values obtained from the two spectra plotted for each mixture. The values obtained for the individual peaks in each mixture were compared to the values obtained for the same individual peaks in the other mixtures.

Several moderate variations in peak positions were observed when the NMR spectrum of pure Thiamine Hydrochloride was compared to the NMR spectrum of Thiamine Hydrochloride + Food Matrix for Vitamin B1 and the NMR spectrum of Real Food™ Vitamin B1. Also, moderate variations were ob-

served in the peak positions of Thiamine Hydrochloride when the Thiamine Hydrochloride + Food Matrix for Vitamin B1 NMR spectrum and the Real Food™ Vitamin B1 spectrum were compared to one another. Several minor variations in peak positions were observed when the IR spectrum of pure Thiamine Hydrochloride was compared to the IR spectrum of Thiamine Hydrochloride + Food Matrix for Vitamin B1 and the IR spectrum of Real Food™ Vitamin B1. When the IR spectrum of Thiamine Hydrochloride + Food Matrix for Vitamin B1 and the IR spectrum of Real Food™ Vitamin B1 were compared, minor variations in the positions of the peaks from the Thiamine Hydrochloride were observed and one moderate-sized shift in a peak belonging to the Food Matrix for Vitamin B1 was observed.

Real Food™ Vitamin B1 Analysis:

A solution containing 10.0 mgs of Riboflavin (obtained from Roche Vitamins and Fine Chemicals) and 2.0 mls of (Methyl Sulfoxide)-d6; a solution obtained from centrifuging a mixture containing 90.0 mgs of Food Matrix for Vitamin B2 and 2.0 mls of (Methyl Sulfoxide)-d6; a solution obtained from centrifuging a mixture containing 10.0 mgs of Riboflavin, 90.0 mgs of Food Matrix for Vitamin B2 and 2.0 mls of (Methyl Sulfoxide)-d6; and a solution obtained from centrifuging a mixture containing 100.0 mgs of Real Food™ Vitamin B2 and 2.0 mls of (Methyl Sulfoxide)-d6 were individually analyzed to obtain their respective NMR spectra. Peak numbers were assigned to each peak which was to be analyzed. The mean value and the standard deviation for each peak were determined by comparing the peak values obtained from the two spectra plotted for each solution. The values obtained for the individual peaks in each solution were compared to the values obtained for the same peaks in the other solutions.

A mixture containing 1.0 mg of Riboflavin and 200.0 mgs of Potassium Bromide; a mixture containing 9.0 mgs of Food Matrix for Vitamin B2 and 200.0 mgs of Potassium Bromide; a mixture containing 1.0 mg of Riboflavin, 9.0 mgs of Food Matrix for Vitamin B2 and 200.0 mgs of Potassium Bromide; and a mixture containing 10.0 mgs of Bio-Interactive Vitamin B2 and 200.0 mgs of Potassium Bromide were individually analyzed in order to determine their IR spectra. Peak numbers were assigned to each peak which was to be analyzed. The mean value and the standard deviation for each peak were determined by comparing the peak values obtained from the two spectra plotted for each mixture. The values obtained for the individual peaks in each mixture were compared to the values obtained for the same peaks in the other mixtures.

Several moderate variations in peak positions were observed when the NMR spectrum of the pure Riboflavin was compared to the NMR spectrum of Riboflavin + Food Matrix for Vitamin B2 and the NMR spectrum of Real Food™ Vitamin B2. Also, moderate-sized peak shifts were observed when the NMR spectrum of Riboflavin + Food Matrix for Vitamin B2 and the NMR spectrum of Bio-Interactive Vitamin B-2 were compared to one another. No variations in peak positions were observed when the IR spectrum of pure Riboflavin was compared to the IR spectrum of Riboflavin + Food Matrix for Vitamin B2 and the IR spectrum of Bio-Interactive Vitamin B2. When the IR spectrum of Riboflavin + Food Matrix for Vitamin B and the IR spectrum of Real Food™ Vitamin B2 were compared, no shifts in the Riboflavin peaks were observed, but one significant shift in a peak from the Food Matrix for Vitamin B2 was observed.

Real Food™ Vitamin B6 Analysis:

A solution containing 10.0 mgs of Pyridoxamine Dihydrochloride and 1.0 ml of Deuterium Oxide; a solution obtained from filtering a mixture containing 80.0 mgs of Food Matrix for Vitamin B6 and 2.0 mls of Deuterium Oxide; a solution obtained from filtering a mixture containing 20.0 mgs of Pyridoxamine Dihydrochloride, 80.0 mgs of Food Matrix for Vitamin B and 2.0 mls of Deuterium Oxide; and

a solution obtained from filtering a mixture containing 100.0 of Real Food™ Vitamin B6 and 2.0 mls of Deuterium Oxide were individually analyzed to obtain their respective NMR spectra. Peak numbers were assigned to each peak which was to be analyzed. The mean value and the standard deviation for each peak were determined by comparing the peak values obtained from the two spectra plotted for each solution. The values obtained for the individual peaks in each solution were compared to the values of the same peaks in the other solutions.

A mixture containing 1.0 mgs of Pyridoxamine Dihydrochloride and 200.0 mgs of Potassium Bromide; a mixture containing 4.0 mgs of Food Matrix for Vitamin B6 and 200.0 mgs of Potassium Bromide; a mixture containing 1.0 mgs of Pyridoxamine Dihydrochloride, 4.0 mgs of Food Matrix for Vitamin B6 and 200.0 mgs of Potassium Bromide; and a mixture containing 5.0 mgs of Real Food™ Vitamin B6 and 200.0 mgs of Potassium Bromide were individually analyzed to obtain their respective IR spectra. Peak numbers were assigned to each peak which was to be analyzed. The mean value and the standard deviation for each peak were determined by comparing the peak values obtained from the two spectra plotted for each mixture. The values obtained for the individual peaks in each mixture were compared to the values of the same peaks in the other mixtures.

Several large variations in peak positions were observed when the NMR spectrum of pure Pyridoxamine Dihydrochloride was compared to the NMR spectrum of Pyridoxamine Dihydrochloride + Food Matrix for Vitamin B6 and the NMR spectrum of Real Food™ Vitamin B6. Also, several large variations in peak positions were observed when the NMR spectrum of Pyridoxamine Dihydrochloride + Food Matrix for Vitamin B6 was compared to the NMR spectrum of Real Food™ Vitamin B6. One major peak shift was observed when the IR spectrum of pure Pyridoxamine Dihydrochloride was compared to the IR spectrum of Pyridoxamine Dihydrochloride + Food Matrix for Vitamin B6 and the IR spectrum of Real Food™ Vitamin B6. When the IR spectrum of Pyridoxamine Dihydrochloride + Food Matrix for Vitamin B6 and the IR spectrum of Real Food™ Vitamin B6 were compared, one moderate-sized peak shift of the Pyridoxamine Dihydrochloride was observed and one major and two moderate-sized peak shifts were observed in the peaks from the Food Matrix for Vitamin B6.

Conclusion:

The results of this study show that the NMR and IR spectra of Real Food™ Vitamin B1, Real Food™ Vitamin B2 and Bio-Interactive Vitamin B6 are not the same as spectra obtained from the combination of the respective vitamins and their corresponding matrices. The NMR spectra reveal real peak shifts in the Real Food™ Vitamins when compared to their respective individual components. The IR spectra show definite peak variations in both peak shape and peak position in the Real Food™ Vitamins when compared to their respective Vitamin and Food Matrix constituents.

This NMR analyses shows that Real Food™ Vitamin B1, Real Food™ Vitamin B2 and Real Food™ Vitamin B6 are:

- A. Not identical to standard free state USP.
- B. Not just a simple mixture of USP Vitamins with food.
- C. Bound in complex formation with constituents of the food matrix.

This FTIR analyses shows that Real Food™ active Vitamin B1, Real Food™ Vitamin B2 and Real Food™ Vitamin B6 are:

- A. Not identical to standard free state USP.
- B. Not just a simple mixture of USP vitamins with food.
- C. Bound in complex formation with constituents of the food matrix.