DISSERTATION

EFFECTS OF ORGANIC, BIOLOGICAL AND CONVENTIONAL PRODUCTION METHODS ON APPLE ANTIOXIDANT LEVELS, SENSORY QUALITIES AND HUMAN GLYCEMIC RESPONSE

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY JANA D. BOGS ENTITLED EFFECTS OF ORGANIC, BIOLOGICAL AND CONVENTIONAL PRODUCTION METHODS ON APPLE ANTIOXIDANT LEVELS, SENSORY QUALITIES AND HUMAN GLYCEMIC RESPONSE BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

EFFECTS OF ORGANIC, BIOLOGICAL AND CONVENTIONAL PRODUCTION METHODS ON APPLE ANTIOXIDANT LEVELS, SENSORY QUALITIES AND HUMAN GLYCEMIC RESPONSE

Different cultivation systems of fruit trees may influence fruit nutrient and phytochemical content, and consequently, human responses. This experiment compared two cultivars of apples (*Malus domestica* Mill.) each under differing cultivation systems. These were evaluated for antioxidant activity, human glycemic response, soluble solids content, shelf life and consumer acceptability by human sensory panels. In addition, soil and leaf tissue tests were performed and correlated to the above results.

‘Braeburn’ (*M. domestica*) apples grown in Washington state under biologically-enhanced organic and conventional methods were evaluated in 2007. Treatments were split to include apples from the outside and inside of the tree canopies. There were no differences ($P > 0.05$) in ABTS or DPPH antioxidant activity between organic and conventional ‘Braeburn’ apples. Organic ‘Braeburn’ apples had a higher level ($P = 0.003$) of total phenolics (TP) than the conventional apples. Outside-canopy apples had higher TP, ABTS and DPPH antioxidant activity levels ($P < 0.01$) than inside-canopy apples. Organically-grown ‘Braeburn’ apples from both outside and inside the canopies had higher soluble solids levels ($P < 0.001$) than those conventionally-grown.

Fruit soluble solids content was higher (P = 0.002) in ‘Braeburn’ apples from outside the canopies than from inside the canopies. There was no difference in shelf life between organic and conventional ‘Braeburn’ apples (P = 0.366), nor between outside-canopy and inside-canopy apples (P = 0.286). The ‘Braeburn’ overall acceptability sensory ratings for organic apples were significantly higher (P < 0.001), than conventional fruits, and outside-canopy fruits surpassed inside-canopy fruits (P < 0.001).

‘Crimson Gala’ (*M. domestica*) apples from Washington state orchards grown under biologically-enhanced conventional management and typical conventional management were evaluated in 2008. The biological apples had higher ABTS antioxidant activity than the conventional (P = 0.0498). The conventional ‘Gala’ apples had higher DPPH antioxidant activity (P = 0.002) than the biological. There was no difference (P = 0.681) between the biological and conventional ‘Gala’ apple total phenolics (TP) levels. None of the values used to compare human glycemic response were statistically different (P > 0.05). The conventionally-grown ‘Gala’ apples had higher soluble solids levels (P = 0.005), greater shelf life (P = 0.035), and a higher overall sensory rating (P = 0.014) than the biologically-grown fruit. The above measured values were also correlated with soil, leaf, and fruit tissue values. It should be noted that the biological ‘Gala’ orchard had a soil with a cation exchange capacity (CEC) of 7.1 meq/100g compared to the conventional control orchard’s CEC of 11.3 meq/100g, which may have negatively affected the quality of the biological apples. Understanding how cultivation practices affect consumer acceptance will encourage growers to further improve production practices.

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DEDICATION

To those who are working to create higher quality foods
while caretaking the environment,
thus making life better for all of us.

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Chapter 1

Introduction

List of Keywords

consumer acceptability, flesh firmness, *Malus domestica*, sensory perception, shelf life, soluble solids concentration, soluble solids content

Introduction

Apples (*Malus domestica* Mill.) are one of the most popular fruits in America and the world (Economic Research Service, USDA, 2009), and a significant source of fiber and phytonutrients that can help prevent degenerative diseases such as cancer and cardiovascular disease (Boyer and Liu, 2004; Hyson et al., 2000; Knekt et al., 2002). Therefore, it is important to increase consumer appeal and intake of apples by improving flavor and nutrient content. A review of the literature has shown differences in antioxidant levels and sensory qualities in apples related to cultivation methods, especially when comparing organic methods to conventional methods (Peck et al., 2006; Weibel et al., 2000).

Overall Objective

An overall objective of this research was to compare biologically-enhanced organic and biologically-enhanced conventional cultivation with typical conventional production practices relative to measurable properties that may contribute to good health, sensory qualities and shelf life, thereby increasing consumer appeal and consumption of fruits.

The regular consumption of fresh produce can have a significantly positive impact on health. The overall aim of this study was to highlight some of the differences production management systems may have on fruit quality, emphasizing nutritional differences and some direct effects on human metabolism. There may also be indirect effects on human health and the environment due to management systems. Although this study was limited to apples, similar principles may be applicable to other fruits and possibly some vegetables.

This study expands on previous research that has shown benefits of organic management (Peck et al., 2006), and then moves beyond USDA organic regulations (USDA, NOP, 2009), which focus on what is not allowed to be used on crops, to biological management which focuses on enhancement of produce quality through promotion of beneficial microbial activity and organic nutrient applications to the soil and trees (Andersen, 2000; Skow and Walters, 1995).

A key hypothesis tested involved a novel concept regarding human blood glucose responses that originated from field observations of diabetic patients who consumed biologically-produced fruit. This hypothesis was also supported with limited preliminary data (collected from samples consumed by Jana Bogs). The impact of management on total phenolic content and fruit antioxidant capacity was examined, as well as fruit soluble solids levels and shelf life over the duration of the experiment. Fruit acceptability was monitored by conducting human taste panel sensory evaluation trials. Linking nutritionally-enhanced production systems directly to improved sensory perception and human health may increase consumer demand for quality produce.

Specific Objectives

To compare the effects of typical conventional apple production to biologically-enhanced organic and biologically-enhanced conventional apple production on:

1. Fruit antioxidant properties
2. Human glycemic response
3. Fruit soluble solids levels
4. Human sensory perception of fruit --flavor, texture, appearance
5. Fruit shelf life

Overview of Health Benefits

Numerous scientific studies support the health benefits of consuming fruits and vegetables due to their vitamin, mineral, fiber, and antioxidant content (La Vecchia et al., 2001; Riboli and Norat, 2003; van't Veer, et al., 2000). As a result of the many studies, dietary guidelines in the United States and most other countries have evolved to recommend a greater intake of fruits and vegetables (Nestle, 1996; USDA, 2005). Unfortunately, the U. S. population has continued to fail to meet the recommended intake levels (Guenther, 2006). Only approximately 40% of Americans consume the USDA recommended five or more servings per day for fruits and vegetables (Guenther, 2006). To complicate matters, there are wide variances in nutrient content within each individual class of produce, due to cultivar variations and growing conditions (Al-Turki and Stushnoff, 2007; Liu et al., 2007). In addition, there have been significant declines in nutrient content of produce, including calcium, magnesium, potassium, iron, copper, carotenoids, riboflavin and ascorbic acid over the past 60-70 years (Andersen, 2006; Davis, 2009; Davis et al., 2004; Mayer, 1997; White and Broadley, 2005). Furthermore, the nutrient profile of the diet may also be affected by the types of fruits and vegetables selected for consumption, as well as preparation methods (Johansson et al., 1992).

Increasing nutrient and antioxidant content in foods can be helpful on a number of fronts. It is generally recognized among health researchers that inflammation is the basis for many disease processes. Inflammation is associated with cancer (Nelson et al., 2002), cardiovascular disease (Paoletti et al., 2006), diabetes (Browning and Jebb, 2006) and obesity (Wang and Cai, 2006), all of which are huge problems facing our society. Full spectrum nutrition, including the full array of bioavailable trace elements (some of which have yet to be studied in depth) and phytonutrients, may help reverse the inflammatory process (Rahman et al., 2006).

Beyond preventing or ameliorating disease conditions is the promotion of ideal health and longevity. Nutrition influences genetic expression, so high quality, full-spectrum nutrition can promote vibrant health as well as longevity (Andersen, 2006).

One plausible reason for the failure of the populace to consume the recommended daily intake of fruits and vegetables is that many people do not greatly enjoy the taste of the produce commonly available (Wansink and Westgren, 2003). Some consumers are also deterred from purchasing the volumes of produce necessary to maintain a readily available supply in their homes due to the poor keeping qualities of the produce (Harrell, 1998). Therefore, research to improve the nutrient content, flavor and shelf life of produce may result in improving the health of the populace.

Apples are a popular fruit in much of the world, and U S citizens consume more fresh and processed apples than any other fruit (Economic Research Service, USDA, 1999). Apples have been shown through numerous scientific studies to decrease disease risk in those who consume them regularly (Boyer and Liu, 2004).

Overview of Production Systems

This study compared the quality of apples grown under biologically-enhanced organic and biologically-enhanced conventional systems with that from typical conventional systems. Nutrition begins in the soil and differences in cultivation techniques may impact the final fruit quality. A search of published scientific literature reveals research supporting the use of microbial (Shrestha et al.,1996) and nutritional soil amendments (Prado et al., 2005) to improve fruit quality. Fruit soluble solids concentration (typically measured in degrees brix) is correlated to sugars, and hence, to sweetness (Lester, 2006). There is limited literature relating fruit soluble solids content to soil properties (Aydin and Yoltas, 2003) and sensory perception (DeEll and Prange, 1992; Lester, 2006). Leaf nutrient content has also been correlated to fruit quality (Blanpied and Silsby, 1992; Godfrey-Sam-Aggrey, et al.,1979; Neilsen et al.,1982). Organic apples have been shown in some studies to have higher soluble solids content (DeEll and Prange, 1992), higher antioxidant levels (Peck et al., 2006; Weibel et al., 2000) and greater nutrient content (Smith, 1993; Weibel et al., 2000) than conventionally-grown apples. However, not all of the comparative studies showed differences (DeEll and Prange, 1993; Reig et al., 2007). Biological cultivation methods with nutrient and microbial enhancements may be particularly beneficial in some poorly performing organic orchards (Andersen, 2000).

Chapter 2

Literature Review

2.1 Human Health Justification for Studying Antioxidant Properties in Food

The antioxidant theory of disease, particularly inflammatory, degenerative diseases such as cancer and cardiovascular disease, is based on free radical molecules, which have unpaired electrons, damaging cell structures. Antioxidants quench the damaging action by donating electrons to the free radicals so that they may become stable, while not becoming unstable themselves. Fruits and vegetables contain many antioxidant phytochemicals which may offer some protection against this damaging oxidation.

Cancer is an enormous disease problem in the USA claiming the lives of over 500,000 people each year (NCI, 2009). Over 1.4 million U. S. residents per year are diagnosed with cancer. It is estimated that over 40% of the people born today will be diagnosed with cancer in their lifetime (NCI, 2009). After heart disease, it is the leading cause of death in the US (CDC, 2009). Fortunately, the risk of these and several other diseases may be decreased through proper nutrition. An estimated 30% of cancers may be prevented with a healthy diet (Willett, 1995).

Oxidative damage to DNA by free radicals can lead to mutations, strand breaks, cross-linking and other rearrangements which may ultimately lead to cancer if repair mechanisms and apoptosis cannot keep up with damage (Liu, 2003). Other degenerative diseases may be explained by free radical damage in various tissues, thus resulting in inflammation and degradation of the specific areas (Rahman et al., 2006). Phytochemicals found in fruits and vegetables have many effects *in vivo*, such as scavenging free radicals, regulating gene expression, and stimulating the immune system, all of which may decrease cancer risk (Liu, 2003).

Apples contain many types of phytochemicals, including strong antioxidant polyphenols such as flavonoids, dihydrochalcones, and chlorogenic acid, which may play roles in human health (Gerhauser, 2008; Lotito and Frei, 2006; Wojdylo et al., 2008). A review by Boyer and Liu (2004) suggests that apple intake may be helpful with numerous diseases, including cancer, cardiovascular disease, lung diseases, diabetes and obesity.

Mayer et al. (2001) performed both *in vitro* fluorescence oxidation assays and *in vivo* experiments with apples, finding both methods to show increased antioxidant capacity. Human subjects (N = 47) each consumed 1 kg of apples at one sitting daily. Antioxidant effects peaked at 3h post consumption and tapered back to baseline values in 24h.

Similarly, Maffei et al. (2007) performed a fresh apple consumption crossover trial with six male subjects. They found significantly increased antioxidant activity 3 and 6 hours post consumption of 600g of apples, tapering back to baseline levels in 24h.

In another crossover study with six subjects (Briviba et al., 2007), a comparison was made between organic and conventionally-produced ‘Golden Delicious’ apples. This double-blind, randomized study utilized 1000g portions. There were no differences in phenolic content or antioxidant capacity in plasma between orchard treatments, however both organic and conventionally-grown apples did provide increased antioxidant activity for DNA protection when challenged by iron chloride.

Nine different fruit juices, including apple juice, were compared for antioxidative effects in 10 male subjects (Ko et al., 2005). Subjects drank 150mL and blood was checked at 0, 30, 60, 90, and 120 min after consumption for plasma antioxidant status. Grape juice showed the greatest activity; while pear juice showed no significant antioxidant activity. Apple juice, along with orange, melon, peach, plum, watermelon, and kiwi juices resulted in significant plasma antioxidant activity increases during the time period from 30 to 90 min.

Another study concerning antioxidant capacity of apple juice used 12 subjects, each consuming 1L of juice (Chrzczanowicz et al., 2008). Blood was drawn 1h post ingestion and serum was checked for activity with DPPH, showing significant increases in antioxidant capacity over baseline values.

While these antioxidant studies with apple juice are interesting, they do not compare juice from different apple cultivars. Al-Turki et al. (2008) compared juice from 21 apple cultivars and 11 apple species for antioxidant activity finding large variations among them. Similarly, Stushnoff et al. (2003) compared juice and fruit tissue from 321 types of apples, also finding very large variations in antioxidant capacity.

Research with ‘Red Delicious’ apples showed that, while they are considered a source of the antioxidant vitamin C, 99.6% of their antioxidant capacity came from other phenolic phytochemicals (Eberhardt et al., 2000). The phytochemicals were more concentrated in the skins of the apples. Colon-cancer cell lines were treated with apple extracts of varying dilutions from 0-50 mg/mL and found to be inhibited in a dose-dependent fashion, with no cytotoxicity (Eberhardt et al., 2000).

A prospective cohort study of 77,283 women nurses (from the Nurses’ Health Study) examined apple and pear intake over 12 years as related to lung cancer risk (Feskanich et al., 2000). An increased intake of one serving of apples or pears per day resulted in a relative risk (RR) of 0.63 with a 95% confidence interval of 0.43-0.91. A relative risk (RR) of 1.0 would indicate that the increased fruit intake resulted in no difference in outcome, whereas a relative risk (RR) of less than 1.0 indicates decreased risk. The same research group examined similar data from a 10-year male health professionals’ prospective cohort study (N = 47,778) and found no effect.

Data from the Nurses’ Health Study (NHS) was also examined for colorectal cancer risk in relation to apple intake (Michels et al., 2006). Data from 34,467 women over a period of 18 years resulted in an approximately 20% less adenoma risk for the highest versus the lowest quintiles of consumption.

A case-control study in Hawaii looked at the effect of dietary flavonoids, which are potent antioxidants, on lung cancer risk in 582 diagnosed patients matched with 582 controls (La Marchand et al., 2000). Apples, onions and white grapefruit were the main dietary sources of flavonoids. Apples and onions have high levels of the flavonoid quercetin, and grapefruit contains high levels of the flavonoid narinigen. Using regression analysis, the intake of these produce items was found to be inversely associated with lung cancer. Apples had an odds ratio for the highest quartile compared to the lowest quartile of intake of 0.6 (P for trend = 0.03). The mechanism of action was thought to be inhibition of a P450 enzyme gene, CYP1A1, which results in decreased bioactivation of carcinogens.

Quercetin's antioxidant activity decreased DNA damage by free radicals (Duthie et al., 1997). In high concentrations, quercetin supressed malignant growth (Yoshida et al., 1999), or caused apoptosis (Csokay et al., 1997). It can block signal transduction pathways through kinase inhibition (Nishioka et al., 1989; Csokay et al., 1997) and decrease oncogene expression (Nishioka et al., 1989). Quercitin has also been shown to decrease estrogen receptor activity in estrogen-sensitive breast cancer cells (Miodini et al., 1999). Researchers at the Mayo Graduate School found quercetin to inhibit the expression and function of the androgen receptor, which is involved with prostate cancer (Xing et al., 2001). These same researchers also found quercetin to down-regulate mRNA levels of androgen-related genes, such as PSA (prostate-specific antigen), a marker commonly used in testing for prostate cancer.

While is it interesting and useful to understand the mechanisms of individual phytochemicals, there appears to be an additive and synergistic effect among them and other components present in fruits and vegetables (Gey, 1995; Liu et al., 2003). There are an estimated 8000 phytochemicals present in foods (Liu et al., 2003). The action of some phytochemicals, such as flavonoids which are associated with increased plasma antioxidant capacity *in vitro*, may not have a direct antioxidant function *in vivo*, but rather work in concert with and lead to the production of other compounds which results in increased antioxidant capacity *in vivo* (Lotito and Frei, 2006). Some isolated phytonutrients, such as beta-carotene and vitamin C, have been shown in human supplementation studies to be counter productive in typically supplemented amounts ([The] Alpha-Tocopherol, Beta-carotene Cancer Prevention Study Group, 1994; FDC Reports, 1996; Podmore et al., 1998). Therefore, it appears to be best to consume phytochemicals in the form of whole foods.

The large amounts of phytochemical antioxidants present in fruits and vegetables may be why the vegan diet, which tends to include substantially more produce than typical diets, has proven effective in decreasing the incidence of cardiovascular disease (CVD), cancer and other disease conditions (Bergesio et al., 2001; Liu, 2003; Ovesen, 2005). Antioxidant status of raw food vegans was compared to that of omnivores, and seen to have higher blood levels of beta-carotene, vitamin C, vitamin E and superoxide dismutase (SOD) (Rauma et al., 1995). Review of large numbers of studies indicates that consumption of dietary antioxidants in the form of fruits and vegetables have cancer and cardioprotective effects (Aviram et al., 2005; Liu, 2003). Beyond the antioxidant content of a plant-based diet rich in fruits and vegetables, the ability of this diet to maintain low levels of insulin and insulin-like growth factor-1 (IGF-1) appears to be linked to lower cancer and cardiovascular risk (Baserga, 2005; Giovannucci, 2003; McCarty, 1999; McCarty, 2004).

Cardiovascular disease (CVD) manifests in several ways including high blood pressure, stroke, heart failure and coronary heart disease, which includes myocardial infarction (heart attack) and angina pectoris (American Heart Association, 2008). Cardiovascular disease is estimated to afflict more than 80 million people in the USA and claims the lives of approximately 870,000 of these people annually (American Heart Association, 2008). The World Health Organization (WHO) estimates that CVD represents 30% of all global deaths, making it the leading cause of death worldwide (World Health Organization, 2008).

In cardiovascular disease, it is thought that the free radicals oxidize LDL cholesterol which is taken up by macrophage scavenger receptors, resulting in cholesterol ester accumulation and foam cell development (Liu, 2003). These in turn promote atherosclerosis. Antioxidants can break this pathway by neutralizing the free radicals.

Currently, many health professionals rely on dietary recommendations similar to those published by the National Heart, Lung and Blood Institute (NHLBI), a division of the National Institutes of Health (NIH), for patients at risk for or diagnosed with cardiovascular disease. The NHLBI launched the National

Cholesterol Education Program (NCEP) in 1985 with the goal of reducing the prevalence of elevated cholesterol, and thereby reducing CVD (NHLBI, 2008). NCEP established dietary intervention programs for cardiovascular disease called Step I and Step II diets (Yu-Poth et al., 1999). Their current cholesterol-lowering diet, termed the “TLC Diet”, evolved from these earlier versions, with the TLC diet being nearly identical to the Step II diet. TLC is an acronym for “Therapeutic Lifestyle Changes.” The National Cholesterol Education Program is associated with 28 national health organizations including the American Dietetic Association (ADA) (2009); therefore, the TLC Diet is widely known and accepted by the medical community. The TLC Diet aims to decrease dietary intake of cholesterol, saturated fat, and trans fat while maintaining a varied diet including five to nine servings of fruits and vegetables per day (NHLBI, 2008). Inclusion of this amount of produce would be a significant change for many Americans who consume much less than the typically recommended five servings per day (Guenther, 2006). A meta-analysis of 37 Step I and Step II dietary intervention studies revealed significant reductions in plasma lipids and lipoproteins (Yu-Poth et al., 1999). The Step II diet resulted in the following decreases: total cholesterol (TC) 13%, low-density lipoprotein (LDL) 16%, triglycerides (TG) 8%, and total cholesterol (TC) to high-density lipoproteins (HDL) ratio 7%. The direction of these science-based recommendations has been derived from a large database of research studies, however the question remains as to how much of each dietary food component is optimal. Further increases in fruit and vegetable intake may be beneficial.

Vegan diets, which eschew all meat, fish, eggs and dairy products, tend to include more servings of fruits and vegetables, and may be even more effective against diseases. For example, the Hallelujah Diet is a vegan diet in which most foods are consumed raw, with only 15% of foods cooked (Hallelujah Acres, 2008). This diet increases the intake of fruits and vegetables well beyond the typically recommended five to nine servings per day. A survey of 141 long-term adherents to the Hallelujah Diet revealed a mean daily consumption of 6.6 servings of fruits and 11.4 servings of vegetables, for a combined total of 18 servings of produce per day (Donaldson, 2001). This quantity of fruits and vegetables provides large amounts of fiber, vitamins, minerals, and naturally-occurring antioxidants including polyphenols, flavonoids, carotenoids, vitamin E and vitamin C, all of which may favorably influence the risk of cancer, CVD and other diseases. A large amount of strong anecdotal evidence exists for this type of diet in relation to improved disease markers (Hallelujah Acres, 2008), as well as several scientific studies. A study of fibromyalgic subjects on this raw, vegan diet for several months resulted in 19 of 30 subjects experiencing significant improvement of symptoms such as pain, fatigue and range of motion (Donaldson et al., 2001).

Osmo Hanninen’s review (Hanninen et al., 1999) of diets studied with uncooked, vegan “living food” showed many favorable changes for subjects. This diet, consisting of germinated seeds/grains, sprouts, vegetables, fruits and nuts, contains a large amount of antioxidants. Consistent with the above results, this diet also showed fibromyalgic subjects improving their joint stiffness, pain and sleep quality (Hanninen et al., 2000). Rheumatoid arthritis patients also experienced decreased pain, stiffness and joint swelling, all of which became worse when they reverted to their previous diets. In addition, a lowering of serum cholesterol in subjects was observed after adoption of the diet (Hanninen et al, 1992).

A serum lipid study reported by Agren et al. in 2001 involved 29 subjects with rheumatoid arthritis. Sixteen subjects were placed on an uncooked vegan diet, while 13 subjects continued with their normal diets, serving as controls. Total serum cholesterol, LDL and phospholipids decreased significantly ($p < 0.001$) after one month in every vegan subject while no changes were realized by the control group.

Rauma et al. (1995) compared the uncooked vegan diet to the typical Finnish diet ($N = 42$) for antioxidant status. The vegans consumed approximately twice the amount of fruits and vegetables as the controls. The vegans had higher blood concentrations of beta-carotene, and vitamins C and E, as well as greater activity of erythrocyte superoxide dismutase (SOD), indicating better overall antioxidant status.

In addition, there have been numerous studies of vegan diets which include cooked foods. These have also demonstrated effectiveness in positively altering blood lipid profiles and antioxidant status (Barnard et al., 2000; Barnard et al., 2006; Bergesio et al., 2001; Bergesio et al., 2005; Fontana et al., 2007; Resnicow et al., 1991).

The study by Resnicow and co-workers, published in 1991, compared blood lipid levels of 31 vegans to values from the Lipid Research Clinics Population Studies (Ernst et al., 1980). The mean lipid levels were lower for the vegans, with mean serum total cholesterol 3.4 mmol/L lower, low-density lipoprotein (LDL) 1.8 mmol/L lower, and triglyceride 0.8 mmol/L lower.

A crossover-design study led by Dr. Neal Barnard examined the effect of a low-fat vegan diet on serum lipids of 35 healthy premenopausal women (Barnard et al., 2000). This diet consisted of grains, legumes, fruits and vegetables, without nuts, oils, or other high fat foods. The five-week vegan diet intervention resulted in significant drops ($p < 0.001$) in total cholesterol (-13.2%) and LDL (-16.9%), with no significant changes during the supplemented (placebo) control phase.

A dietary study of chronic renal failure patients (Bergesio et al., 2001) resulted in reducing lipid peroxidation (LP), triglycerides (TG), and lipoprotein A (LpA) in the vegan group as compared with the control subjects. In addition to being a risk for cardiovascular disease, lipid peroxidation is considered to result in progression of renal disease.

Another study by Bergesio et al. (2005) of cardiovascular risk factors in severe chronic renal failure patients on a vegan diet (n=29) compared with 31 control patients showed numerous improved markers for those following the vegan diet. HDL cholesterol increased ($p < 0.005$), LDL decreased ($p < 0.01$), oxidized LDL (OxLDL) decreased ($p < 0.05$), thiobarbituric acid-reactive substances (TBARS), which is a measure of antioxidant activity, decreased (p <

0.01), homocysteine (Hcy), an oxidizing agent, decreased ($p < 0.002$), lipoprotein A (LpA) decreased ($p < 0.002$), and C-reactive protein (CRP), a marker of inflammation, decreased ($p < 0.05$) in vegan diet subjects compared with those on the conventional control diet.

A study by Barnard et al. (2006) which examined lipid levels in type 2 diabetics (N=99) compared a low-fat vegan diet with the American Diabetes Association (ADA) diet. The ADA diet contained five or more servings per day of fruits and vegetables, while the vegan diet included seven or more servings per day. The ADA diet improved lipid values (total cholesterol decreased 9.7% and LDL decreased 9.3%), however the vegan diet performed better, with reductions in total cholesterol of 17.6% ($p = 0.013$) and LDL of 21.2% ($P = 0.023$) over 22 weeks of intervention. In addition to improving lipids levels, both diets reduced hemoglobin A1C, a marker of blood glucose control. Again the vegan diet outperformed the ADA diet with a decrease of 0.96 percentage point versus 0.56 point ($p = 0.089$).

Fontana et al. (2007) examined subjects on a long-term, low-calorie, low-protein vegan diet (n=21) with healthy controls on a conventional Western diet (n=21) for cardiovascular disease risk. Plasma lipids, lipoproteins, glucose, insulin, C-reactive protein (CRP), and blood pressure were all lower ($p < 0.05$) in the vegan group as compared to those on the Western diet.

An epidemiological study of the effect of flavonoid intake on cardiovascular disease mortality showed an inverse relationship (Mink et al., 2007). The study involved 34,489 postmenopausal women from the Iowa

Women's Health Study tracked over a 16-year period. Intakes of several flavonoid-rich foods, including apples, were each associated with decreased CVD mortality.

After cardiovascular disease and cancer, stroke is the third major cause of mortality in the USA, with over 160,000 deaths annually (CDC, 2009). More than 700,000 strokes occur annually in US citizens causing debility, if not death. A dose-response correlation has been made between fruit and vegetable consumption and stroke incidence (Joshipura et al., 1999). This was shown in a prospective study with follow-up data on 77,283 women subjects from the Nurses' Health Study over a 12-year period (Feskanich et al., 2000). A difference of one additional serving of fruit or vegetables per day resulted in a 6% decreased risk of stroke. A particularly protective relationship was seen with consumption of cruciferous vegetables, leafy greens and citrus fruits.

High blood pressure, a major risk factor for stroke, can be decreased through dietary intervention. A six-month trial with 690 healthy subjects randomly assigned to dietary intervention and control groups, with the intervention group consuming 1.4 servings more of fruits or vegetables daily, resulted in a significant decrease in blood pressure in the intervention group (John et al., 2002). An eight-week clinical trial of the DASH (Dietary Approaches to Stop Hypertension) diet, which includes five to nine servings per day of fruits and vegetables, also resulted in significant decreases in blood pressure readings (Appel et al., 1997).

Blood glucose and insulin levels have been correlated with inflammation and disease risk (Brand-Miller, 2004). Diabetes, a serious disorder in which there is a large amount of circulating blood glucose, is a major cause of morbidity and mortality in the USA (CDC, 2009). Left untreated it can result in poor circulation, neuropathy, retinopathy leading to blindness, nephropathy leading to kidney failure, and heart disease (ADA, 2009a). Many of the complications are due to glycosylation of the tissues and collagen-linked advanced glycosylation end products (AGEs) (Beisswenger et al., 1993).

The incidence of type 2 diabetes has risen sharply over the past decade. Between 1997 and 2003 alone there was a 41% increase ($p < 0.01$) in incidence of diagnosed diabetes, rising from 4.9 to 6.9 per 1000 population (Geiss et al., 2006). By the survey period of 2005-2007, the incidence had risen to 9.1 per 1000 population, which equates to a 90% increase in 10 years (CDC, 2009). These data appear to parallel the rise in obesity, which is a risk factor for diabetes (Sturm, 2007).

Currently available information on the glycemic index (GI) of fruit is limited. A search of the literature shows that some work has been done with blood glucose and insulin scoring (Holt et al., 1997), and establishment of a glycemic index (Foster-Powell et al., 2002) for apples as a group, however these studies did not address differences due to variations in soluble solids content values or cultivation methods within the same cultivar. The International Table of Glycemic Index and Glycemic Load Values (Foster-Powell et al., 2002) contains six GI listings for raw apples of mixed, largely unspecified, cultivars ranging from 28 to 44. Five of these studies were performed on type 2 diabetics. The other study involved healthy subjects, and reported a GI of 39. Cherries have only one listing in the glycemic index (GI = 22); grapes only three listings and large variability (Foster-Powell et al., 2002). There is no indication of how the fruits were produced, or information on differences in SSC or nutrient levels, hence the need for additional data. This study examined the correlation of glycemic responses to soluble solids concentration (SSC) levels in apple fruit.

Clinical and field observations of diabetic patients have surprisingly shown flatter blood glucose response curves to high soluble solids content biologically-grown fruits than to lower soluble solids content conventionally-grown fruits of the same cultivar (Tainio, 2007a). Diabetics who had been previously restricted on fruit consumption by their physicians were able to consume the high soluble solids content, biologically-produced fruit ad libitum without glycemic concerns. Subsequent consumer demand for these high soluble solids content fruits brought exceptional returns for the producers.

Before starting our main body of research, preliminary blood glucose trials conducted by Jana Bogs (N = 1) were performed with biologically-produced apples and cherries with results showing a markedly lower glycemic response than that from conventionally-produced fruit, even though the biologically-produced fruit had higher soluble solids content levels.

Possible reasons for the decreased glycemic response may be differences in amounts and types of sugars (MacDonald et al., 1978; Ha et al., 1992; Lunetta et al., 1995; Wills et al., 1998), varying levels of organic acids (Wills et al., 1998), amounts and types of fiber (Haber et al., 1977), varying levels of minerals (Wills et al., 1998), and/or delayed gastric emptying due to high osmolality (Bolton et al., 1981) and nutrient concentration (Wills et al., 1998). Another reason may involve levels of phytochemicals. Apples particularly, but also some other fruits, contain a polyphenolic dihydrochalcone compound, phlorizin, which lowers blood glucose (Ehrenkranz et al., 2005). If biologically-produced fruit contains a higher level of this compound, the lower glycemic response may thereby be at least partially explained.

A benefit of consuming fresh fruits in place of more calorically-dense foods is weight normalization. Obesity and overweight conditions contribute to inflammatory conditions and are considered risk factors for several disease conditions, including CVD, cancer and diabetes (Finkelstein et al., 2003). There has been an alarming rise in obesity in the USA over the past decade, with resulting health care costs estimated at over $92 billion per year (Finkelstein et al., 2003). A 24% increase in obesity, defined as BMI over 30, occurred between 2000 and 2005 alone (Sturm, 2007). Worse, the prevalence of morbid obesity (BMI > 50) increased by 75% during the same time period. The increase in obesity has essentially paralleled, and is considered a major factor in, the increased incidence of type 2 diabetes, making obesity a genuine medical concern (Geiss et al., 2005).

Various types of food in portions of equal caloric content have varying abilities to produce satiety (Holt et al., 1995). Due to an abundance of fiber and nutrients, consumption of large amounts of fresh produce can lead to satiety without supplying an overabundance of calories. A 12-week weight-loss study with 411 women randomly assigned to consume three apples, three pears, or three oat cookies as a supplement to their daily diets found significantly more weight loss with the fruit-supplemented diets (Conceicao de Oliveira et al., 2003). A two-year weight loss trial by Turner-McGrievy et al. (2007) compared a low-fat vegan diet with the National Cholesterol Education Program (NCEP) diet. The trial used 62 overweight premenopausal women randomly assigned to a diet for 14 weeks. The NCEP diet contained five or more servings of fruit and vegetables per day, while the vegan diet included seven or more servings of fruit and vegetables per day. Both groups lost weight, however the vegan group lost more weight ($p < 0.05$).

Blood glucose levels and the glycemic index have been correlated with weight concerns (Zavaroni et al., 1994). Realizing the value of fruits, the American Dietetic Association (ADA) and the Dietary Guidelines for Americans (USDA, 2005) recommend an average of two cups of fruit per day, which varies with caloric needs. The American Diabetes Association (ADA) also follows the same recommendation, but in addition advises carbohydrate counting to help regulate blood glucose. The widespread availability of low-glycemic fruit could be helpful for many individuals, especially diabetics.

2.2 **Production Systems**

Management systems typically fall along a spectrum from conventional, utilizing manufactured chemical fertilizers, herbicides, fungicides and pesticides, to certified organic, which is a tightly regulated system that prevents the use of manufactured chemical additives. Our research compares typical conventional management to biologically-enhanced organic and biologically-enhanced conventional management. Biological management is an environmentally-friendly system which may be applied within certified organic systems, but goes beyond merely remaining within organic regulations to specifically promoting beneficial microbial activity and providing nutrient applications to the soil and plants with the goals of increasing yield, quality and nutrient density (Andersen, 2000; Sait, 2003; Skow and Walters, 1995).

Some growers have implemented biological protocols with great success, yielding abundant, even award-winning crops of supreme quality (Beyersdorf, 2007). Biological growers have also reported greatly extended shelf life and higher soluble solids content (Tainio, 2007a). Contrary to popular opinion that high soluble solids content in apples equates to short shelf life, nutritionally-balanced fruits are not subject to rapid senescent breakdown (Skow and Walters, 1995). Furthermore, growers have reported substantial decreases in insect pest and disease problems (Beyersdorf, 2007; Sait, 2003).

2.2.1 Organic Production vs. Conventional Production

A considerable amount of research has been done comparing the quality of production from various management systems (Peck et al., 2006; Worthington, 2001). Organically grown produce often has higher amounts of nutrients such as vitamin C and some minerals, and higher levels of various antioxidant compounds as compared with conventionally-grown produce (Peck et al., 2006; Weibel et al., 2000). A meta-analysis of 41 studies comparing nutritional quality of organic versus conventional produce showed overall results of organic crops containing significantly more vitamin C, iron, magnesium and phosphorus, and significantly less nitrates than conventional crops (Worthington, 2001). Research reported in the Journal of Applied Nutrition (Smith,1993) showed higher levels of essential minerals and lower levels of toxic elements in organically-grown apples, pears, potatoes and corn as compared with conventionally-grown produce.

Peck et al. (2006) at Washington State University reported consumer panels rating organically-grown Gala apples superior in overall acceptability, firmness, and texture over conventionally-grown Gala apples. The organic apples also had higher total antioxidant activity (TAA).

In addition to vitamin C, some plant-based polyphenols are potent antioxidants (Prakash et al., 2007). Organically-produced grapes had higher amounts of polyphenol oxidase than those conventionally grown (Nunez-Delicado et al., 2005), which may increase disease resistance in plants (Ohazurike and Arinze, 1996). Organically-grown kiwi fruits had higher concentrations of minerals and antioxidants (ascorbic acid and total phenols) than conventionally-grown kiwi fruits from the same farm (Amodio et al., 2007).

A peer-reviewed German study showed biodynamically-raised (similar to biological/organic management) carrots to have higher dry matter and sugar content, lower nitrate content, and longer shelf life than conventionally-grown carrots (Fleck et al., 1998). A U.S. study comparing organic with biodynamic management showed biodynamically-grown grapes to have a higher soluble solids concentration than the organically-grown control (Reeve et al., 2005). Worthington (1999) also compared conventional crops to biodynamic and organic crops, using 1230 comparisons. The organic and biodynamic crops had higher nutrient levels or lower nitrate levels 56 and 59 percent of the time, respectively, compared with the conventionally-grown crops. The average organic crop had 14 to 30% more vitamin C,iron, calcium, potassium and magnesium, than the corresponding conventional crop.

Brandt and Molgaard (2001) reviewed the differences between organic and conventionally-raised foods as related to human health, noting that the seemingly most important difference is higher levels of phytochemicals in organic produce.

Zhao et al. (2006) reviewed literature comparing organic with conventional production effects on phytochemicals, also finding higher levels in organically-grown produce. Gyorene et al. (2006) also reviewed studies comparing organically-raised food with conventionally-raised foods finding organic foods to have higher antioxidant (vitamin C, flavonoids, polyphenols) levels, higher dry matter and mineral content, and lower levels of contaminants (pesticides, heavy metals, nitrates).

A review by Srivastava et al. (2002) found several areas in which organic fruit production is superior to conventional production—better storage due to lower moisture content, more nutritious, better flavor, greater resistance to pests and diseases, suppression of soil-borne pathogens, and better soil structure. Excess nitrogen from inorganic fertilizers can result in decreases in produce flavor and biological value of protein, as well as decreased resistance to pests and diseases (Chaboussou, 2004; Sankarum, 1996). Fallahi and Mohan (2000) tested four levels of nitrogen application in Scarlet Gala apples finding the lowest level of nitrogen application resulting in the best fruit quality and yield, as well as the least incidence of fire blight. Apple fruit coloring is negatively correlated to fruit nitrogen levels (Fallahi et al., 2006). Amiri et al. (2008) found urea (46% N) applications, disallowed by organic standards, decreased soluble solids content (sugars) and resulted in russeting in Golden Delicious apples. Dris et al. (1999) also found a decrease in fruit soluble solids concentration with increasing leaf nitrogen. Excess phosphorus application results in a “dilution effect” where phosphorus acts to increase dry matter and effectively displace other nutritionally-important minerals in the plants (Davis, 2009; Jarrell and Beverly, 1981).

Apple research in Washington state comparing organic and conventional management systems showed organic systems to have higher soil quality and potentially lower negative environmental impact (Reganold et al., 2001). The organic apples were sweeter and less tart. The authors concluded that the organic system was more economically sustainable.

Not all studies of organic production versus conventional production have shown beneficial effects associated with organic production (DeEll and Prange, 1993). This may be due to the organic system failing to address plant nutrient needs satisfactorily, especially when fields have been recently changed from conventional to organic production (Andersen, 2000). Clearly, successful organic fruit production is multi-factorial (Weibel et al., 2007). Biological cultivation methods with nutrient and microbial enhancements may be particularly beneficial in some poorly performing organic orchards (Andersen, 2000). Fortunately, biological management focuses on practical, cost-effective methods of rebuilding soil health for maximum yield and quality production (Andersen, 2000; Sait, 2003).

2.2.2 Biological Production vs. Conventional Production

Biological management is a system that promotes beneficial microbial activity and nutrient applications to the soil and plants with the goals of increasing yield, quality and nutrient density while simultaneously caretaking the environment.

Soil microbial activity increases mineral availability to plants (Morgan et al., 2005; Shrestha et al., 1996). Soil microbes also increase plant growth, and improve water regulation and carbohydrate metabolism (Srivastava et al., 2002). A greater volume of soil microorganisms in organic systems contributes to soil stability by binding soil particles and decreasing wind (Williams et al., 1995a) and water (Williams et al., 1995b) erosion. Citrus fruit trees inoculated with mycorrhizal fungi grew larger and produced larger fruit with a higher sugar content and better peel color (Shrestha et al., 1996).

Biological management involves testing of soil and plant tissues, and application of nutrients, both on the soil and foliage. For example, spraying apple trees with calcium and boron solutions may result in firmer fruits with decreased incidence of bitter pit (Wojcik, 2002). Porro et al. (2002) experimented with foliar nutrient sprays containing Ca, N, B, Zn and Fe on ‘Golden Delicious’ apples, and found improvements in fruit quality, including increased fruit size and decreased incidence of bitter pit. Lotze and Theron (2006) also worked with ‘Golden

Delicious' apples using foliar calcium sprays and also found decreased incidence of bitter pit. Stampar et al. (2003) examined nutrient status in four apple cultivars over a four year period utilizing soil, leaf and fruit tissue. Needed nutrients were applied which resulted in increased yields while maintaining fruit quality at optimal levels. Marcon and Ferraro (2001) monitored 'Golden Delicious' apple orchards from 1989 to 2000 fertilized according to soil and leaf analyses, which led to a reduction in nitrogen and phosphorus inputs while maintaining fruit quality and yield. Awasthi et al. (1998) assessed nutrient status in 20 apple orchards using soil and leaf analyses, finding positive correlations between N, Mg and K, and fruit yield. Neilsen and Neilsen (2006) used potassium fertigation on potassium-deficient apple orchards to increase fruit size, titratable acidity and fruit color in several cultivars.

2.2.3 Environmental Impacts

Biological production of nutrient-rich foods can have many positive environmental impacts (Andersen, 2000; Chaboussou, 2004; Sankaram, 1996; Skow and Walters, 1995; Srivastava et al., 2002; Tainio, 2007b). First, these foods can only be produced from soil that is properly managed without excessive use of inorganic nitrogen or other chemical fertilizers which pollute ground water. Second, healthy, nutrient-rich plants do not experience the disease and pest pressure that deficient plants do, hence reducing or eliminating the use of chemicals such as fungicides and pesticides. Third, nutrient-rich foods deliver more nutrition in a compact fashion, decreasing shipping costs in both monetary and environmental ways. Fourth, produce with extended shelf life is more likely to be consumed than discarded, which would prevent huge losses on several fronts.

Giuffre et al. (2003) studied the nutritional status of apple orchard soils finding greater carbon sequestration in older orchards. Adequate soil potassium was found to be critical for fruit quality. Proper monitoring and fertilization may result in longer orchard life and environmental benefits due to greater carbon sequestration.

Reganold et al. (2001) examined the comparative sustainability of three apple production systems—organic, conventional and integrated. They compared a number of parameters including yield, fruit quality, costs, environmental impacts, and energy requirements. Organic production was found to be the most energy efficient, profitable, and environmentally sound. It also produced the best tasting apples. Integrated management was second best overall, while conventional management ranked last.

2.3 **Soluble Solids Content**

Measurements of soluble solids content (SSC), also known as total soluble solids (TSS), with a refractometer provides a convenient method of estimating sugar levels (Gutzwiler, 2007). Refractometers are inexpensive (typically $100-400), simple-to-use, hand-held instruments. The refractive indexes for the total soluble solids content are measured and often reported as % brix (or ° brix). Refractometers have been used for many years in various aspects of the food industry (Harrell, 1998). Refractometers have also been used by some commercial food producers and agriculturalists to help them determine crop quality (Andersen, 2000). Checking SSC in fruit is one method producers use to determine when to harvest fruit. For many years USDA standards in the table grape industry have included minimum SSC (USDA, 2007). For apples, SSC is measured along with firmness (using a penetrometer), color, and starch content, using the starch-iodine test (Blanpied and Silsby, 1992; Fadanelli et al., 2005). During ripening, starch is converted to sugars. Firmness and starch generally decrease as SSC and color increase. There have been notable correlations between high soluble solids concentration and improved taste and shelf life across a large variety of fruits and vegetables (Andersen, 2000; Beyersdorf, 2007; Lester, 2006; Skow and Walters, 1995). A difference of 1.5 ° brix is distinguishable by human sensory perception (Fujiwara and Sakakura, 1999).

Optimal plant nutrition is essential for producing high SSC fruit (Skow and Walters, 1995; Andersen, 2000; Tainio, 2007b). Clay soils hold nutrients better than sandy soils (Andersen, 2000). Lester (2006) found that growing melons in clay, as opposed to sandy soils, resulted in higher SSC. Dr. Carey Reams (an agronomist with a large nutrition and agricultural laboratory) compared the SSC values to overall produce quality including nutrient content, taste and shelf life. He published a chart of refractive indices for 52 fruits and vegetables (Reams, 1972). This chart lists apples with a brix of 6 as poor quality, a brix of 10 as average quality, a brix of 14 as good quality, and a brix of 18 as excellent quality. It is very difficult to find an 18 brix apple in today's marketplace. Reams claimed that as the SSC values of fruits and vegetables rise, so does their nutritional content, sweetness and length of shelf life. The juice SSC comprises minerals, vitamins, sugars, proteins and various phytochemicals which lend to the quality and taste parameters. Another observation was that nitrate levels decrease with increasing SSC (Reams, 1972). Nitrates are considered potentially toxic with implications for increasing cancer through formation of nitrosamines (Onyesom and Okoh, 2006), and causing methemoglobinemia in infants (Greer and Shannon, 2005).

Organic fertilizers were compared with chemical (NPK) fertilizers at various rates of application on orange trees by Tayeh (2003). The organic applications (chicken manure plus bio-fertilizer) resulted in the best quality fruit with the highest soluble solids and ascorbic acid content.

DeEll and Prange (1992) found that organically grown ‘McIntosh’ and ‘Cortland’ apples had higher soluble solids than those conventionally grown. Reig et al. (2007) compared organically and conventionally grown ‘Fuji’ and ‘Golden Delicious’ apples finding higher soluble solids in those grown organically, as well as other parameters of better quality, including increased firmness and acidity.

2.4 **Consumer Preferences**

Current standards of produce quality, i.e. “extra fancy, grade A apples”, focus on size and appearance, with little regard for nutritional value or taste. To bring about a change in standards there must ultimately be a demand by the consumer. It stands to reason that every consumer would enjoy better quality produce, and many will spend extra money to purchase what they perceive as better quality. As an example, the sales of organically-grown fruits and vegetables continue to increase approximately 20% per year despite the higher cost (Organic Trade Association, 2005). Overall organic sales have increased nearly 20% annually since 1990, with current sales at approximately $23 billion annually (Organic Trade Association, 2009).

In a recent presentation before a crowd of 2,000 people, John Mackey, founder and CEO of Whole Foods Market, explained that he is developing a tiered rating system for organic farms in response to the public's demand for the use of improved agroecological methods (Powell, 2007). This rating system, which includes soil health, will be managed by established organic certifiers.

In most cases, farmers are not rewarded for producing fruits and vegetables which have a high nutrient density, taste good and have a long shelf life, but are paid only for volume (weight), with premiums for appearance and preferable size(s). Changing grading standards to include quality parameters of nutrient content, taste and shelf life, for which farmers would be paid premiums, will encourage farmers to rise to these better standards.

Near infrared (NIR) spectroscopy is used to predict SSC in some apple packing houses to determine fruit sweetness without damaging the fruit (Iyo and Kawano, 2001). Zude et al. (2005) worked with apples specifically, using an acoustic impulse resonance frequency sensor and a VIS (visible wavelength range)/NIR spectrometer, finding good correlations with these non-destructive tests to fruit firmness and SSC. Some commercial produce buyers are currently using refractometers to help them in purchasing decisions (Marler, 2006). They “buy on brix and sell on brix”. A grocery store in Seattle displays brix readings on individual produce items for its customers. It is a method that is working well to increase produce sales. The customers are being educated to look for quality beyond size and appearance.

Appropriate farming inputs may create produce with a greater nutrient density, but producers need simple methods to use in the fields to help them determine input needs (Andersen, 2000; Sait, 2003). Growers may be able to use SSC tests on fruit to help determine overall quality, and use the information to improve crops. Some farmers are already using refractometers to test their crops at various stages of growth and help determine appropriate nutrient and microbial applications to increase quality, disease/pest resistance and yield (Andersen, 2000; Sait, 2003). Soil properties of mineral availability, organic matter levels and pH have been positively correlated to SSC of tomatoes (Aydin and Yoltas, 2003). Ubi et al. (2006) correlated SSC in pineapple to various levels of nitrogen application to determine ideal application rates.

Research on stone fruit quality involving sensory panels and shelf life has measured SSC in an attempt to create a flavor code classification (Crisosto et al., 2006). Melon research correlated flavor with sweetness and sweetness with SSC (Lester, 2006). Azodanlou et al. (2003) positively correlated brix (total sugar content) with overall sensory appreciation of tomatoes and apricots.

DeEll and Prange (1992) reported that organically-grown apples had a higher soluble solids concentration than conventionally-grown apples. Sensory panels in their study also perceived the organically-grown ‘McIntosh’ apples to be firmer at harvest than those conventionally grown.

Consumers have reported more rapid and sustained satiety with high SSC fruit versus low SSC fruit (Harrell, 1998). This may be due to a variation in composition or increased density of components such as sugars and/or fiber (Bolton et al., 1981; Haber et al., 1977). Maintenance of appropriate body weight has been shown to decrease disease risk and increase longevity, however this has been difficult for many people to achieve due to lack of satiety. It has been observed that nutrient-rich produce satisfies consumers’ appetites much more readily than consumption of lesser quality produce (Harrell, 1998). This may have huge implications for prevention and treatment of obesity. Nutrient-rich foods may provide the solution of satisfying hunger and nutrient requirements while keeping caloric intake low.

2.5 **Shelf Life**

Fruit firmness is a primary goal in the fruit industry (Peck et al., 2006). Quality control evaluations for apples involve physical force penetration and assessment of starch levels because apples lose firmness and convert starch to sugars over time (Blanpied and Silsby, 1992; Fadanelli et al., 2005). Acoustic sensors are also used to determine stiffness, a function of cell turgidity, which correlates with water content (Roth et al., 2005). Apple shelf life can be related to mineral content. Low concentrations of calcium and phosphorus, and high concentrations of nitrogen are correlated with decreased shelf-life (DeEll and Prange, 1992). For example, browning of fruit flesh has been correlated to lowered calcium content (Neilsen et al., 1982). Leaf and fruit content of calcium was shown to increase when calcium was added to the soil (Prado et al., 2005). Approximately 60% of cellular calcium is contained in the cell wall, increasing the cell wall integrity (Tobias et al., 1993). This lends to improved firmness and longer shelf life (Prado et al., 2005). Field and postharvest treatments with calcium have been effective in increasing apple fruit calcium content and improving storability (Mignani and Bassi, 2005). Casero et al. (2004) showed a positive correlation between fruit calcium and firmness, and a negative correlation between fruit calcium and bitter pit, in 'Golden Smoothee' apples. Fallahi et al. (2006) found the same correlations in other apple cultivars. Porro et al. (2006) recommends foliar calcium sprays on apples during the growing season to lessen the incidence of bitter pit. Increased cell wall integrity may also lend to increased disease and pest resistance (Follett et al., 1981).

Consumers enjoy firm fruit, but it must also be mature, so that it has developed its sweetness, color and flavor (Blanpied and Silsby, 1992; Neri et al., 2005). Maturity has also been judged by measuring ethylene gas from the core of apples. A rise in ethylene gas is correlated to increased maturity, and tends to be associated with senescent breakdown, however this is not seen in all cases (Blanpied and Silsby, 1992). The ability of fruit to ripen to an excellent level of sweetness, yet remain firm for an extended period of time may be a function of nutrient balance, such as the above mentioned calcium content.

A Swiss consumer study on apple freshness utilized 4,758 data sets obtained on six apple cultivars (Peneau et al., 2006). Consumers rated flavor, aroma and freshness as the most important parameters for apple selection. Freshness was also judged by crispness and juiciness.

Peck et al. (2006) examined Washington state ‘Gala’ apples for firmness under varying cultivation systems, using both consumer panels and laboratory measurements. Consumers rated apples produced by organic and integrated management better in firmness, texture and overall acceptability than conventionally-produced apples. Laboratory measurements coincided with consumer ratings, with organic apples being firmest, integrated management second, and the conventional least firm. After six months of controlled atmosphere (CA) storage, 90% of the organic apples were still considered to be of acceptable firmness compared with only 64% of conventional apples and 42% of integrated apples.

Fadanelli et al. (2005) examined controlled atmosphere (CA) storage of ‘Golden Delicious’ apples for eight months on several parameters. They found, surprisingly, that ascorbic acid and polyphenols increased over time. Low crop load (low production by trees) also tended to result in increased polyphenols after storage.

2.6 **Justification for this Research**

Consumption of fruit is important for human health due to its content of vitamins, minerals, fiber and antioxidants. Intake of fruits and vegetables is negatively correlated to the incidence of degenerative diseases. Organically-grown fruits have grown in popularity due to environmental and health concerns, and in some cases, consumer taste preferences. Research efforts continually strive to produce better quality fruit with longer shelf life, produced under environmentally-sensitive management practices.

This study compares biologically-enhanced organic and biologically-enhanced conventional management of apples to typical conventional management examining differences in antioxidant activity, fruit quality and human glycemic response. Organic management is restricted to specific guidelines which disallow manufactured chemical applications. Biological management focuses on practical, cost-effective methods of rebuilding soil health and the use of foliar nutrient applications for maximum yield and quality production. Conventional methods employ manufactured fertilizers, pesticides and herbicides which may be detrimental to the environment.

One goal of this study was to correlate soil microbial activity levels to fruit quality and seek to enhance the understanding of the relationships of soil health to plant health to human health. Soil tests included values for microbial activity, organic matter, cation exchange capacity (CEC), pH, nitrogenous compounds and various minerals. Comparative leaf and fruit tissue analyses measured nitrogen compounds and mineral levels.

If it is possible to produce higher quality fruit containing more antioxidants and other nutrients through alterations in management systems, more nutrition would be derived from the amounts of fruit currently eaten. Furthermore, if fruit tasted better, people may be encouraged to eat more of it, and consequently less of poor nutritional quality foods. Fruits that have a long shelf life are more likely to be consumed than discarded due to rotting. Finally, greater consumption of higher quality fruits should result in a healthier populace. Such is the aim of biological agriculture.

In summary, this research may be a step toward supplying better quality produce to consumers, increasing consumption of fruits and vegetables, and improving the general health of the population, while caring for our environment.

Chapter 3

Antioxidant Levels in Apples from Biologically-enhanced Organic, Biologically-enhanced Conventional andTypical Conventional Systems

3.1 Abstract

Two apple cultivars, each grown under differing management systems, were evaluated for antioxidant activity using three separate assays, ABTS 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), DPPH (1,1-diphenyl-2-picrylhydrazyl), and total phenolics (TP) (Folin-Ciocalteu phenol reagent). In 2007, Washington ‘Braeburn’ *(Malus domestica* Mill.*)* apples raised under biologically-enhanced organic and conventional methods were evaluated. Treatments were split to include apples from the outside and inside of the tree canopies. There was no difference in ABTS antioxidant activity between organic and conventional ‘Braeburn’ apples (P = 0.102). Outside-canopy apples had higher ABTS antioxidant activity than inside-canopy apples (P = 0.002). There was no difference in DPPH antioxidant activity between organic and conventional ‘Braeburn’ apples (P = 0.190). Outside-canopy apples had higher DPPH antioxidant activity than the inside-canopy fruit (P = 0.0002). Organic ‘Braeburn’ apples showed a higher level of total phenolics (TP) than the conventional apples (P = 0.003). Outside-canopy apples had higher TP levels than inside-canopy fruits (P = 0.0001). In 2008, Washington ‘Crimson Gala’ apples *(M. domestica)*

from orchards under biologically-enhanced conventional and typical conventional management were evaluated. The biological apples had higher ABTS antioxidant activity than the conventional ($P = 0.0498$). The conventional ‘Gala’ apples had higher DPPH antioxidant activity than the biological ($P = 0.002$). There was no difference between the biological and conventional apple total phenolics levels ($P = 0.681$). It should be noted that the biological ‘Gala’ apples were grown on a sandier soil type than the ‘Gala’ conventional apples which may have resulted in lower antioxidant levels.

3.2 **Introduction**

Apples contain many naturally-occurring compounds displaying antioxidant activity that may be important to human health in terms of decreasing incidence of degenerative diseases, such as cancer and cardiovascular disease (Boyer and Liu, 2004). Scientific literature supports differences in antioxidant levels in apple cultivars related to cultivation methods, especially when comparing organic and conventional management practices (Peck et al., 2006; Weibel et al., 2000). Biological cultivation focuses on improving fruit quality through applications of nutrients and beneficial microflora to soil and trees.

3.2.1 Objectives

The objectives of this study were to examine antioxidant activity in apples grown under biologically-enhanced organic, biologically-enhanced conventional, and typical conventional growing methods, using the following assays:

1. ABTS-- 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid),

2. DPPH-- 1,1-diphenyl-2-picrylhydrazyl radical,
3. TP-- total phenolic content (Folin-Ciocalteu phenol reagent).

3.3 **Materials and Methods**

3.3.1 2007 'Braeburn' Apples, Biologically-enhanced Organic vs. Conventional, and Outside-canopy vs. Inside-canopy

Two adjacent Royal City, Washington state 'Braeburn' apple orchards on the same Royal loamy fine sand soil type (NRCS, 2009) and trees of the same rootstock, but under differing cultivation methods were compared.

3.3.1.1 Biologically-enhanced organic 'Braeburn'

The approximately eight year old experimental biologically-enhanced organic orchard had transitioned from conventional production and had been certified organic for one year. The tree planting density was 407 trees per acre with 15 feet between rows and seven feet between trees within rows. The cover crop consisted of mowed grass. Weeds were controlled by cultivation. A wire trellis system was used along with a tubing/spray irrigation system. No soil amendments were used in 2007, but certified organic Rhizoboost®, a probiotic soil conditioner from BIO-CAT Microbials (BCM) of Shakopee, Minnesota, and Organic Restoration, a fertilizer blend from Soil Restoration, LLC, Culver, Oregon, were sprayed on the ground. Microbiology-enhancing Bio-N-Liven Answer® and The Carbon Answer® sprays (Environmental Care and Share, Inc., Golden, Colorado) (appendix), containing various enzymes, mineral electrolytes, vitamin precursors and sugars were applied to the trees and soil in 2006 and

2007. Bio-N-Liven Answer® and The Carbon Answer® were applied foliarly at rates of 16oz and 8oz, respectively, in 100 gallons of water per acre of trees. These were utilized every two weeks starting at bloom for three applications. Additionally, Bio-N-Liven Answer® and The Carbon Answer® were sprayed on the soil in the fall of 2006 at rates of 8oz each in 100 gallons of water per acre. Blossom thinning was accomplished with lime, sulfur and fish oil, along with hand thinning. Certified organic Entrust® (Dow AgroSciences, LLC, Indianapolis, Indiana) at a rate of 6g per 100L of water per hectare, and pheromone confusion were used to decrease leafroller and codling moth infestations. Sulfur was used before fruiting to discourage powdery mildew.

3.3.1.2 Conventional ‘Braeburn’

The approximately 15 year old conventional control orchard had been continuously conventionally managed. The tree planting density was 242 trees per acre with 18 feet between rows and 10 feet between trees within rows. The cover crop consisted of mowed grass, with glyphosate used as an herbicide at a rate of one quart per acre. There was no evidence of a trellis system. A sprinkler irrigation system was utilized. Ammonium sulfate, calcium nitrate and NPK were used as fertilizers. A calcium-containing foliar spray was applied five times per year. Blossom thinning was accomplished by spraying with Sevin® carbamate (Bayer CropScience LP, Research Triangle Park, NC) at 2 quarts per acre. Various pesticides were used every two to three weeks throughout the season to control insects and diseases. These included Diazinon 50 WSB, an organophosphate from Gowan Company, and Success® from Dow AgroSciences.

3.3.1.3 'Braeburn' sampling

At October harvest time, four rows of apples, five rows apart and at least five rows from the service road which divided the two treatment orchards, were randomly selected for sampling each treatment. Ten trees were selected (trees 10 through 19 from the beginning of each row) in each of the selected four rows from each orchard. From each selected tree, four apples from the outside tree canopy at mid-canopy height and four apples from inside the canopy at mid-canopy height were picked. Apples were separately bagged and labeled for each cultivation and canopy treatment, but pooled by row. They were shipped in cardboard apple boxes via flatbed truck. The apples arrived in Colorado one week post harvest. They were stored in the shipping boxes at Colorado State University's cold storage units in the Shepardson building, in regular atmosphere, refrigerated at 2°C.

3.3.2 2008 'Crimson Gala' Apples, Biologically-enhanced Conventional vs. Typical Conventional

Two southern Washington state 'Crimson Gala' apple orchards on the same Malling 26 dwarf rootstock, but under differing cultivation methods were compared. The biologically-managed orchard was located near Pasco, Washington on sandy loam soil (cation exchange capacity [CEC] 7.1 meq/100g). The conventionally-managed orchard was located approximately 60 miles away near Mattawa, Washington on predominantly Kennewick silty loam soil (NRCS, 2009) with a CEC of 11.3 meq/100g.

3.3.2.1 Biologically-enhanced conventional ‘Gala’

The approximately 15 year old experimental biologically-managed orchard, while still classified as conventionally-managed, had been treated with biological enhancement products for approximately four years. The tree planting density was 453 trees per acre with 16 feet between rows and six feet between trees within rows. The cover crop consisted of mowed mixed grasses, with one 0.5 gallon per acre application of Goal and Surflan pre-emergent herbicide (Dow Chemical Company) and one quart per acre of RoundUp® (Monsanto Company) used to control weeds. A single-pole, two-wire trellis system was used along with a permanent under-tree irrigation system and overhead sprinklers, which were used for hydrocooling the orchard. No soil amendments were used in 2008, but Arbor T & O, a fertilizer blend from Soil Restoration, LLC, Culver, Oregon, was applied through the sprinkler system at a rate of one quart per acre. Microbiology-enhancing Bio-N-Liven Answer® and The Carbon Answer® sprays (Environmental Care and Share, Inc., Golden, Colorado), containing various enzymes, mineral electrolytes, vitamin precursors and sugars had been applied to the ground and trees with a tractor sprayer two to five times per season for four years. Bio-N-Liven Answer® and The Carbon Answer® were applied foliarly at rates of 8oz and 16oz, respectively, in 100 gallons of water per hectare. These were typically utilized approximately every two weeks starting at the ½” green leaf stage. Neither was applied in the two months prior to the 2008 harvest, even though it was recommended by the manufacturer. Blossom thinning was accomplished with three separate chemical treatments (initially with lime-sulfur at a rate of six gallons per acre, then twice with Kalo Regulaid at one pint per acre and Sevin® XLR Plus at one quart per acre). Some hand thinning was also needed. Foli-Gro Link Calcium (Wilbur-Ellis, Fresno, CA) and Vigor-Cal (Agro-K Corp., Minneapolis, MN), foliar calcium products, were applied once and twice, respectively, in 2008 at a rate of 2 quarts per acre per application. Cuprofix (United Phosphorus, Inc., King of Prussia, PA), a copper sulfate product, was applied twice a year at rates of 7.5 and 10 lbs to decrease incidence of fire blight. Two gallons per acre of liquid NPK 3-18-18 fertilizer was mixed into the spray regimens. Pristine® fungicide (BASF Company) at 4.5 oz per acre and Procure® (Crompton Corp., Middlebury, CT) at 12 oz per acre were used to decrease fungal infections. Lorsban® 4E (Dow AgroSciences), Delegate™ (Dow AgroSciences), Diazinon 50 WSB (an organophosphate from Gowan Company), and pheromone confusion were used to decrease insect infestations such as wooly aphid and codling moth. Raynox® (Pace International, LLC, Seattle, WA), a carnauba coating, was sprayed from a helicopter three times during the season at a rate of 2.5 gallons per acre to prevent sunburn of the fruit. It was expected that 90% of the 2008 crop load would bring premiums as Washington Extra Fancy due to color and size.

3.3.2.2 Typical conventional 'Gala'

The approximately 15 year old conventional control orchard had been continuously conventionally managed. The tree planting density was 484 trees per acre with 15 feet between rows and six feet between trees within rows. The cover crop consisted of mowed mixed grasses, with glyphosate used as an herbicide. A single-pole, one-wire trellis system was used along with a permanent under-tree irrigation system. Approximately 100 lbs./acre of calcium nitrate was applied in the spring. A calcium-containing foliar spray was applied five times per year. NPK was applied three times during spring/summer. Blossom thinning was accomplished by spraying with Sevin® carbamate (Bayer CropScience LP, Research Triangle Park, NC), and hand thinning late in the season. Various pesticides, including organophosphates, were used throughout the season to control insects and diseases. The 2008 crop achieved premium color ratings, but were smaller than ideal.

3.3.2.3 'Gala' sampling

At late August harvest time, four rows of apples, five rows apart were randomly selected for sampling each treatment. Ten trees were selected (trees 10 through 19 from the beginning of each row) in each of the selected four rows from each orchard. From each selected tree, four apples of similar size were picked from the outside tree canopy at mid-canopy height. Apples were separately bagged and labeled for each cultivation method, but pooled by row. They were shipped in cardboard apple boxes via unrefrigerated truck. The apples arrived in Colorado one week post harvest. They were stored in the shipping boxes at Colorado State University's cold storage units in the Shepardson building, in regular atmosphere, refrigerated at 2°C.

3.3.3 Preparation for Assays

Three apples were randomly chosen from each row of each experimental and control treatment to be utilized for assays. The apples were sliced equatorially and the inedible parts were removed. The peel was left intact. Several 0.5 cm slices from the equatorial region were weighed, freeze-dried using a VirTis Genesis Series 25LL freeze dryer (The VirTis Company, Gardiner, NY). The slices were reweighed to determine dry matter content, powdered by grinding with a coffee grinder, and stored in plastic screw-top tubes at -20^{o} C until further testing.

Sample extraction was accomplished by combining 400 mg of apple powder with 10 mL of 80% acetone (Mallinckrodt Chemicals, Phillipsburg, NJ) and mixing with an auto-rotator for 60 minutes in a refrigerator at 4 ^{o}C. Tubes were centrifuged at 6000 RPM for 15 min at 4 ^{o}C, aliquoted into 1 mL plastic snap-top Eppendorf tubes, and vacufuged at 45 ^{o}C until dry. The tubes were stored at – 20 ^{o} C until testing.

Antioxidant activities were determined spectrophotometrically using three assays, an ABTS (2,2'-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid]) antioxidant assay, a DPPH (1,1-diphenyl-2-picrylhydrazyl radical) assay to evaluate radical scavenging capacity, and the Folin-Ciocalteu assay (Sigma-Aldrich, St. Louis, MO) to detect total phenolics (TP).

3.3.4 ABTS Assay

ABTS is 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, which is combined with manganese dioxide to form radical cations that capture electrons from antioxidants present in the extract. This is a decolorization assay which begins as a dark green color and becomes lighter/clearer as the reaction proceeds. The more antioxidants present, the greater the clearing of the color. Briefly, by the method described by Miller and Rice Evans (1997), the assay was performed with a 96-well SpectraMax spectrophotometric microplate reader (Molecular Devices, Sunnyvale, CA) with absorbance set at 734 nm. ABTS (40 mg) was dissolved in 15 mL water, mixed with 150 mg manganese oxide, filtered, and adjusted to 0.7 absorbance units with 5.0 mmol phosphate buffer (PBS). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble analog of vitamin E, was used to establish a standard curve. The standards were plated at 25 μL per well, with 250 μL of ABTS solution added to each well via a multichannel pipetter (VWR International, West Chester, PA), and read after exactly 60 seconds at 25 °C. Apple extracts were reconstituted with 1 mL aliquots of 80% acetone and further diluted to fall within the standard curve after ABTS solution was added. The absorption values were adjusted with dry matter percentages, compared to the Trolox standard calibration curve, and results reported in μM TEAC (Trolox equivalent antioxidant capacity)/100 g fresh weight.

3.3.5 DPPH Assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) is a decolorization assay used to evaluate radical scavenging capacity. DPPH is an unstable free radical with an unpaired electron, causing it to be very reactive. Sample extracts containing antioxidants will become oxidized as they donate electrons to DPPH, which then becomes reduced and thus more stable. In this assay, microplate well color changes from dark purple to lighter purple as DPPH is reduced by antioxidants. The stronger the antioxidant activity, the clearer the wells become. Briefly, after the method described by Brand-Williams, et al. (1995), the dried apple extracts in the 1 mL Eppendorf tubes were reconstituted with 1 mL 5.0 mmol phosphate buffer (PBS). DPPH was dissolved in 100% methanol, and adjusted to approximately 0.90 absorption at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) standards and extracted samples were plated in 15 μL amounts in a 96-well plate. DPPH solution was added (285 μL per well) with a multichannel pipetter, and read with a SpectraMax spectrophotometric microplate reader (Molecular Devices, Sunnyvale, CA) at 515 nm after 3 minutes at 25 °C. Values were adjusted with dry matter percentages, compared to the Trolox standard calibration curve, and results reported in μM TEAC (Trolox equivalent antioxidant capacity)/100 g fresh weight.

3.3.6 Total Phenolics (TP) Assay

Phenolic compounds are naturally-occurring in plants, including apple fruit, and serve as antioxidants, among other functions. The polyphenolic content of apples is responsible for approximately 90% of the antioxidant activity (Lamperi et al., 2008). The total phenolics (TP) assay (Spanos and Wrolstad, 1990) is a colorimetric assay that utilizes the Folin-Ciocalteu reagent, a mixture of phosphomolybdate and phosphotungstate. Reagents in a 96-well microplate becomes darker in color as –OH groups from phenolic compounds in the sample react with the Folin-Ciocalteu reagent. Briefly, dried apple extract samples in

Eppendorf tubes were reconstituted with 1 mL aliquots of 80% acetone and diluted 1 to 9 with nanopure water. Standards were prepared with gallic acid (a strong antioxidant), acetone, and water. Folin-Ciocalteu reagent was mixed 1 to 9 with water to produce a 0.2 M solution. Samples and standards were pipetted in triplicates at 35 μL per well, and 150 μL of the Folin-Ciocalteu solution was then added to each well. The plate was mixed for 30 sec at 400 RPM, placed on a heating pad for 30 min at 45 ^{o} C, cooled at room temperature for 1 h, and read in a SpectraMax spectrophotometric microplate reader (Molecular Devices, Sunnyvale, CA) at 765 nm. Values were adjusted with dry matter percentages, compared to the Trolox standard calibration curve, and results reported in mg GAE (gallic acid equivalents)/100 g fresh weight.

3.4 **Statistical Analysis**

Data were analyzed for significance and correlations using Microsoft Excel and SAS statistical software (SAS Institute, Cary, NC). Data were subjected to t-tests, paired t-tests, Analysis of Variance (ANOVA) and/or Pearson correlation analyses as required. Observation-related differences were calculated, with $P < 0.05$ considered significant.

3.5 **Results and Discussion**

3.5.1 'Braeburn'—Biologically-enhanced Organic vs. Conventional, Outside-canopy vs. Inside-canopy

3.5.1.1 'Braeburn' ABTS

Overall, the two-factor ANOVA showed no difference ($P = 0.102$) in ABTS antioxidant activity between organic and conventional orchards (Fig. 3.0). The organic apples averaged 667.6 μM TEAC/100g fresh wt. The conventional apples averaged 721.4 μM TEAC/100g fresh wt. Also shown in Figure 3.0, there was an overall difference ($P = 0.002$) in ABTS between fruit location on the tree, with outside-canopy fruit having a higher antioxidant activity of 747.0 μM TEAC/100g fresh wt. and inside-canopy fruit averaging 641.9 μM TEAC/100g fresh wt. There was also interaction ($P = 0.006$) between the cultivation treatment and the canopy location factors. The inside-canopy conventional apples had higher ABTS antioxidant activity than the inside-canopy organic apples, while there was no difference between the management systems on the outside-canopy apples.

More specifically, t-tests revealed that, while there was no difference ($P = 0.331$) between organic and conventional outside-canopy fruits, there was a difference ($P = 0.009$) between organic and conventional inside-canopy fruits. The conventional inside fruits had a greater antioxidant activity of 715.2 μM TEAC/100g fresh wt., whereas the organic inside fruits had an antioxidant activity of 568.6 μM TEAC/100g fresh wt. Furthermore, there was no difference ($P > 0.330$) between convention outside-canopy (727.5 μM TEAC/100g fresh wt.), conventional inside-canopy (715.2 μM TEAC/100g fresh wt.), or organic outside-canopy (766.6 μM TEAC/100g fresh wt.) fruits.

Peck et al. (2006) reported higher levels of ABTS antioxidant activity in organic apples as compared to those from conventional and integrated systems. The apple trees in their study had been under their respective systems since planting. The organic orchard in our study had been converted from conventional to organic, having been certified for only one year. Perhaps longer-term organic management would result in higher ABTS antioxidant levels.

3.5.1.2 'Braeburn' DPPH

Overall, the two-factor ANOVA showed no difference ($P = 0.190$) in DPPH antioxidant activity between organic (247.3 μM TEAC/100g fresh wt.) and conventional (225.6 μM TEAC/100g fresh wt.) orchards. (Fig. 3.1). This was similar to the findings of Reig et al. (2007) with 'Fuji' and 'Golden Delicious' apples showing no difference in DPPH antioxidant activity between organic and conventional production systems. There was no report of how long the orchards had been under their respective management systems.

Also shown in Figure 3.1, there was an overall difference ($P = 0.0002$) in DPPH between fruit location on the tree, with outside-canopy fruit having a higher antioxidant activity (269.5 μM TEAC/100g fresh wt.) than the inside-canopy fruit (203.4 μM TEAC/100g fresh wt.). There was no interaction ($P = 0.811$) between the cultivation treatment and the canopy location factors.

More specifically, t-testing revealed that, while there was an overall difference in DPPH antioxidant activity between outside and inside-canopy fruits, there was no difference (P = 0.070) between conventional outside-canopy (256.7 μM TEAC/100g fresh wt.) and organic inside-canopy (212.3 μM TEAC/100g fresh wt.) fruits. This is not surprising given the large difference (P = 0.0009) between the outside-canopy organic fruits (282.4 μM TEAC/100g fresh wt.) and the inside-canopy conventional fruits (194.5 μM TEAC/100g fresh wt.).

3.5.1.3 'Braeburn' total phenolics (TP)

Overall, the two-factor ANOVA showed a higher level (P = 0.003) of total phenolics in the organic apples (143.5 mg GAE/100g fresh wt.), compared to the conventional apples (126.0 mg GAE/100g fresh wt.) (Fig. 3.2). These findings correlate with the findings of Rembiakowska et al. (2006), who compared five apple cultivars for total polyphenols and antioxidant activity finding organically managed apples to have higher levels than the conventional counterparts. Weibel et al. (2000) also found organic 'Golden Delicious' apples to have higher levels of phenolic compounds than those grown in integrated systems.

Also shown in Figure 3.2, there was an overall difference (P = 0.0001) between fruit location on the tree, with outside fruits (146.5 mg GAE/100g fresh wt.) having higher levels than the inside fruits (123.0 mg GAE/100g fresh wt.). There was no interaction between the cultivation treatment and the canopy location factors (P = 0.488).

More specifically, t-testing revealed that, while there was an overall difference in total phenolics between organic and conventional, there was no difference (P = 0.460) between conventional outside-canopy fruits (135.8 mg GAE/100g fresh wt.) and organic inside-canopy fruits (129.9 mg GAE/100g fresh wt.). This is not surprising given the large difference between the outside-canopy organic fruits (157.2 mg GAE /100g fresh wt.) and the inside-canopy conventional fruits (116.2 mg GAE /100g fresh wt.).

3.5.2 'Gala'—Biologically-enhanced Conventional vs. Typical Conventional

3.5.2.1 'Gala' ABTS

There was a difference (P = 0.0498) between the biologically-enhanced conventional and the typical conventional control apple ABTS antioxidant activity, with the biological having a higher level of 624.4 μM TEAC/100g fresh wt. compared to the conventional control at 558.9 μM TEAC/100g fresh wt. (Fig. 3.3). Similarly, Peck et al. (2006) found greater levels of antioxidant activity in organic 'Galaxy Gala' apples with ABTS assays as compared to apples from a conventional system.

3.5.2.2 'Gala' DPPH

There was a difference (P = 0.002) between the biological and conventional control apple DPPH antioxidant activity, with the conventional control having a higher level of 849.0 μM TEAC/100g fresh wt. compared to the biological at 742.8 μM TEAC/100g fresh wt. (Table 3.4). The lower cation exchange capacity (CEC) of the biological orchard soil may have resulted in lower antioxidant activity.

3.5.2.3 ‘Gala’ total phenolics (TP)

There was no difference (P = 0.681) between the biological (143.3 mg GAE/100g fresh wt.) and conventional control apple (146.5 mg GAE/100g fresh wt.) total phenolics levels (Table 3.5).

3.6 **Summary**

Under the conditions of this study, biologically-enhanced organic ‘Braeburn’ apples had higher levels of total phenolics (TP) than those conventionally grown. There was no difference in antioxidant activity as measured by ABTS and DPPH assays between organic and conventional Braeburn apples. Apples from the outside-canopy, where they receive more sunlight and develop better color, had more antioxidant activity than apples from inside the canopy as measured by ABTS and DPPH assays, and a higher level of total phenolics (TP). (These results are summarized in Table 3.0.)

The ‘Gala’ apples showed mixed results (Table 3.1). While the biologically-enhanced ‘Gala’ apples had higher ABTS antioxidant activity than the typical conventional, the conventional ‘Gala’ apples had higher DPPH antioxidant activity than the biological. There was no difference between the biological and conventional apple total phenolics (TP) levels. It should be noted that the biological ‘Gala’ apples were grown on soil with a cation exchange capacity (CEC) of 7.1 meq/100g compared to the conventional control orchard’s CEC of 11.3 meq/100g. Furthermore, the biological apple trees had been under that system for only the last four of their 15 years. The management had not always applied the biological enhancements according to directions, with none applied in the two months before harvest, which may have affected results. This orchard continued to use conventional pesticides, herbicides, and chemical thinning agents. According to product suppliers, biological management should be performed carefully over a long-term period, to achieve expected results.

3.7 **Conclusions/Implications**

Producing apples in a biologically-enhanced organic system may result in apples with greater antioxidant activity, with potentially greater human health benefits and lowered environmental impacts. Well-colored apples from the outside tree canopy have greater antioxidant activity, therefore producers should be careful to pick according to color, and consumers may use color as an indicator of greater antioxidant activity. However, due to the limitations of these studies, more tightly-controlled research should be performed in these areas before drawing firm conclusions.

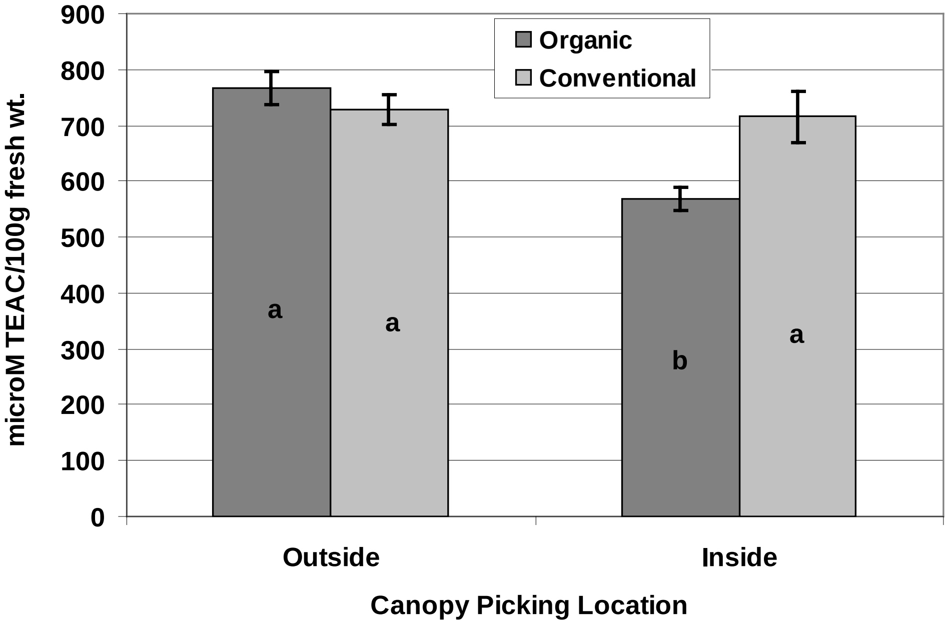

Fig. 3.0 Effects of orchard management and canopy location on ‘Braeburn’ apple ABTS antioxidant activity, measured in μM Trolox Equivalent Antioxidant Capacity (TEAC) per 100g fresh weight of apple. Overall, biologically-enhanced organic apples were not different from conventional apples in ABTS antioxidant activity ($P = 0.102$). Overall, outside-canopy apples had greater ABTS antioxidant activity than inside-canopy apples ($P = 0.002$). There was significant interaction between orchard management and canopy location ($P = 0.006$). Treatments with the same letter are not significantly different at $P < 0.05$ level. Error bars indicate SEM; N = 48, n = 12 (four rows, three reps each). ANOVA table: Appendix page 139.

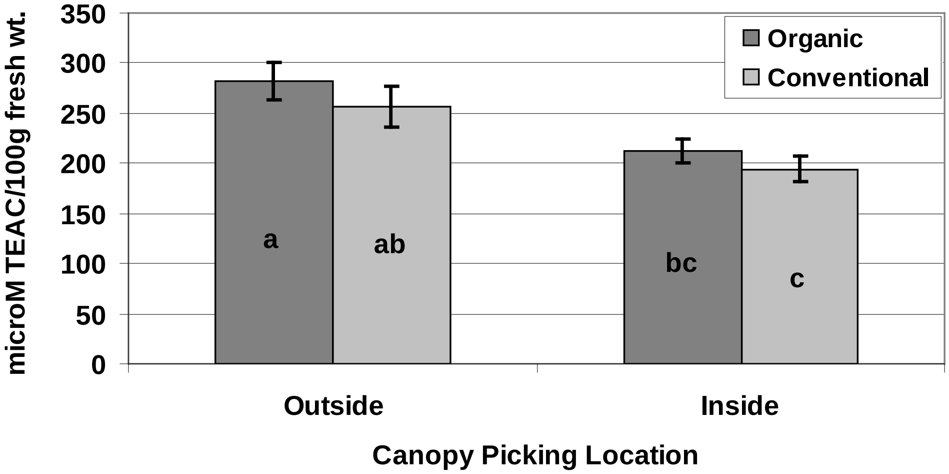

Fig. 3.1 Effects of orchard management and canopy location on ‘Braeburn’ apple DPPH antioxidant activity, measured in μM Trolox Equivalent Antioxidant Capacity (TEAC) per 100g fresh weight of apple. Overall, biologically-enhanced organic apples were not different from conventional apples in DPPH antioxidant activity ($P = 0.190$). Overall, outside-canopy apples had more ABTS antioxidant activity than inside-canopy apples ($P = 0.0002$). There was no interaction between orchard management and canopy location ($P = 0.811$). Treatments with the same letter are not significantly different at $P < 0.05$ level. Error bars indicate SEM; N = 48, n = 12 (four rows, three reps each). ANOVA table: Appendix page 139.

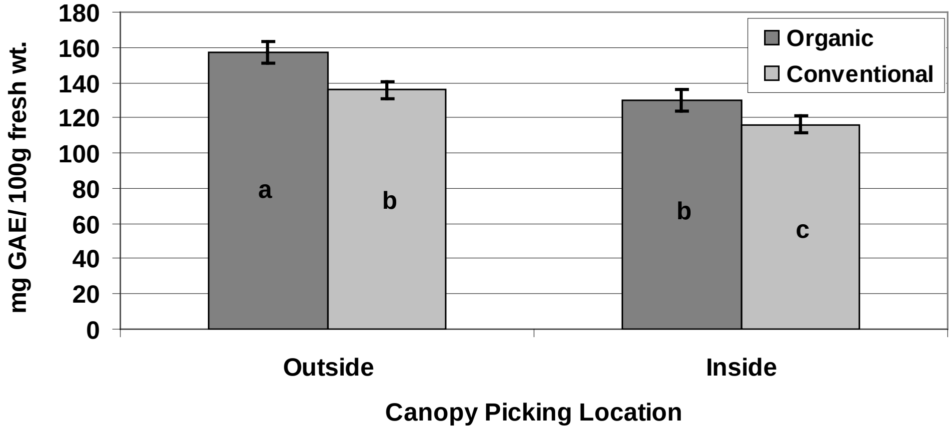

Fig. 3.2 Effects of orchard management and canopy location on ‘Braeburn’ apple Total Phenolics (TP), measured in mg Gallic Acid Equivalents (GAE) per 100g fresh weight of apple. Overall, biologically-enhanced organic apples contained more phenolic compounds than conventional apples ($P = 0.003$). Overall, outside-canopy apples contained more phenolic compounds than inside-canopy apples ($P = 0.0001$). There was no interaction between orchard management and canopy location ($P = 0.488$). Treatments with the same letter are not significantly different at $P < 0.05$ level. Error bars indicate SEM; N = 48, n = 12 (four rows, three reps each). ANOVA table: Appendix page 139.

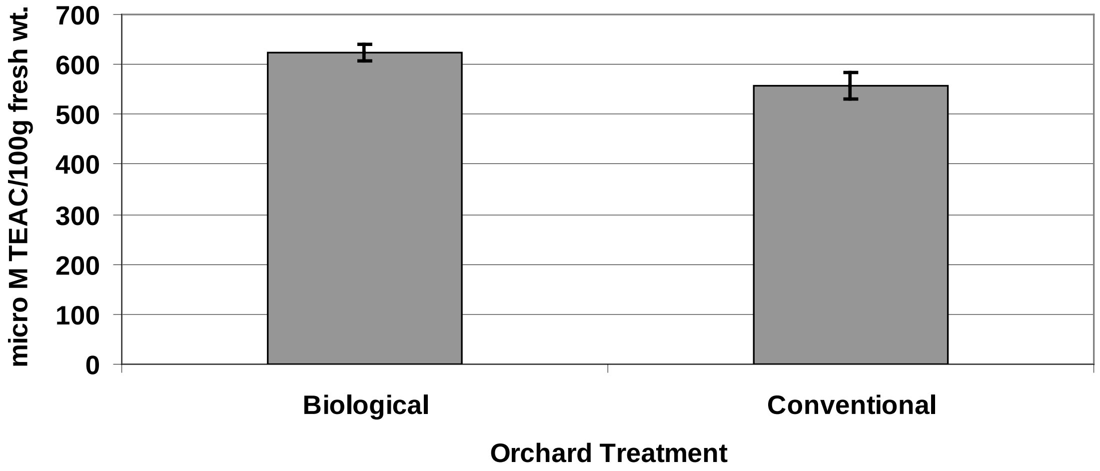

Fig. 3.3 Effects of orchard management on ‘Crimson Gala’ apple ABTS antioxidant activity, measured in μM Trolox Equivalent Antioxidant Capacity (TEAC) per 100g fresh weight of apple. Biologically-enhanced conventional apples had greater ABTS antioxidant activity than typically-managed conventional apples ($P = 0.0498$). Error bars indicate SEM; N = 24, n = 12 (four rows, three reps each).

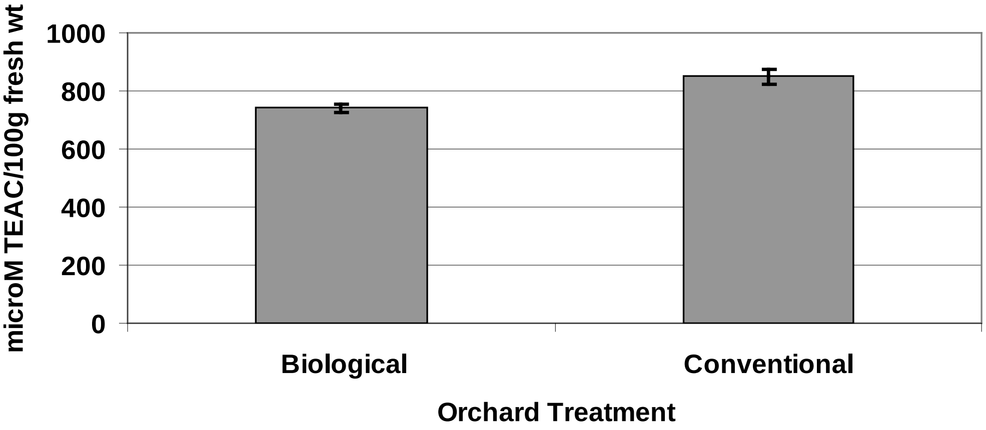

Fig. 3.4 Effects of orchard management on ‘Crimson Gala’ apple DPPH antioxidant activity, measured in μM Trolox Equivalent Antioxidant Capacity (TEAC) per 100g fresh weight of apple. Biologically-enhanced conventional apples had lower DPPH antioxidant activity than typically-managed conventional apples (P = 0.002). Error bars indicate SEM; N = 24, n = 12 (four rows, three reps each).

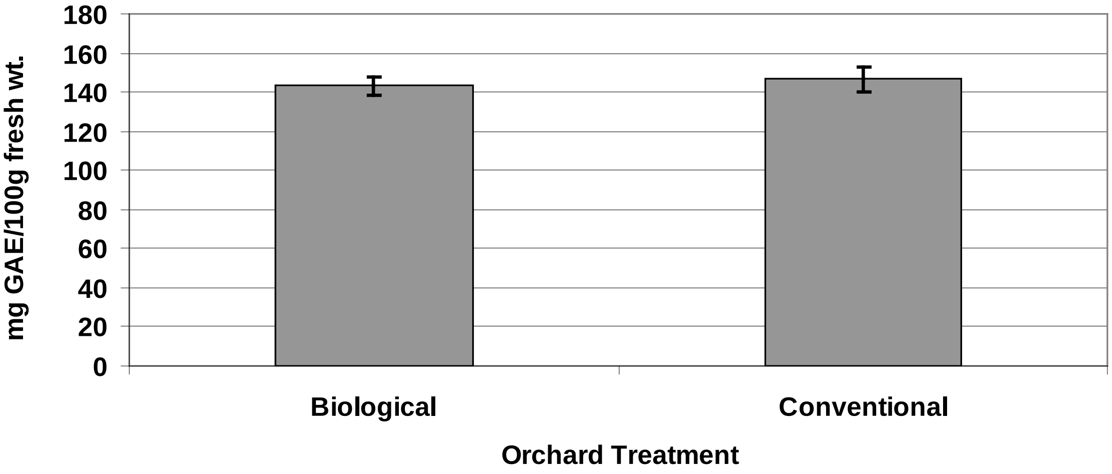

Fig. 3.5 Effects of orchard management on ‘Crimson Gala’ apple Total Phenolics (TP), measured in mg Gallic Acid Equivalents (GAE) per 100g fresh weight of apple. Biologically-enhanced conventional apples were not different in TP content than typically-managed conventional apples (P = 0.68). Error bars indicate SEM; N = 24, n = 12 (four rows, three reps each).

Table 3.0 '**Braeburn' apple antioxidant summary**

	Organic Outside	Conventional Outside	Organic Inside	Conventional Inside
ABTS (μM TEAC /100g fresh wt.)	767 ± 28a	728 ± 20a	569 ± 27b	715 ± 47a
DPPH (μM TEAC /100g fresh wt.)	282 ± 19a	257 ± 12ab	212 ± 20bc	194 ± 12c
TP (mg GAE/100g fresh wt.)	157 ± 6a	136 ± 6b	130 ± 5bc	116 ± 5c

± SEM; a,b,c different letters within rows indicate differences at $P < 0.05$ level

Table 3.1 '**Crimson Gala' apple antioxidant summary**

	Biologically-enhanced Conventional	Typical Conventional
ABTS (μM TEAC/100g fresh wt.)	624 ± 17a	559 ± 27b
DPPH (μM TEAC/100g fresh wt.)	743 ± 14b	849 ± 26a
TP (mg GAE/100g fresh wt.)	143 ± 5a	147 ± 6a

± SEM; a,b different letters within rows indicate differences at $P < 0.05$ level

Chapter 4

Human Glycemic Response to Crimson Gala Apples from Biologically-enhanced Conventional and Typical Conventional Systems

4.1 **Abstract**

Preliminary and field data suggest that equal amounts of high soluble solids fruits grown under a biological management system paradoxically resulted in a lesser glycemic response in humans than lower brix conventionally-grown fruits. To test this hypothesis, ‘Gala’ apples from biologically-enhanced conventional and typical conventional orchards were tested in a single-blind cross-over human study (N = 15). None of the values used to compare glycemic response were statistically different ($P > 0.05$). The highest blood glucose values for each treatment were averaged, resulting in 128.6 mg/dl (range 112-160 mg/dl) for the biological apples and 129.6 mg/dl (range 108-163 mg/dl) for the conventional control apples ($P = 0.816$). The differences from base to peak for each subject under each treatment were averaged, resulting in 37.2 mg/dl (range 14-62 mg/dl) for the biological apples and 36.5 mg/dl (range 22-61 mg/dl) for the conventional control ($P = 0.874$). We also measured time to peak in 10 minute increments, with an average of 28 minutes (range 20-50 minutes) for the biological apples and 30 minutes (range 20-50 minutes) for the conventional control ($P = 0.271$). It should be noted that the biological ‘Gala’ apples had lower soluble solids ($P < 0.001$) than the conventional ‘Gala’ apples, which may be due

to the biological apples having been grown on a sandier soil type than the conventional apples.

4.2 **Introduction**

The alarming rise in obesity, type 2 diabetes and related diseases is a problem that may be at least partially addressed through biological agriculture. Excessive blood glucose levels are related to inflammation (Browning and Jebb, 2006), and inflammation is related to many diseases such as diabetes (Browning and Jebb, 2006), cancer (Nelson et al., 2002), and cardiovascular disease (Paoletti et al., 2006). High intake of fruits and vegetables is positively correlated with disease prevention (van't Veer, et al., 2000; La Vecchia et al., 2001; Riboli and Norat, 2003; Boyer and Liu, 2004). This study involved Washington 'Gala' apples grown with biological and nutrient enhancements which may influence antioxidant and nutrient content. Field observations and preliminary data suggest that equal amounts of nutritionally-enhanced fruits with high soluble solids concentrations (SSC) paradoxically shows less of a glycemic response in humans than conventionally-grown fruits with lower SSC (Fig. 4.0). This research may open new doors to disease treatment and health maintenance.

Human satiety/hunger response tends to follow glycemic response (Bolton et al., 1981; Holt et al., 1996; Holt et al., 1997; Kolset, 2003). Fasting blood glucose, measured after abstaining from food and beverages other than water for eight hours or more, is considered normal when below 100 mg/dL of human blood (ADA, 2009a). When blood glucose rises to a high peak in healthy individuals, a strong insulin response ensues that may cause the glucose level to plummet rapidly and possibly dip below baseline (a hypoglycemic response). This drop in blood glucose elicits a strong hunger response resulting in a desire to consume a large amount of calories. Such events may lead to weight gain. Therefore, apples which result in a flatter glycemic response may deter weight gain and obesity.

A search of the literature shows that some work has been done with blood glucose and insulin scoring (Brand-Miller, 2004), and establishment of a glycemic index for apples as a group (Foster-Powell et al., 2002); however, these studies did not address differences due to variations in SSC values or cultivation methods within the same cultivar. Distinguishing biologically-grown high SSC apples as an aid to regulating blood glucose would be advantageous to diabetics and other individuals with blood glucose dysfunction, as well as appealing to the general health-conscious population.

For this study, testing was done in a cross-over design with 15 healthy adult human subjects in a single-blind trial. For each apple treatment, multiple capillary blood values were obtained in 10 minute intervals by finger-prick samples applied to glucose test strips on glucose meters. This procedure was approved by the CSU Institutional Review Board for human subjects testing (See Appendix pages 143 and 144).

4.2.1 Objective

To compare human glycemic response following ingestion of ‘Gala’ apples from a biologically-enhanced conventional system versus ‘Gala’ apples from a typical conventional system.

4.3 **Materials and Methods**

4.3.1 2008 ‘Crimson Gala’ Apples, Biologically-enhanced Conventional vs. Typical Conventional

Two southern Washington state ‘Crimson Gala’ apple orchards on the same Malling 26 dwarf rootstock, but under differing cultivation methods were compared. The biologically-managed orchard was located near Pasco, Washington on sandy loam soil (cation exchange capacity [CEC] 7.1 meq/100g). The conventionally-managed orchard was located approximately 60 miles away near Mattawa, Washington on predominantly Kennewick silty loam soil (NRCS, 2009) with a CEC of 11.3 meq/100g.

4.3.1.1 Biologically-enhanced conventional

The approximately 15 year old experimental biologically-managed orchard, while still classified as conventionally-managed, had been treated with biological enhancement products for approximately four years. The tree planting density was 453 trees per acre with 16 feet between rows and six feet between trees within rows. The cover crop consisted of mowed mixed grasses, with one 0.5 gallon per acre application of Goal and Surflan pre-emergent herbicide (Dow Chemical Company) and one quart per acre of RoundUp® (Monsanto Company) used to control weeds. A single-pole, two-wire trellis system was used along with a permanent under-tree irrigation system and overhead sprinklers, which were used for hydrocooling the orchard. No soil amendments were used in 2008, but Arbor T & O, a fertilizer blend from Soil Restoration, LLC, Culver, Oregon, was applied through the sprinkler system at a rate of one quart per acre. Microbiology-enhancing Bio-N-Liven Answer® and The Carbon Answer® sprays (Environmental Care and Share, Inc., Golden, Colorado), containing various enzymes, mineral electrolytes, vitamin precursors and sugars had been applied to the ground and trees with a tractor sprayer two to five times per season for four years. Bio-N-Liven Answer® and The Carbon Answer® were applied foliarly at rates of 8oz and 16oz, respectively, in 100 gallons of water per hectare. These were typically utilized approximately every two weeks starting at the ½" green leaf stage. Neither was applied in the two months prior to the 2008 harvest, even though it was recommended by the manufacturer. Blossom thinning was accomplished with three separate chemical treatments (initially with lime-sulfur at a rate of six gallons per acre, then twice with Kalo Regulaid at one pint per acre and Sevin® XLR Plus at one quart per acre). Some hand thinning was also needed. Foli-Gro Link Calcium (Wilbur-Ellis, Fresno, CA) and Vigor-Cal (Agro-K Corp., Minneapolis, MN), foliar calcium products, were applied once and twice, respectively, in 2008 at a rate of 2 quarts per acre per application. Cuprofix (United Phosphorus, Inc., King of Prussia, PA), a copper sulfate product, was applied twice a year at rates of 7.5 and 10 lbs to decrease incidence of fire blight. Two gallons per acre of liquid NPK 3-18-18 fertilizer was mixed into the spray regimens. Pristine® fungicide (BASF Company) at 4.5 oz per acre and Procure® (Crompton Corp., Middlebury, CT) at 12 oz per acre were used to decrease fungal infections. Lorsban® 4E (Dow AgroSciences), Delegate™ (Dow AgroSciences), Diazinon 50 WSB (an organophosphate from

Gowan Company), and pheromone confusion were used to decrease insect infestations such as wooly aphid and codling moth. Raynox® (Pace International, LLC, Seattle, WA), a carnauba coating, was sprayed from a helicopter three times during the season at a rate of 2.5 gallons per acre to prevent sunburn of the fruit. It was expected that 90% of the 2008 crop load would bring premiums as Washington Extra Fancy due to color and size.

4.3.1.2 Typical Conventional

The approximately 15 year old conventional control orchard had been continuously conventionally managed. The tree planting density was 484 trees per acre with 15 feet between rows and six feet between trees within rows. The cover crop consisted of mowed mixed grasses, with glyphosate used as an herbicide. A single-pole, one-wire trellis system was used along with a permanent under-tree irrigation system. Approximately 100 lbs./acre of calcium nitrate was applied in the spring. A calcium-containing foliar spray was applied five times per year. NPK was applied three times during spring/summer. Blossom thinning was accomplished by spraying with Sevin® carbamate (Bayer CropScience LP, Research Triangle Park, NC), and hand thinning late in the season. Various pesticides, including organophosphates, were used throughout the season to control insects and diseases. In 2008, this orchard's apples achieved premium color ratings, but were smaller than ideal.

4.3.1.3 Sampling and laboratory analyses

At late August harvest time, four rows of apples, five rows apart were randomly selected for sampling each treatment. Ten trees were selected (trees 10 through 19 from the beginning of each row) in each of the selected four rows from each orchard. Soil core samples (0 to 15 cm deep) were obtained from the midpoints between the selected apple trees in each selected row and pooled by mixing in a bucket to obtain four pooled soil samples from each orchard described above. These were sent to International Ag Labs, Fairmont, MN for formazan and soil nutrient testing which included humus, pH, ergs, ORP, nitrates, ammonia, phosphorus, potassium, calcium, magnesium, sodium, copper, iron, zinc, and manganese.

Additionally, a pooled soil sample was taken from each orchard by the method described above, except that the rows were pooled to result in only one sample per orchard. These were sent for analyses by Midwest Laboratories, Inc., Omaha, NE. These analyses included organic matter, minerals, pH, and cation exchange capacity (CEC).

From each selected tree, four apples of similar size were randomly picked from the outside tree canopy at mid-canopy height. Apples were separately bagged and labeled as they were picked for each cultivation method, but pooled by row.

Fruit tissue analyses were performed for each row, using randomized (randomly picked from the outside canopies), pooled samples of eight apples per row, by International Ag Labs, Fairmont, Minnesota. These apple samples were sent by priority mail directly from Washington state to the laboratory in Minnesota. The assays included free nitrates, nitrogen, calcium, phosphorus, magnesium, potassium, sodium, boron, copper, iron, zinc and manganese (analyses by atomic absorption and spectrophotometer).

The remaining apples were shipped in cardboard apple boxes via unrefrigerated truck. The apples arrived in Colorado one week post harvest. They were stored in the shipping boxes at Colorado State University's cold storage units in the Shepardson building, in regular atmosphere, refrigerated at 2^{o}C.

4.3.2 Human Glycemic Testing

Randomized, pooled apple samples were obtained from each experimental (biologically-enhanced conventional) and typical conventional control treatment for human glycemic testing one month post harvest. These samples were obtained on each of six testing days by randomly selecting three apples from each of the four rows from the orchard treatment being evaluated on any given day. Every apple used for human glycemic testing was assayed for soluble solids content (SSC) by extracting a juice aliquot from an equatorial slice with a hand press (Pike Agri-Lab Supplies, Jay, ME). The SSC was measured with a digital refractometer (Pike Agri-Lab Supplies, Jay, ME) and reported as ^{o} brix.

Human glycemic responses were determined on 15 healthy adult subjects using a single-blind, cross-over design study. Approval was obtained through the CSU Institutional Review Board for human subjects testing. With the exception of pregnant women, any healthy adult human subject who was not allergic to apples, had the ability and willingness to fast overnight, and had normal blood glucose responses was allowed take part in this study. Persons taking medications that altered blood glucose regulation such as steroids or metformin were disallowed. Diabetics, pre-diabetics, and other individuals with blood glucose regulatory problems were not allowed to participate. The maximum fasting blood glucose level allowed was 107 mg/dL. The same 15 subjects were used for testing of both orchard treatments, which gave paired data sets. For ease of testing, the 15 subjects were split into three groups of five subjects each. For each group, 12 apples (three per row) were randomly selected from each orchard treatment, and were assayed for soluble solids content immediately before they were consumed by the subjects. The apples were cored, but the peel was left intact.

Glycemic values were determined via testing capillary blood obtained by finger pricks. Portable glucose monitors and corresponding reagent strips (Roche Diagnostics, Indianapolis, Indiana) were utilized. Subjects were fasted overnight (8+ hours) and baseline blood glucose values were determined. In all instances, the subjects' baseline blood glucose values were below the acceptable limit of 107 mg/dL. Bowls were filled with 300g portions of fruit, with each portion composed of longitudinal slices from each of the 12 apples for uniformity. Apples were consumed within a 15-minute period. Blood samples were taken at 10-minute intervals after the subjects began consumption, and continued until the subjects' blood glucose values returned to within 5% of their respective baseline values. (See glycemic testing form in appendix, page145.) The multiple measures of blood glucose from each feeding session were graphed and analyzed for differences using paired t-tests. Peak values, time-to-peak values, and baseline-to-peak values were used to establish and compare glycemic responses.

4.3.3 Statistical Analysis

Data were analyzed for significant differences using Microsoft Excel and SAS (SAS Institute, Cary, NC) statistical software. Data were subjected to t-tests, paired t-tests and Analysis of Variance (ANOVA) as required. Observation-related differences were calculated, with $P < 0.05$ considered significant.

4.4 **Results and Discussion**

4.4.1 Fruit Nutrient Analyses

Fruit nutrient analyses were performed on apples from each row by International Ag Labs, Inc. (Table 4.0). Fruit density ([mass/volume] x 100) was measured on the fresh fruit before drying (compared to the density of water at 100). There was no density difference between the treatments ($P = 0.906$), with the biologically-raised fruit scoring 93.25 and the conventional at 93.5. There were also no differences in percentage of dry matter (%DM), SSC, percentage of protein, percentage of calcium, percentage of phosphorus, percentage of magnesium, PPM copper, PPM iron, PPM zinc, or PPM manganese. There were, however, differences in potassium, sodium and pH. The conventional control apples had a higher percentage of potassium (P = 0.011), lower percentage of sodium (P = 0.010), and a lower pH (P = 0.001).

It was not originally expected that all tested fruit mineral levels would be statistically different between treatments, especially considering there were only four replicates (rows) from each treatment. It was expected, however, that more compositional differences would have been seen. The preliminary data were collected on apples and cherries from other biological and control orchards in Washington state. In the preliminary experiments, the biological fruits had higher SSC and paradoxically lower glycemic responses. Due to factors beyond our control, fruit from these same orchards was not available for this study. Furthermore, fruits were not able to selected from adjacent orchards with the exact same soil type due to an orchard of choice being harvested before samples were able to be obtained. The orchards able to be used were approximately 60 miles apart.

4.4.2 Fruit Soluble Solids Content

Soluble solids content (SSC) measurements were taken on all apples (N = 72) used for glycemic testing (Table 4.1). The overall biological apple average was 11.9 brix, while the overall conventional control average was 12.4 brix. While this 0.5 brix difference was statistically significant (P < 0.001), the ability of this SSC difference to affect human glycemic response appeared nil. There was no statistical difference in SSC within the biological treatment (P = 0.151), with the largest measured mean difference between any two of the three days being 0.4 brix. There was a statistical and measured mean difference of 0.7 brix in

SSC over the three testing dates within the conventional control treatment (P = 0.014), however, again, the actual effect on human glycemic response appeared nil. Our original intent was to obtain biological apples with a higher soluble solids content, however this was not possible for reasons described above.

4.4.3 Soil Quality Assays

Soil quality may have affected the SSC and other nutrient qualities of the fruits. Beneficial soil microbes help the trees uptake nutrients, which may result in better quality fruit. Formazan microbial soil testing of the ‘Gala’ orchards by International Ag Labs, Inc. showed greater microbial activity in the conventional control orchard (P = 0.017), with an average of 570.75 PPM in the biological orchard and an average of 959.75 PPM in the conventional control orchard. It was originally expected that the soil microbial activity would be greater in the biologically-managed soil, however the biological orchard had a sandier soil with a cation exchange capacity (CEC) of 7.1 meq/100g compared to the conventional control orchard’s CEC of 11.3 meq/100g. Both orchards had low levels of organic matter, however the biological orchard had a very low organic matter level of 1.2 % compared to the conventional control orchard at 1.7 %. Low levels of these factors may have decreased microbial survival and proliferation.

The soil analyses performed on each row by International Ag Labs, Inc. (Table 4.2) were summarized by the laboratory into a soil index rating, based on a scale of 0 to 100, with 100 being ideal. The conventional control orchard average was higher (P < 0.001) at 58, with the biological orchard at 22.5. There were no differences in humus levels, nitrates, ammonium, or manganese, with both treatments averaging in the very low range. There was a trend ($P = 0.082$) toward lower sodium in the biological soil, with the biological treatment showing a low reading and the conventional control in the medium range. There was no difference between the calcium to phosphorus ratios (Ca:P), with both treatments showing slightly high according to International Ag Labs' standards. There were differences ($P < 0.05$) in phosphorus, calcium, magnesium, ergs (electrical conductivity), the phosphorus to calcium ratio (P:Ca), and the calcium to magnesium ratio (Ca:Mg), with the biological treatment being in the very low ranges. There were differences ($P < 0.05$) in potassium, copper, iron, and zinc, with the biological treatment running very high (excessive) levels. Excessive salt levels may have interfered with the uptake of other nutrients.

Given the poorer quality soil, it is not surprising that the biological fruits had lower SSC values.

4.4.4 Human Glycemic Response

None of the values used to compare glycemic response were statistically different (Table 4.3). The highest blood glucose values for each treatment were averaged, resulting in 128.6 mg/dl (range 112-160 mg/dl) for the biological apples and 129.6 mg/dl (range 108-163 mg/dl) for the conventional control apples ($P = 0.816$). The differences from base to peak for each subject under each treatment were averaged, resulting in 37.2 mg/dl (range 14-62 mg/dl) for the biological apples and 36.5 mg/dl (range 22-61 mg/dl) for the conventional control ($P = 0.874$). Time to peak was measured in 10-minute increments, with an average of 28 minutes (range 20-50 minutes) for the biological apples and 30 minutes (range 20-50 minutes) for the conventional control (P = 0.271).

4.5 **Summary**

Preliminary and field data suggested that equal amounts of fruits with a high SSC grown under a biological management system paradoxically resulted in a lesser glycemic response in humans than conventionally-grown fruits with lower SSC. To test this hypothesis, 'Crimson Gala' apples from biologically-enhanced conventional and typical conventional orchards were tested in a single-blind cross-over human study (n = 15). To compare glycemic response, blood glucose curves were examined for peak values, baseline to peak differences, and times from baseline to peak. None of these values used to compare glycemic response were statistically different (P > 0.05). It should be noted that the biological Gala apples had lower soluble solids (P < 0.001) than the conventional Gala apples, which may have be due to the biological apples having been grown on a sandier soil type than the conventional apples. Furthermore, the biological apple trees had been under that system for only the last four of their 15 years. The management had not always applied the biological enhancements according to directions, with none applied in the two months before harvest, which may have affected results. This orchard continued to use conventional pesticides, herbicides, and chemical thinning agents.

4.6 **Conclusions/Implications**

Due to the limitations of this study, more research should be performed in these areas. Repeating the experiment with fruit from a well-managed biological orchard and fruit of the same cultivar from an adjacent conventional orchard is imperative before reaching any conclusions.

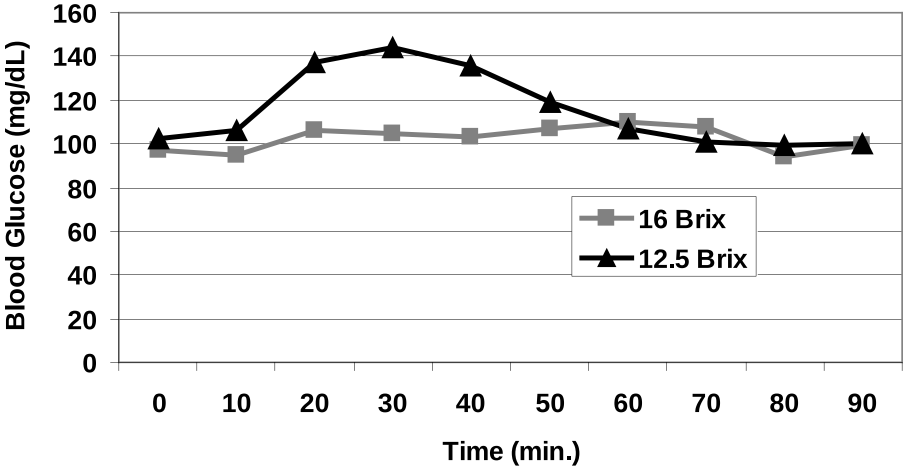

Fig. 4.0 Preliminary data on human glycemic response to 300g portions of ‘Fuji’ apples of differing soluble solids content (reported as degrees brix). Testing was performed on two different days, with baseline data taken after overnight fasting. N = 1.

Table 4.0 **Fruit nutrient analyses**

	Biological	**Conventional**	**P Value**
Density (water = 100)	93.25	93.5	ns
%DM	13.45	14.1	ns
Soluble Solids (Brix)	12.2	12.4	ns
% Protein	0.07	0.06	ns
% Ca	0.01	0.01	ns
% P	0.02	0.02	ns
% Mg	0.01	0.01	ns
% K	0.15	0.17	0.01
Na (PPM)	16.0	10.8	0.01
Cu (PPM)	1.45	0.89	ns
Fe (PPM)	4.01	3.41	ns
Zn (PPM)	1.06	1	ns
Mn (PPM)	0.18	0.14	ns
pH	3.68	3.48	0.001

N = 8, n = 4 (rows)

Table 4.1 **Apple soluble solids content (measured in brix)**

	Biological	**Conventional**	**P Value**
Section 1 Means	11.8 ± 0.2	12.8 ± 0.1	< 0.001
Section 2 Means	11.7 ± 0.1	12.1 ± 0.1	ns
Section 3 Means	12.1 ± 0.2	12.3 ± 0.2	ns
Overall Means	11.8 ± 0.1	12.4 ± 0.1	< 0.001

±SEM; P Values for rows; N = 72, n =12

Table 4.2 **'Crimson Gala' soil test results**

	Biological	**Rating**		**Conventional**	**Rating**
Formazan, PPM	570.75b*	Low		959.75a*	Good
Humus Index	5	Low		5	Low
Nitrates, kg/ha	9	Very Low		8	Very Low
Ammonia, kg/ha	3	Very Low		6	Very Low
Phosphorus, kg/ha	33b***	Very Low		157.5a***	Low
Potassium, kg/ha	310.75a*	High		261b*	High
Calcium, kg/ha	788.75b*	Very Low		3394.75a*	Good
Magnesium, kg/ha	162.25b***	Very Low		329.5a***	Low
Sodium. PPM	11	Low		21.5	Good
Ergs, microS/cm	70.5b***	Very Low		169.25a***	Low
ORP	27b**	Good		28.25a**	Good
pH	7.4a***	High		8.35b***	Very High
Copper, PPM	4.875b***	Very High		0.825a***	Good
Iron, PPM	27.325b***	Very High		5.6a***	Low
Zinc, PPM	6.825b***	Very High		3.375a***	Good
Mn, PPM	2.075	Very Low		2.525	Very Low
P:K	0.1075b***	Very Low		0.605a***	Low
Ca:Mg	4.86b*	Low		10.145a*	Good
Ca:P	24.375	High		22.465	High
Soil Index, 0-100	22.5b***	Poor		58a***	Medium

N = 8, n = 4 (rows)
a, b Significant differences * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 4.3 **Summary of human glycemic results**

	Biological, 11.9 brix	Conventional, 12.4 brix	P value
Peak Values	128.6 ± 3.8 mg/dL	129.6 ± 3.7 mg/dL	0.816
Base to Peak	37.2 ± 3.6 mg/dL	36.5 ± 2.8 mg/dL	0.874
Time to Peak	28.0 ± 2.0 min	30.0 ± 2.2 min	0.271

± SEM**;** N = 15

Chapter 5

Soluble Solids, Shelf Life and Human Sensory Perception of Apples from Biologically-enhanced Organic, Biologically-enhanced Conventional and Typical Conventional Systems

5.1 **Abstract**

Orchard management systems may affect soluble solids levels in apples. Increasing fruit soluble solids levels may improve flavor. Orchard management systems may also affect the shelf life of apples. In this experiment, two apple cultivars, each grown under differing management systems, were evaluated for soluble solids content (SSC), shelf life and consumer acceptability by human sensory panels. In 2007, we evaluated Washington *(Malus domestica)* *'Braeburn'* apples raised under biologically-enhanced organic and typical conventional methods. We further split treatments to include apples from the outside and inside of the tree canopies. Organically-grown 'Braeburn' apples from both outside and inside the canopies had higher SSC ($P < 0.001$) than those conventionally-grown. Fruit SSC was higher ($P = 0.002$) overall in 'Braeburn' apples from outside the canopies (11.5 brix) than from inside the canopies (10.7 brix). There was no overall difference ($P = 0.366$) in shelf life as measured by penetrometer between organic and conventional 'Braeburn' apples. Nor was there an overall shelf life difference ($P = 0.286$) between 'Braeburn' apples from outside of the canopy versus inside the canopy. The 'Braeburn'

overall acceptability sensory ratings showed highly significant differences (P < 0.001), with organic surpassing conventional fruits, and outside-canopy fruits surpassing inside-canopy fruits. In 2008, we evaluated Washington *(M. domestica) 'Crimson Gala'* from orchards under biologically-enhanced conventional and typical conventional management. The conventionally-grown 'Gala' apples had higher (P = 0.005) soluble solids levels than the biologically-grown fruit. 'Gala' penetrometer readings for shelf life showed a difference (P = 0.035) between treatments, with the conventional being firmer than the biological. There was also a difference (P = 0.014) in the 'Gala' overall acceptability sensory rating, with the conventional being favored over the biological. The above measured values were also correlated with soil, leaf, and fruit tissue values. It should be noted that the biological 'Gala' apples were grown on a sandier soil type than the 'Gala' conventional apples. Understanding how cultivation practices affect consumer acceptance will encourage growers to further improve production practices.

5.2 **Introduction**

Sensory evaluation is a scientific method to evaluate products using the five senses of vision, smell, taste, touch and hearing (Lawless and Heymann, 1999; Stone and Sidel, 1993). Improved human sensory perception of fruit may lead to increased consumer demand. Differing cultivation practices may result in differences in fruit quality. For example, Peck et al. (2006) compared apples grown under organic, conventional, and integrated systems finding organically-grown being preferred by human sensory panelists. The organic apples were sweeter and less tart. Within the same cultivar, our preliminary data showed that higher SSC apples tend to be preferred over lower SSC apples in sensory evaluations. High SSC fruit is also reported to be more satisfying (Harrell,1998). Apple firmness is another important aspect of consumer acceptance, as well as an indicator of shelf life (Peck et al., 2006). Consumers rated apples produced by organic and integrated management better in firmness, texture and overall acceptability than conventionally-produced apples (Peck et al., 2006). Laboratory measurements coincided with consumer ratings, with organic apples being firmest, integrated management second, and the conventional least firm.

This experiment involved ‘Braeburn’ apples from biologically-enhanced organic and typical conventional systems, and ‘Gala’ apples from biologically-enhanced conventional and typical conventional systems. Biological systems emphasize enhancement of soil microbiology, and soil and foliar nutrient applications. In addition to soil and leaf analyses, fruit soluble solids were measured with a digital refractometer, and fruit firmness (shelf life) was measured with a penetrometer over a period of five months. A minimum of 100 human subjects tasted samples from each treatment of the two cultivars of apples and responded to a questionnaire regarding visual appearance, flavor, texture, and overall acceptability.

The aim of this research was to examine environmentally-favorable ways to improve fruit quality and consumer acceptance. Biological production of nutrient-rich foods may have many positive environmental impacts. First, high quality foods can only be produced from soil that is properly managed without excessive use of inorganic nitrogen or other chemical fertilizers which may create excess nitrate levels in foods and pollute ground water (Andersen, 2000; Chaboussou, 2004; Skow and Walters, 1995). Second, healthy, nutrient-rich plants do not experience the disease and pest pressure that deficient plants do (Tainio, 2007b). Third, nutrient-rich foods deliver more nutrition in a compact fashion, decreasing shipping costs in both monetary and environmental ways. Fourth, produce with extended shelf life is more likely to be consumed than discarded, which would prevent huge losses on many fronts.

With increased consumer demand for high quality products, conscientious growers may expect steady market growth and increases in premiums for improved quality.

5.2.1 Objectives

Measure and compare the following factors--

1. ‘Braeburn’ soluble solids, biologically-enhanced organic vs. conventional, and outside-canopy vs. inside-canopy
2. ‘Braeburn’ shelf life, biologically-enhanced organic vs. conventional, and outside-canopy vs. inside-canopy
3. Human sensory perception of ‘Braeburn’ apples, biologically-enhanced organic vs. conventional, and outside-canopy vs. inside-canopy
4. ‘Gala’ soluble solids, biologically-enhanced conventional vs. typical conventional apples
5. ‘Gala’ shelf life, biologically-enhanced conventional vs. typical

conventional apples

6. Human sensory perception of ‘Gala’ apples, biologically-enhanced conventional vs. typical conventional

5.3 **Materials and Methods**

5.3.1 2007 ‘Braeburn’ Apples, Biologically-enhanced Organic vs. Conventional, Outside-canopy vs. Inside-canopy

Two adjacent Royal City, Washington state ‘Braeburn’ apple orchards on the same Royal loamy fine sand soil type (NRCS, 2009) and trees of the same rootstock, but under differing cultivation methods, were compared.

5.3.1.1 Biologically-enhanced organic ‘Braeburn’

The approximately eight year old experimental biologically-enhanced organic orchard had transitioned from conventional production and had been certified organic for approximately one year. The tree planting density was 407 trees per acre with 15 feet between rows and seven feet between trees within rows. The cover crop consisted of mowed grass. A wire trellis system was used along with a tubing/spray irrigation system. In addition to being an organically-managed orchard, this orchard was considered to be an experimental orchard due to the biology-enhancing products used. No soil amendments were used in 2007, but certified organic Rhizoboost®, a probiotic soil conditioner from BIO-CAT Microbials (BCM) of Shakopee, Minnesota, and Organic Restoration, a fertilizer blend from Soil Restoration, LLC, Culver, Oregon, were sprayed on the ground. Microbiology-enhancing Bio-N-Liven Answer® and The Carbon Answer®

sprays (Environmental Care and Share, Inc., Golden, Colorado), containing various enzymes, mineral electrolytes, vitamin precursors and sugars were applied to the ground and trees in 2006 and 2007. These were utilized every two weeks starting at bloom for three applications. Blossom thinning was accomplished with lime, sulfur and fish oil, along with hand thinning. Certified organic Entrust® (Dow AgroSciences, LLC, Indianapolis, Indiana) and pheromone confusion were used to decrease leafroller and codling moth infestations. Sulfur was used before fruiting to discourage powdery mildew.

5.3.1.2 Conventional ‘Braeburn’

The approximately 15 year old conventional control orchard had been continuously conventionally managed. The tree planting density was 242 trees per acre with 18 feet between rows and 10 feet between trees within rows. The cover crop consisted of mowed grass, with glyphosate used as an herbicide. There was no evidence of a trellis system. A sprinkler irrigation system was utilized. Ammonium sulfate, calcium nitrate and NPK were used as fertilizers. A calcium-containing foliar spray was applied five times per year. Blossom thinning was accomplished by spraying with carbamate (Sevin) (Bayer CropScience LP, Research Triangle Park, NC). Various pesticides were used every two to three weeks throughout the season to control insects and diseases.

5.3.1.3 ‘Braeburn’ sampling

At October harvest time, four rows of apples, five rows apart and at least five rows from the service road which divided the two treatment orchards, were randomly selected for sampling each treatment. Ten trees were selected (trees 10 through 19 from the beginning of each row) in each of the selected four rows from each orchard.

Soil core samples (0 to 6 inches deep) were obtained from the midpoints between the selected apple trees in each selected row and pooled by mixing in a bucket to obtain four pooled soil samples from each orchard described above. These were sent to International Ag Labs, Fairmont, MN for formazan testing. The formazan test is a colorimetric assay of dehydrogenase activity (Camina et al.,1998), to determine microbial activity. Typical formazan values for conventionally-farmed soil are 100-200 PPM, whereas biologically-farmed soils typically range 300-600 PPM (International Ag Labs, 2006).

From each selected tree, four apples from the outside tree canopy at mid-canopy height and four apples from inside the canopy at mid-canopy height were picked. Apples were separately bagged and labeled for each cultivation and canopy treatment, but pooled by row. They were shipped in cardboard apple boxes via flatbed truck. The apples arrived in Colorado one week post harvest. They were stored in the shipping boxes at Colorado State University's cold storage units in the Shepardson building, in regular atmosphere, refrigerated at 2^{o}C.

5.3.2 2008 'Crimson Gala' Apples, Biologically-enhanced Conventional vs. Typical Conventional

Two southern Washington state 'Crimson Gala' apple orchards on the same Malling 26 dwarf rootstock, but under differing cultivation methods were compared. The biologically-managed orchard was located near Pasco, Washington on sandy loam soil (cation exchange capacity [CEC] 7.1 meq/100g). The conventionally-managed orchard was located approximately 60 miles away near Mattawa, Washington on predominantly Kennewick silty loam soil (NRCS, 2009) with a CEC of 11.3 meq/100g.

5.3.2.1 Biologically-enhanced conventional 'Gala'

The approximately 15 year old experimental biologically-managed orchard, while still classified as conventionally-managed, had been treated with biological enhancement products for approximately four years. The tree planting density was 453 trees per acre with 16 feet between rows and six feet between trees within rows. The cover crop consisted of mowed mixed grasses, with one 0.5 gallon per acre application of Goal and Surflan pre-emergent herbicide (Dow Chemical Company) and one quart per acre of RoundUp® (Monsanto Company) used to control weeds. A single-pole, two-wire trellis system was used along with a permanent under-tree irrigation system and overhead sprinklers, which were used for hydrocooling the orchard. No soil amendments were used in 2008, but Arbor T & O, a fertilizer blend from Soil Restoration, LLC, Culver, Oregon, was applied through the sprinkler system at a rate of one quart per acre. Microbiology-enhancing Bio-N-Liven Answer® and The Carbon Answer® sprays (Environmental Care and Share, Inc., Golden, Colorado), containing various enzymes, mineral electrolytes, vitamin precursors and sugars had been applied to the ground and trees with a tractor sprayer two to five times per season for four years. Bio-N-Liven Answer® and The Carbon Answer® were applied foliarly at rates of 8oz and 16oz, respectively, in 100 gallons of water per hectare. These were typically utilized approximately every two weeks starting at the ½" green leaf stage. Neither was applied in the two months prior to the 2008 harvest, even though it was recommended by the manufacturer. Blossom thinning was accomplished with three separate chemical treatments (initially with lime-sulfur at a rate of six gallons per acre, then twice with Kalo Regulaid at one pint per acre and Sevin® XLR Plus at one quart per acre). Some hand thinning was also needed. Foli-Gro Link Calcium (Wilbur-Ellis, Fresno, CA) and Vigor-Cal (Agro-K Corp., Minneapolis, MN), foliar calcium products, were applied once and twice, respectively, in 2008 at a rate of 2 quarts per acre per application. Cuprofix (United Phosphorus, Inc., King of Prussia, PA), a copper sulfate product, was applied twice a year at rates of 7.5 and 10 lbs to decrease incidence of fire blight. Two gallons per acre of liquid NPK 3-18-18 fertilizer was mixed into the spray regimens. Pristine® fungicide (BASF Company) at 4.5 oz per acre and Procure® (Crompton Corp., Middlebury, CT) at 12 oz per acre were used to decrease fungal infections. Lorsban® 4E (Dow AgroSciences), Delegate™ (Dow AgroSciences), Diazinon 50 WSB (an organophosphate from Gowan Company), and pheromone confusion were used to decrease insect infestations such as wooly aphid and codling moth. Raynox® (Pace International, LLC, Seattle, WA), a carnauba coating, was sprayed from a helicopter three times during the season at a rate of 2.5 gallons per acre to prevent sunburn of the fruit. It was expected that 90% of the 2008 crop load would bring premiums as Washington Extra Fancy due to color and size.

5.3.2.2 Typical conventional ‘Gala’

The approximately 15 year old conventional control orchard had been continuously conventionally managed. The tree planting density was 484 trees per acre with 15 feet between rows and six feet between trees within rows. The cover crop consisted of mowed mixed grasses, with glyphosate used as an herbicide. A single-pole, one-wire trellis system was used along with a permanent under-tree irrigation system. Approximately 100 lbs per acre of calcium nitrate was applied in the spring. A calcium-containing foliar spray was applied five times per year. NPK was applied 3 times during spring/summer. Blossom thinning was accomplished by spraying with Sevin® carbamate (Bayer CropScience LP, Research Triangle Park, NC), and hand thinning late in the season. Various pesticides, including organophosphates, were used throughout the season to control insects and diseases. This orchard’s apples achieved premium color ratings, but were smaller than ideal.

5.3.2.3 ‘Gala’ sampling

At late August harvest time, four rows of apples, five rows apart were randomly selected for sampling each treatment. Ten trees were selected (trees 10 through 19 from the beginning of each row) in each of the selected four rows from each orchard.

Soil core samples (0 to 6 inches deep) were obtained from the midpoints between the selected apple trees in each selected row and pooled by mixing in a bucket to obtain four pooled soil samples from each orchard described above. These were sent to International Ag Labs, Fairmont, MN for formazan and soil nutrient testing which included humus, pH, ergs, ORP, nitrates, ammonia, phosphorus, potassium, calcium, magnesium, sodium, copper, iron, zinc, and manganese. These values were compared to desired levels and summarized by International Ag Labs with soil index ratings, range 0-100, with 100 being the ideal score.

Additionally, a pooled soil sample was taken from each orchard by the method described above, except that the rows were pooled to result in only one sample per orchard. These were sent for analyses by Midwest Laboratories, Inc., Omaha, NE. These analyses included organic matter, minerals, pH, and cation exchange capacity (CEC).

Also at harvest time, pooled leaf samples (10 mid-terminal shoot leaves from each of 10 trees from each of four rows) were collected from each orchard. These two pooled leaf samples were submitted to Midwest Laboratories, Inc. by Priority Mail for mineral and nitrogen analyses.

From each selected tree, four apples of similar size were randomly picked from the outside tree canopy at mid-canopy height. Apples were separately bagged and labeled as they were picked for each cultivation method, but pooled by row. Some commercial produce packers drench fruits with various chemicals such as calcium chloride or fungicide, and apply wax to improve shelf life. To avoid skewing the data, fruits were picked directly from the trees thus avoiding post-harvest treatments.

Fruit tissue analyses were performed for each of the selected four rows, using randomized, pooled samples of eight apples per each row, by International

Ag Labs, Fairmont, Minnesota. These apple samples were sent by Priority Mail directly from Washington state to the laboratory in Minnesota. The assays included free nitrates, nitrogen, calcium, phosphorus, magnesium, potassium, sodium, boron, copper, iron, zinc and manganese (analyses by atomic absorption and spectrophotometer).

The remaining apples were shipped in cardboard apple boxes via unrefrigerated truck. The apples arrived in Colorado one week post harvest. They were stored in the shipping boxes at Colorado State University's cold storage units in the Shepardson building, in regular atmosphere, refrigerated at 2^{o}C.

5.3.3 Soluble Solids and Shelf Life Testing of 'Braeburn' and 'Gala' Apples

Soluble solids and shelf life were measured on multiple occasions (over time) for both the 'Braeburn' and the 'Gala' orchards. For the 'Braeburn' orchards, three inside-canopy and three outside-canopy apples were randomly selected from the labeled bags as representatives from each selected row of both the organic and conventional orchards for each testing session. For the 'Gala' orchards, three randomized (randomly selected), biological replicates (apples) from each of the selected four rows of each experimental (biological) and conventional control treatment were utilized for tests and assays.

The selected apples were first tested for firmness using an apple penetrometer (Hunter Spring Mechanical Force Gauge, Ametek, Inc., Hatfield, PA). The apples were tested at their equators. Shelf life for both varieties was evaluated after harvest, and approximately two and four months later. The evaluation was based on loss of firmness over time as measured with the penetrometer. Notations of additional indicators of shelf life such as molding, browning and rotting were recorded.

Second, a juice aliquot was obtained from a representative edible portion near the equator of each selected fruit, tested for soluble solids concentration (SSC), and reported as °brix. Brix values were obtained from juice samples using a hand-held, digital refractometer (Pike Agri-Lab Supplies, Jay, ME).

Third, only during the first testing sessions for each cultivar, these same selected apples were prepared for freeze drying. The apples were sliced and had inedible parts removed, but the peel was left intact. The slices were weighed, freeze-dried, re-weighed for determining dry matter content, powdered by grinding in a coffee grinder, and stored in screw-top plastic tubes at -20 ^{o}C awaiting additional testing.

5.3.4 Human Sensory Perception of ‘Braeburn’ and ‘Gala’ Apples

For sensory evaluation, each category of fruit was prepared (washed, sliced, cored, peel left intact) in a manner to provide similar samples, labeled only with numbers. Sensory perception tests were performed with 100 and 102 untrained human panelists, for ‘Braeburn’ and ‘Gala’ apples, respectively. This testing was performed in various locations on the Colorado State University (CSU) campus in single-blind trials, so that the panelists did not know what treatments had been applied to the apples. Approval was obtained through the CSU Institutional Review Board for human subjects testing. Each panelist received approximately equal portions of fruit (1/12 of an apple) from each treatment, water and unsalted crackers for cleansing their palate between samples, a napkin and a score sheet. The evaluators tasted and rated the samples for test parameters, including ratings for appearance, flavor, texture and overall acceptability on a 9-point hedonic scale.

5.4 **Statistical Analyses**

Data were analyzed for significance and correlations using Microsoft Excel and SAS statistical software (SAS Institute, Cary, NC) statistical software. Data were subjected to t-tests, Analysis of Variance (ANOVA) and/or Pearson correlation analyses as required. Observation-related differences were calculated, with $P < 0.05$ considered significant.

5.5 **Results and Discussion**

5.5.1 ‘Braeburn’ Apples

5.5.1.1 ‘Braeburn’ soluble solids, biologically-enhanced organic vs. conventional, outside-canopy vs. inside-canopy

‘Braeburn’ fruit soluble solids content (SSC) was measured on two occasions, at 12 days after picking and four months later (day 138). Overall, the organic fruit from both outside and inside the canopies had higher soluble solids levels ($P < 0.001$) than the conventionally-grown fruits, with the organic fruit averaging 11.9 brix and the conventional fruit averaging 10.3 brix (Fig. 5.0). This was consistent with the findings of DeEll and Prange (1992) who also found higher soluble solids in organic apples compared with those conventionally grown.

Fruit SSC was higher (P = 0.002) overall in fruit from outside the canopies (11.5 brix) than from inside the canopies (10.7 brix), possibly due to greater sun exposure (Fig. 5.0). Fruit SSC increased (P < 0.001) over time as fruit starches were converted to sugars. The overall soluble solids, disregarding treatments, was 10.6 brix on day 12 and 11.6 brix on day 138.

Examining individual dates, on day 12 the organic fruit (11.4 brix) had higher soluble solids (P < 0.001) than the conventional fruit (9.8 brix). The outside-canopy fruit (11.2 brix) had higher soluble solids (P < 0.001) than the inside-canopy fruit (10.0 brix). There was no interaction between the cultivation treatment and the canopy location (P = 0.271). Not surprisingly, fruit soluble solids levels were highly correlated (r = .996, P = 0.004) to fruit dry matter levels.

On day 138, the organic fruit (12.4 brix) continued to show higher soluble solids (P < 0.001) than the conventional fruit (10.7 brix). There was no difference (P = 0.181) between outside-canopy fruit (11.7 brix) and inside-canopy fruit (11.4 brix). There was, however, interaction between the cultivation treatment and the canopy location (P = 0.005), with the inside-canopy fruits showing a greater difference between the organic and conventional treatments than the outside-canopy fruits.

Reig et al. (2007) also found organically-grown apples higher in soluble solids than conventionally-grown. Organic high SSC apples may be marketed as better tasting apples. Indeed, SSC is already being used as a marketing tool by some grocery stores, where they “buy on brix and sell on brix” (Marler, 2006). This equates to higher premiums for growers and greater incentives for conventional growers to convert to organic production. It is encouraging that environmentally-sensitive management practices can produce as good or better apples than conventional systems. It is hoped that there will be an overall shift in production practices to those that produce more healthful apples and are more environmentally friendly.

Examining SSC-quality correlations in our study and others may prove interesting enough to lead to additional studies to affirm or reject the use of the refractometer for use with many varieties of fruits and vegetables. Ultimately, refractometers may be promoted as an easy-to-use tool for any purchaser of produce, whether commercially or for home consumption, to estimate quality. Wider use of refractometers may help raise awareness of differences in produce quality. Produce buyers will demand higher quality which farmers, in turn, will seek to provide. It is beyond the scope of our study to thoroughly explore refractometer application methods, but produce quality may be improved through appropriate nutrient and microbial applications, and this opens the door for further research with refractometers as useful monitoring devices.

5.5.1.2 'Braeburn' percentage dry matter (%DM) , biologically-enhanced organic vs. conventional, outside-canopy vs. inside-canopy

Two-factor ANOVA testing showed an overall difference ($P < 0.001$) between the percentage of dry matter in the organic and conventional apples, with the organic apples being higher (Fig. 5.1). The organic apples averaged 13.9% DM, while the conventional apples averaged 12.3% DM. There was also an overall difference ($P < 0.001$) in the percentage of dry matter due to canopy location, with the outside-canopy fruits being higher (Fig. 5.1). The outside-canopy fruits averaged 13.8% DM, while the inside-canopy fruits averaged 12.4% DM. There was no interaction between the cultivation treatment and the canopy location ($P = 0.144$).

More specifically, t-testing revealed that, while there was an overall difference in the percentage of dry matter between organic and conventional, there was no difference ($P = 0.633$) between conventional outside-canopy fruits (average 13.2% DM) and organic inside-canopy fruits (average 13.5% DM). Also, even though overall there was a higher percentage of dry matter in outside-canopy fruits, within the organic treatment there was no difference ($P = 0.070$) between outside (average 14.4% DM) and inside-canopy (average 13.5% DM) fruits.

5.5.1.3 'Braeburn' shelf life, biologically-enhanced organic vs. conventional, outside-canopy vs. inside-canopy

'Braeburn' apples were evaluated for shelf life at days 12, 72, and 138. There were the expected decreases ($P < 0.05$) in firmness over time in all 'Braeburn' treatments, with 19.2 pound test on day 12, 15.0 pound test on day 72, and 13.8 pound test on day 138 (Fig. 5.2). As shown in Figure 5.2, penetrometer readings in this study showed no overall difference ($P = 0.366$) between organic (15.7 pound test) and conventional (16.3 pound test). Nor was there an overall difference ($P = 0.286$) between fruits from outside of the canopy (15.7 pound test) versus inside the canopy (16.3 pound test) (Fig. 5.2). Some studies have shown organic apples being more firm than their conventional or integrated management counterparts (DeEll and Prange, 1992; Weibel et al., 2000; Peck et al., 2006; Reig et al., 2007). The ‘Braeburn’ apples in this study had been certified organic for only one year. Perhaps longer term organic management makes a difference in firmness.

There were no differences ($P > 0.05$) seen at day 12 between organic (19.5 pound test) and conventional (18.9 pound test) fruit, or between outside (19.3 pound test) and inside (19.1 pound test) canopy fruit. However, at day 72, higher readings ($P = 0.002$) were seen in the conventional apples (15.8 pound test) than the organic apples (14.2 pound test). Inside-canopy fruits (15.8 pound test) were firmer ($P = 0.003$) than outside-canopy fruits (14.2 pound test). There was no interaction between the cultivation treatment and the canopy location ($P = 0.855$). On day 138, no difference ($P = 0.146$) was seen between organic (13.5 pound test) and conventional (14.1 pound test) apples. Nor was there a difference ($P = 0.180$) between outside-canopy (13.5 pound test) and inside-canopy (14.1 pound test) fruits. However, there was interaction between the cultivation treatment and the canopy location ($P = 0.003$). The inside-canopy conventional apples had a higher pound test than the inside-canopy organic apples, while there was no difference between the management systems on the outside-canopy apples.

The conventional apples had some incidence of black-colored mold and bitter pit. The organic apples, especially those from the inside canopy, tended to have a greater incidence of bitter pit than the conventional.

5.5.1.4 ‘Braeburn’ soil test—microbial activity, biologically-enhanced organic vs. conventional

Formazan microbial soil testing of the ‘Braeburn’ orchards showed numerically higher, but not statistically significant, levels of microbial activity in the organically-managed orchard ($P = 0.144$). Formazan was an average of 1756.25 PPM in the organic orchard and an average of 1490.3 PPM in the conventional control orchard. There was a significant correlation ($r = .957$, $P = 0.043$) between formazan levels and penetrometer measurements, implying that increased soil microbial activity may result in increased fruit firmness and longer shelf life, which may be expected due to microbially-enhanced uptake of nutrients into the trees.

5.5.1.5 Human sensory perception of ‘Braeburn’ apples, biologically-enhanced organic vs. conventional, outside-canopy vs. inside-canopy

‘Braeburn’ sensory testing was performed approximately two months after picking using 100 untrained panelists from CSU. The apples had been continuously stored in regular atmosphere at 2^{o}C since receipt, one week after harvest. A hedonic scale of 1-9 was used, with 9 being the top score. As shown in Figure 5.3, there were highly significant differences ($P < 0.001$) in ‘Braeburn’ apple sensory appearance with organic fruits (score 7.92) being preferred over the conventional fruits (score 7.29), and outside-canopy fruits (score 8.09) being preferred over the inside-canopy fruits (score 7.12). There was also significant interaction between the cultivation treatment and the canopy location ($P = 0.012$).

The inside-canopy organic apples had a higher appearance score than the inside-canopy conventional apples, while there was no difference in the score between the management systems on the outside-canopy apples. These data agree with a study by Weibel et al. (2000) showing organic apples being preferred by sensory panelists over integrated management apples.

'Braeburn' sensory flavor data (Fig. 5.4) was also highly significantly different ($P < 0.001$), with organic fruits (score 7.36) surpassing conventional fruits (score 6.69), and outside-canopy fruits (score 7.56) surpassing inside-canopy fruits (score 6.48). There was also highly significant interaction between the cultivation treatment and the canopy location ($P < 0.001$). The inside-canopy organic apples had a higher flavor score than the inside-canopy conventional apples, while there was no difference in the score between the management systems on the outside-canopy apples.

The 'Braeburn' sensory texture ratings (Fig. 5.5) showed no difference ($P = 0.438$) between organic (score 7.53) and conventional (score 7.41) fruits. Nor was there a difference ($P = 0.522$) between outside-canopy (score 7.42) and inside-canopy (score 7.52) fruits. There was, however, a significant ($P = 0.002$) interaction between the cultivation treatment and the canopy location. The inside-canopy organic apples had a higher texture score than the inside-canopy conventional apples, while there was no difference in the score between the management systems on the outside-canopy apples.

The overall acceptability sensory scores showed highly significant differences ($P < 0.001$), with organic (score 7.50) surpassing conventional (score

6.89) fruits, and outside-canopy (score 7.60) surpassing inside-canopy (score 6.79) fruits (Fig. 5.6). There was highly significant interaction between the cultivation treatment and the canopy location ($P < 0.001$). The inside-canopy organic apples had a higher overall acceptability score than the inside-canopy conventional apples, while there was no difference in the score between the management systems on the outside-canopy apples. This data agrees with the findings of sensory panelists in the study by Peck et al. (2006) where organic apples were preferred over conventional apples regarding texture, flavor, firmness and overall acceptability.

Panelists were asked to rank each treatment from 1 to 4, with 1 being their favorite. There was no difference ($P > 0.05$) between outside conventional (score 2.095), outside organic (score 2.135), and inside organic (score 2.36) apples, however there was a difference ($P < 0.001$) between these top three treatments and the inside conventional apples, which scored a distant last (score 3.41).

Pearson correlations showed some significant correlations ($P < 0.05$) between several factors. A positive correlation ($r = 0.957$, $P = 0.043$) was seen between appearance and percentage of dry matter (%DM). Flavor was positively correlated with appearance ($r = 0.952$, $P = 0.048$). Appearance was positively correlated with the overall ratings ($r = 0.959$, $P = 0.041$). Flavor was also positively correlated with the overall ratings ($r = 0.996$, $P = 0.004$). Flavor was understandably negatively correlated with rank ($r = -0.963$, $P = 0.037$). These correlations are summarized in Table 5.0.

5.5.2 ‘Gala’ Results, Biologically-enhanced Conventional vs. Typical Conventional

5.5.2.1 ‘Gala’ soluble solids

‘Crimson Gala’ fruit soluble solids were measured on three occasions, at 12 days after picking, two months (day 68) and four months (day 128). Considering all dates tested, the conventionally-grown fruit (12.3 brix) had higher (P = 0.005) soluble solids levels than the biologically-grown fruit (11.7 brix) (Fig. 5.7). As shown in Figure 5.7, there were no differences over time of the three dates tested (P = 0.193). There were no differences between treatments at day 12 (P = 0.533) or day 68 (P =0.053), however there was a difference (P = 0.028) at day 128, with the conventional control apples (12.3 brix) having higher soluble solids than the biological apples (11.6 brix). There was no interaction between treatment and time (P = 0.395).

5.5.2.2 ‘Gala’ percentage dry matter (%DM)

There was no difference (P = 0.053) between the percentage of dry matter of the biological apples (average 13.8% DM) and the conventional control apples (average 14.5% DM).

5.5.2.3 ‘Gala’ fruit nutrient analyses

Fruit nutrient density analyses were performed on apples from each row by International Ag Labs, Inc. (Table 4.0). Fruit density ([mass/volume] x 100) was measured on the fresh fruit before drying (compared to the density of water at 100). There was no density difference between the treatments (P = 0.906), with the biologically-raised fruit scoring 93.25 and the conventional at 93.5. There were also no differences in percentage of dry matter (%DM), soluble solids content (brix), percentage of protein, percentage of calcium, percentage of phosphorus, percentage of magnesium, PPM copper, PPM iron, PPM zinc, or PPM manganese. There were, however, differences in potassium, sodium and pH. The conventional control apples had a higher percentage of potassium (P = 0.011), lower percentage of sodium (P = 0.010), and a lower pH (P = 0.001).

It was not expected that all tested fruit mineral levels would be statistically different between treatments, especially considering there were only four replicates (rows) from each treatment. It was expected, however, that more compositional differences would have been seen. Fruits were not able to selected from adjacent orchards with the exact same soil type due to an orchard of choice being harvested before samples were able to be obtained. The orchards able to be used were approximately 60 miles apart.

5.5.2.4 'Gala' soil tests—microbial activity, CEC, organic matter

Soil quality may have affected the SSC and other nutrient qualities of the fruits. Beneficial soil microbes help the trees uptake nutrients, which may result in better quality fruit. Formazan microbial soil testing of the 'Gala' orchards showed greater microbial activity in the conventional control orchard (P = 0.017), with an average of 570.75 PPM in the biological orchard and an average of 959.75 PPM in the conventional control orchard. It was originally expected that the soil microbial activity would be greater in the biologically-managed soil, however the biological orchard had a soil with a cation exchange capacity (CEC) of 7.1 meq/100g compared to the conventional control orchard's CEC of 11.3 meq/100g. The biological orchard also had a very low organic matter level of 1.2 % compared to the conventional control orchard at 1.7 %. Low levels of these factors decrease microbial survival and proliferation.

5.5.2.5 'Gala' soil analyses

Soil analyses were performed on each 'Gala' row by International Ag Labs, Inc. (Table 4.2). The various factors were summarized by the laboratory into a soil index rating, based on a scale of 0 to 100, with 100 being ideal. The conventional control orchard average was higher ($P < 0.001$) at 58, with the biological orchard at 22.5. There were no differences ($P > 0.05$) in humus levels, nitrates, ammonium, or manganese, with both treatments averaging in the very low ranges. There was a trend ($P = 0.082$) toward lower sodium in the biological soil, with the biological treatment showing a low reading (11 PPM) and the conventional control in the medium range (21.5 PPM). There was no difference ($P = 0.776$) between the calcium to phosphorus ratios (Ca:P), with both treatments showing slightly high. There were differences ($P < 0.05$) in phosphorus, calcium, magnesium, ergs (electrical conductivity), the phosphorus to calcium ratio (P:Ca), and the calcium to magnesium ratio (Ca:Mg), with the biological treatment being in the very low ranges. There were differences ($P < 0.05$) in potassium, copper, iron, and zinc, with the biological treatment running very high (excessive) levels. The oxidation-reduction potential (ORP) was near the ideal level in both treatments. Soil quality may affect apple production and quality (Awasthi et al., 1998; Marcon and Ferraro, 2001). Given the poorer quality soil, as shown by the soil index ratings, it is not surprising that the biological fruits had lower SSC levels.

5.5.2.6 'Gala' leaf tissue analyses

Leaf tissue analyses by Midwest Laboratories, Inc. included nitrogen, phosphorus, potassium, magnesium, calcium, sulfur, sodium, iron, manganese, boron, copper, and zinc. The analyses showed low nutrient levels on several items from both treatments, especially nitrogen, manganese, copper and zinc. With only one pooled sample from each orchard, these are unable to be compared statistically. However, they do reflect the low soil nitrogen and manganese values, and show the inability of the trees to uptake copper and zinc.

5.5.2.7 'Gala' shelf life

The Gala apples were tested with a penetrometer for firmness/shelf life at days 12, 68, and 128 (Fig. 5.8). There were the expected decreases ($P < 0.001$) in firmness over time in both treatments. The biological apples dropped over time from 24.7 to 18.5 to 15.6 pound test. The conventional control apples dropped over time from 27.2 to 20.0 to 14.8 pound test. As shown in Figure 5.8, there were no differences ($P > 0.05$) between treatments on any individual date. However, when examined over all dates with two-factor ANOVA, penetrometer readings showed a difference ($P = 0.035$) between treatments, with the conventional control (20.7 pound test) being firmer than the biological (19.6 pound test) (Fig. 5.8). There was also a significant interaction between the treatments and time (P = 0.029), with the conventional being slightly more firm than the biological on the first two dates tested.

5.5.2.8 Human sensory perception of ‘Gala’ apples

Gala sensory testing was performed approximately five weeks after picking using 102 untrained panelists from CSU. A hedonic scale of 1-9 was used, with 9 being the top score. As shown in Figure 5.9, there were no differences (P > 0.05) between biologically-raised apples and the conventional control apples regarding appearance (average score 8.2), flavor (average score 8.0), or ranking of one treatment over the other. There was a difference (P = 0.039) in texture, with the conventional control (score 7.9) being favored over the biological (score 7.5) (Fig. 5.9). Also shown in Figure 5.9, there was also a difference (P = 0.014) in the overall rating, with the conventional control (score 8.1) again being favored over the biological (score 7.8).

5.6 **Summary**

Biologically-enhanced organic ‘Braeburn’ apples from both outside and inside the canopies had higher soluble solids content (SSC) (P < 0.001) than those conventionally-grown. Fruit SSC was higher (P = 0.002) in ‘Braeburn’ apples from outside the canopies than from inside the canopies. There was no difference in shelf life between organic and conventional ‘Braeburn’ apples (P = 0.366), nor between outside-canopy and inside-canopy apples (P = 0.286). The ‘Braeburn’ sensory ratings showed highly significant differences (P < 0.001), with organic surpassing conventional fruits, and outside-canopy fruits surpassing inside-canopy fruits.

The typical conventional ‘Gala’ apples had higher soluble solids levels (P = 0.005), greater shelf life (P = 0.035), and a higher overall sensory rating (P = 0.014) than the biologically-enhanced fruit. The above measured values were also correlated with soil, leaf, and fruit tissue values. It should be noted that the biological ‘Gala’ apples were grown on a lower cation exchange capacity (CEC) soil than the ‘Gala’ conventional apples, which may have affected the quality. Furthermore, the biological apple trees had been under that system for only the last four of their 15 years. The management had not always applied the biological enhancements according to directions, with none applied in the two months before harvest, which may have affected results. This orchard continued to use conventional pesticides, herbicides, and chemical thinning agents.

5.7 **Conclusions/Implications**

Biologically-enhanced organic production methods resulted in ‘Braeburn’ apples with higher soluble solids levels and a greater percentage of dry matter than those conventionally produced. The organic ‘Braeburn’ apples were also preferred by humans in sensory evaluation trials.

When comparing outside-canopy ‘Braeburn’ apples with inside-canopy apples, the outside-canopy apples had higher soluble solids levels and a higher percentage of dry matter. The outside-canopy apples were also preferred by human sensory panelists.

Understanding how cultivation practices affect fruit quality and shelf life, consumer acceptance, and the environment will encourage growers to further improve production practices. As conscientious producers are well compensated with premiums for producing excellent products, more producers will be encouraged to expend efforts to improve quality.

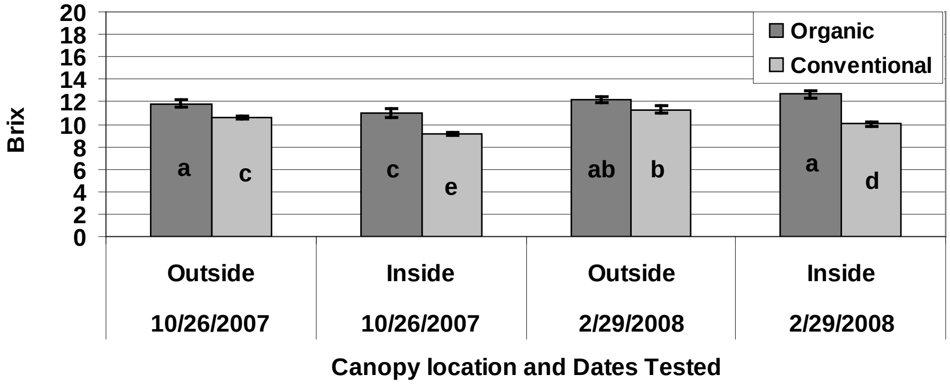

Fig. 5.0 Effects of orchard management, canopy location and time on ‘Braeburn’ apple soluble solids content (SSC), measured in degrees brix. Overall, biologically-enhanced organic apples had a higher SSC than conventionally-managed apples ($P < 0.001$). Overall, outside-canopy apples had a higher SSC than inside-canopy apples ($P = 0.002$). Overall, SSC increased over time ($P < 0.001$). Treatments with the same letter are not significantly different at $P < 0.05$ level. Error bars indicate SEM. Per testing date, N = 48, n = 12 (four rows, three reps each). ANOVA table: Appendix page 139.

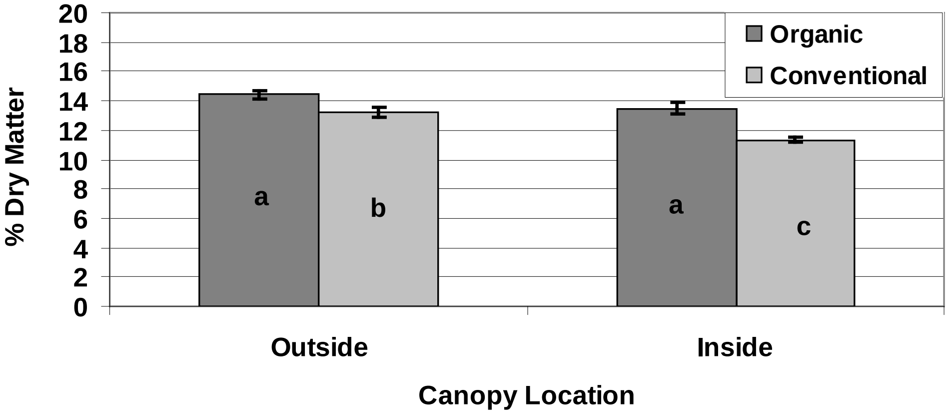

Fig. 5.1 Effects of orchard management and canopy location on ‘Braeburn’ apple dry matter content (%DM). Overall, biologically-enhanced organic apples had higher %DM than conventionally-managed apples ($P < 0.001$). Overall, outside-canopy apples had higher %DM than inside-canopy apples ($P < 0.001$). There was no interaction between orchard management and canopy location ($P = 0.144$). Treatments with the same letter are not significantly different at $P < 0.05$ level. Error bars indicate SEM; N = 48, n = 12 (four rows, three reps each). ANOVA table: Appendix page 140.

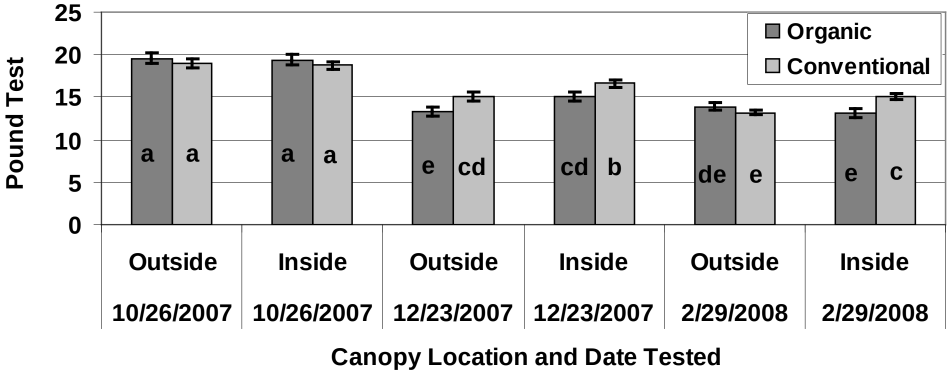

Fig. 5.2 Effects of orchard management, canopy location and time on 'Braeburn' apple firmness, measured with a penetrometer. Overall, there was no difference between the firmness of biologically-enhanced organic apples and conventional apples ($P < 0.366$). Overall, there was no difference between the firmness of outside-canopy apples and inside-canopy apples ($P = 0.286$). Overall, firmness decreased over time ($P < 0.001$). Treatments with the same letter are not significantly different at $P < 0.05$ level. Error bars indicate SEM. Per testing date, N = 48, n = 12 (four rows, three reps each). ANOVA table: Appendix page 140.

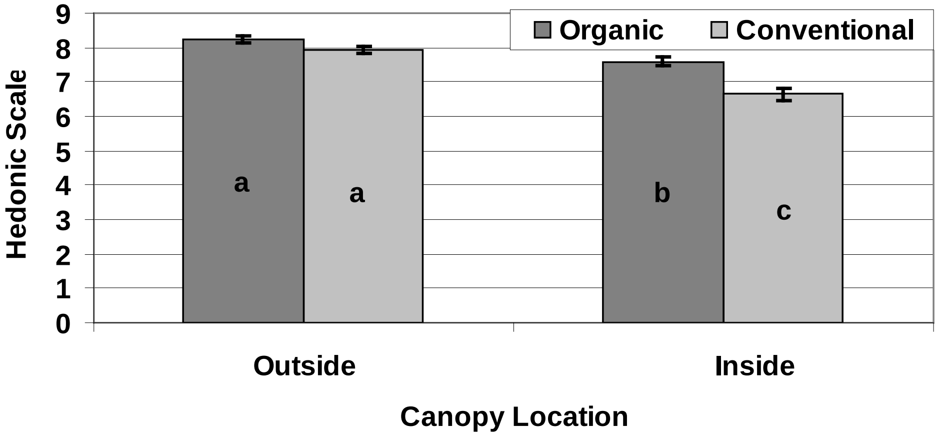

Fig. 5.3 Effects of orchard management and canopy location on ‘Braeburn’ apple human sensory perception of appearance. Overall, biologically-enhanced organic apples had higher scores for appearance than conventionally-managed apples ($P < 0.001$). Overall, outside-canopy apples had higher scores for appearance than inside-canopy apples ($P < 0.001$). There was interaction between orchard management and canopy location ($P = 0.012$). Treatments with the same letter are not significantly different at $P < 0.05$ level. Error bars indicate SEM; N = 100. ANOVA table: Appendix page 141.

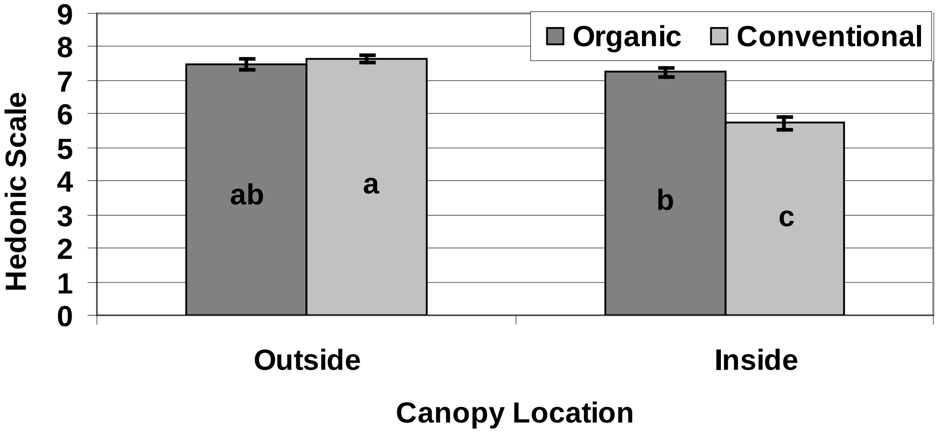

Fig. 5.4 Effects of orchard management and canopy location on ‘Braeburn’ apple human sensory perception of flavor. Overall, biologically-enhanced organic apples had higher scores for flavor than conventionally-managed apples ($P < 0.001$). Overall, outside-canopy apples had higher scores for flavor than inside-canopy apples ($P < 0.001$). There was interaction between orchard management and canopy location ($P = 0.001$). Treatments with the same letter are not significantly different at $P < 0.05$ level. Error bars indicate SEM; N = 100. ANOVA table: Appendix page 141.

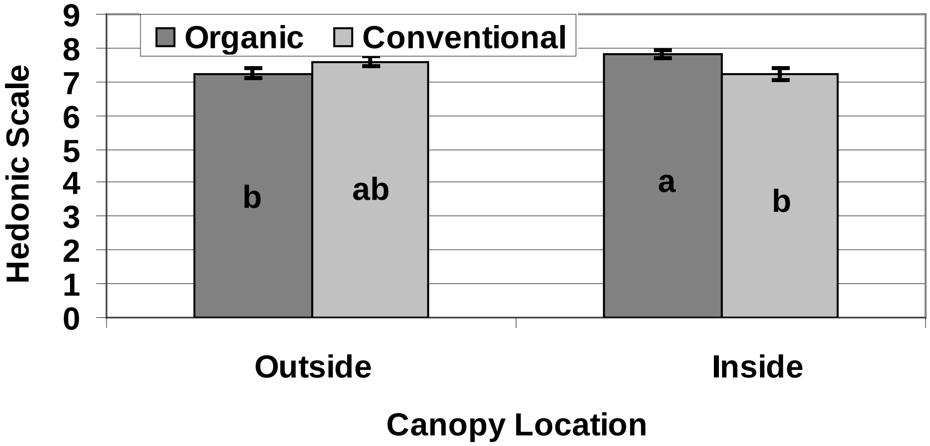

Fig. 5.5 Effects of orchard management and canopy location on ‘Braeburn’ apple human sensory perception of texture. Overall, there was no difference in texture scores between biologically-enhanced organic apples and conventionally-managed apples (P = 0.438). Overall, there was no difference in texture scores between outside-canopy apples and inside-canopy apples (P = 0.522). There was interaction between orchard management and canopy location (P = 0.002). Treatments with the same letter are not significantly different at P < 0.05 level. Error bars indicate SEM; N = 100. ANOVA table: Appendix page 141.

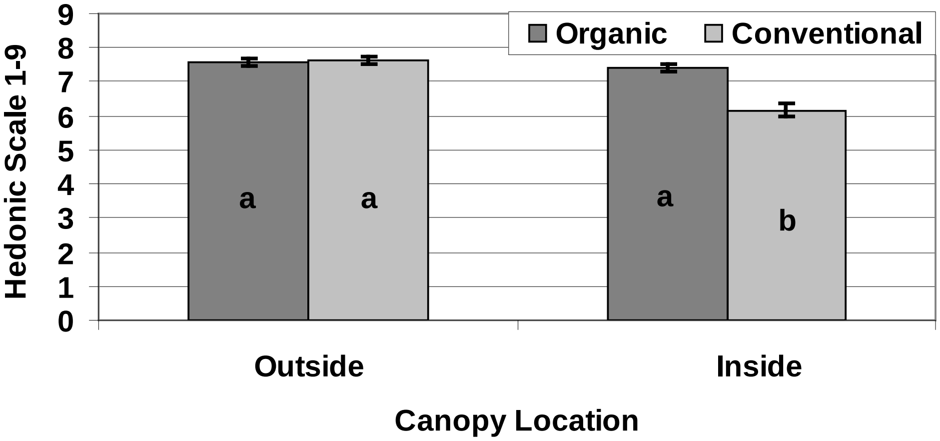

Fig. 5.6 Effects of orchard management and canopy location on ‘Braeburn’ apple human sensory perception of overall acceptability. Overall, biologically-enhanced organic apples had higher scores for overall acceptability than conventionally-managed apples ($P < 0.001$). Overall, outside-canopy apples had higher scores for overall acceptability than inside-canopy apples ($P < 0.001$). There was interaction between orchard management and canopy location ($P = 0.001$). Treatments with the same letter are not significantly different at $P < 0.05$ level. Error bars indicate SEM; N = 100. ANOVA table: Appendix page 141.

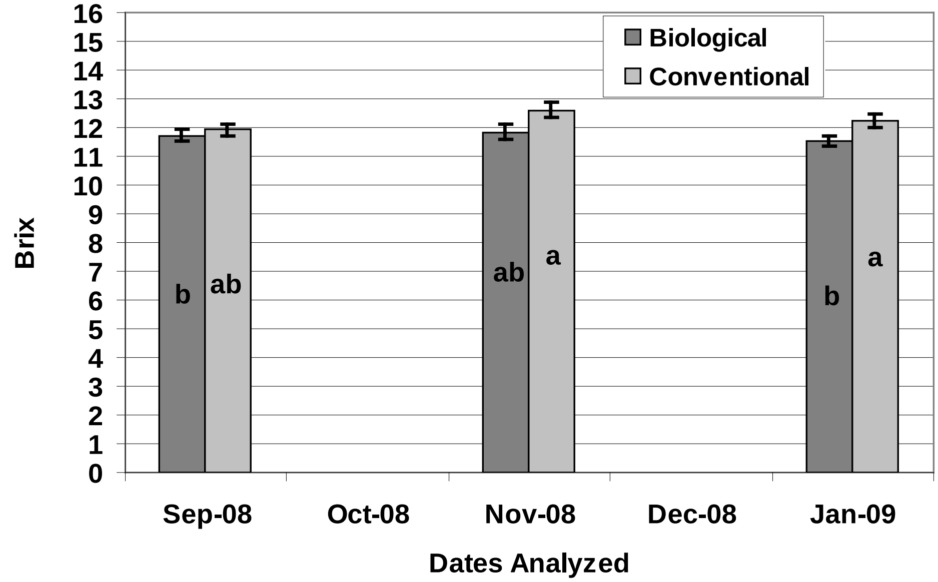

Fig. 5.7 Effects of orchard management and time on ‘Crimson Gala’ apple soluble solids content (SSC), measured in degrees brix. Biologically-enhanced conventional apples had a lower SSC than conventionally-managed apples (P = 0.005). Overall, SSC did not change over time (P = 0.193). There was no interaction between orchard management and time (P = 0.395). Treatments with the same letter are not significantly different at $P < 0.05$ level. Error bars indicate SEM. Per testing date, N = 24, n = 12 (four rows, three reps each). ANOVA table: Appendix page 142.

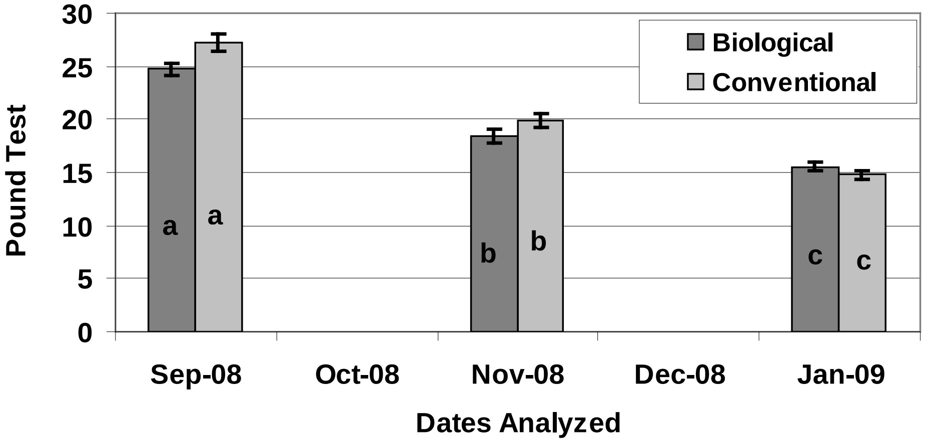

Fig. 5.8 Effects of orchard management and time on ‘Crimson Gala’ apple firmness, measured with a penetrometer. Overall, biologically-enhanced conventional apples were less firm than conventional apples (P = 0,035). Overall, firmness decreased over time (P < 0.001). There was interaction between orchard management and time (P = 0.029). Treatments with the same letter are not significantly different at P < 0.05 level. Error bars indicate SEM. Per testing date, N = 24, n = 12 (four rows, three reps each). ANOVA table: Appendix page 142.

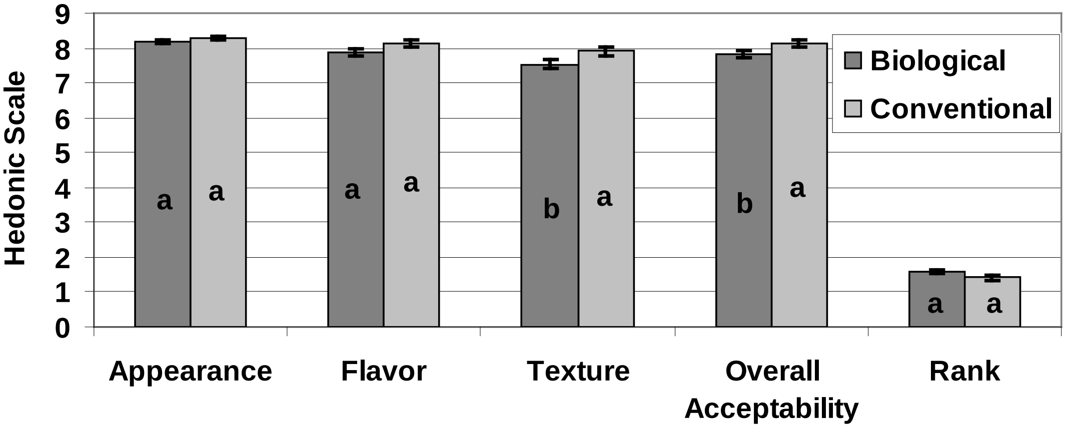

Fig. 5.9 Effects of orchard management on ‘Crimson Gala’ apple human sensory perception. There was no difference in appearance, flavor or rank between biologically-enhanced conventional apples and typical conventional apples ($P < 0.05$). Biologically-enhanced conventional apples scored lower than typical conventional apples for texture ($P = 0.039$) and overall acceptability ($P = 0.014$). Treatments with the same letter are not significantly different at $P < 0.05$ level. Error bars indicate SEM; N = 103.

Table 5.0 **'Braeburn' apple Pearson correlations**

	% DM	Appearance	Flavor	Texture	Overall Acceptability
Appearance	r = 0.957, P = 0.043				
Flavor	r = 0.891, ns	r = 0.952, P = 0.048			
Texture	r = 0.245, ns	r = 0.222, ns	r = 0.493, ns		
Overall Acceptability	r = 0.922, ns	r = 0.959, P = 0.041	r = 0.996, P = 0.004	r = 0.488, ns	
Rank	r = -0.740, ns	r = -0.868, ns	r = -0.963, P = 0.037	r = -0.531, ns	r = -0.937, ns

ns = not significant

Chapter 6

Recommendations for Further Studies

This current study focused on the effects of variations in management on nutrient uptake into the fruit and formation of healthful antioxidant compounds, changes in human sensory qualities and shelf life, as well as effects on human glycemic responses. Further study is merited in these areas due to the potential impact on human health, however related topics of plant management and environment should also be investigated.

Confirming human health benefits of a biological cultivation approach to production may increase marketability of fruit. Verification of increased fruit nutrient content, increased antioxidant levels, and improved human glycemic response may be used as marketing points, especially to individuals with health concerns such as diabetes or obesity.

The glycemic effect observed in preliminary data appears to be related to the SSC level, however it is not a function of SSC alone. It may be due to nutritional balancing, which may result in a fuller genetic expression and development various types of sugars or other compounds which are metabolized more slowly by humans. The reasons for the effect need to be explored through laboratory testing. The phenomenon is not limited to apples. The preliminary data on cherries showed similar results. With continuing research, a similar effect may be expected with other fruits and possibly with some vegetables that have high sugar content.

Comparative laboratory analyses of fruit components, such as sugar, acid and fiber profiles, antioxidants and minerals, will give insight into the reasons for the differences in human responses. Apples contain a polyphenolic compound, phlorizin, which lowers blood glucose (Ehrenkranz et al., 1999). Weibel et al. (2000) found higher levels of phloretin glycosides in organic apples compared to those raised under integrated management. Similarly, Garnweidner et al. (2007) found higher levels of phloridzin in organic apples as compared to those conventionally produced. The ability of biological management to enhance various health-promoting phytochemicals, such as quercitin and phlorizin, and nutrient content needs to be explored.

Additional research on correlation of SSC levels to overall produce quality could help consumers and commercial produce purchasers to use this simple testing method to make wise buying decisions, and help farmers in their quest to produce higher quality.

Publishing research on health benefits directly related to nutritionally-enhanced agricultural systems could greatly increase marketability of the resulting high quality produce, financially rewarding competent producers for assisting in caretaking our populace.

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APPENDICES

Supporting Statistical Analyses

ANOVA ‘Braeburn’ ABTS

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Organic vs. Conv.	34733.28	1	34733.28	2.795171	0.101647	4.061706
Outside vs. Inside	132657.2	1	132657.241	10.67563	0.002109	4.061706
Interaction	103509.2	1	103509.188	8.329932	0.006022	4.061706
Within	546751.7	44	12426.1741			
Total	817651.4	47				

ANOVA ‘Braeburn’ DPPH

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Organic vs. Conv.	5663.708	1	5663.708	1.772346	0.189948874	4.061706
Outside vs. Inside	52483.41	1	52483.41	16.42365	0.000203022	4.061706
Interaction	185.6533	1	185.6533	0.058097	0.81064976	4.061706
Within	140606.4	44	3195.599			
Total	198939.1	47				

ANOVA ‘Braeburn’ Total Phenolics (TP)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Organic vs. Conv.	3673.6	1	3673.6	9.997387	0.00283802	4.061706
Outside vs. Inside	6590.859	1	6590.859	17.93646	0.00011461	4.061706
Interaction	179.336	1	179.336	0.488048	0.48847578	4.061706
Within	16168.06	44	367.456			
Total	26611.86	47				

ANOVA 'Braeburn' Soluble Solids 10/26/2007

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Organic vs. Conv.	29.29688	1	29.296875	34.64523	4.96E-07	4.0617063
Outside vs. Inside	16.45021	1	16.450208	19.45331	6.56E-05	4.0617063
Interaction	1.050208	1	1.0502083	1.241932	0.27115	4.0617063
Within	37.2075	44	0.845625			
Total	84.00479	47				

ANOVA 'Braeburn' Soluble Solids 2/29/2008

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Organic vs. Conv.	36.92521	1	36.925208	37.81251	2.04E-07	4.0617063
Outside vs. Inside	1.801875	1	1.801875	1.845174	0.18127	4.0617063
Interaction	8.585208	1	8.5852083	8.791509	0.004873	4.0617063
Within	42.9675	44	0.9765341			
Total	90.27979	47				

ANOVA 'Braeburn' % Dry Matter

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Organic vs. Conv.	33.20013	1	33.20013	29.2331	2.4907E-06	4.061706
Outside vs. Inside	24.33901	1	24.33901	21.43078	3.245E-05	4.061706
Interaction	2.511675	1	2.511675	2.211559	0.14411182	4.061706
Within	49.97095	44	1.135703			
Total	110.0218	47				

ANOVA 'Braeburn' Penetrometer Values 12/23/2007

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Organic vs. Conv.	34.155	1	34.155002	10.62527	0.00215579	4.0617063
Outside vs. Inside	31.997	1	31.997002	9.953939	0.002893	4.0617063
Interaction	0.109252	1	0.1092521	0.033987	0.85458112	4.0617063
Within	141.4383	44	3.2145066			
Total	207.6995	47				

ANOVA 'Braeburn' Penetrometer Values 2/29/2008

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Organic vs. Conv.	4.718802	1	4.7188021	2.187349	0.14627273	4.0617063
Outside vs. Inside	3.996302	1	3.9963021	1.852442	0.18043004	4.0617063
Interaction	20.73755	1	20.737552	9.612666	0.00336605	4.0617063
Within	94.92188	44	2.1573153			
Total	124.3745	47				

ANOVA 'Braeburn' Penetrometer Values over Time

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups (Dates)	63.2594431	2	31.6297	34.05615	6.3392E-05	4.25649
Within Groups (Dates)	8.35876823	9	0.92875			
Total	71.6182113	11				

ANOVA 'Braeburn' Sensory Appearance

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Organic vs. Conv.	39.0625	1	39.0625	22.47091	2.98054E-06	3.865048
Outside vs. Inside	93.1225	1	93.1225	53.56921	1.40269E-12	3.865048
Interaction	11.2225	1	11.2225	6.455802	0.01143987	3.865048
Within	688.39	396	1.73835858			
Total	831.7975	399				

ANOVA 'Braeburn' Sensory Flavor

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Organic vs. Conv.	44.89	1	44.89	16.67489	5.3699E-05	3.865048
Outside vs. Inside	116.64	1	116.64	43.32724	1.47221E-10	3.865048
Interaction	72.25	1	72.25	26.83807	3.53081E-07	3.865048
Within	1066.06	396	2.692070			
Total	1299.84	399				

ANOVA 'Braeburn' Sensory Texture

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Organic vs. Conv.	1.3225	1	1.3225	0.60280	0.43797	3.86504
Outside vs. Inside	0.9025	1	0.9025	0.4113652	0.52164	3.86504
Interaction	22.5625	1	22.5625	10.28413	0.00145	3.86504
Within	868.79	396	2.193914			
Total	893.5775	399				

ANOVA 'Braeburn' Sensory Overall Ratings

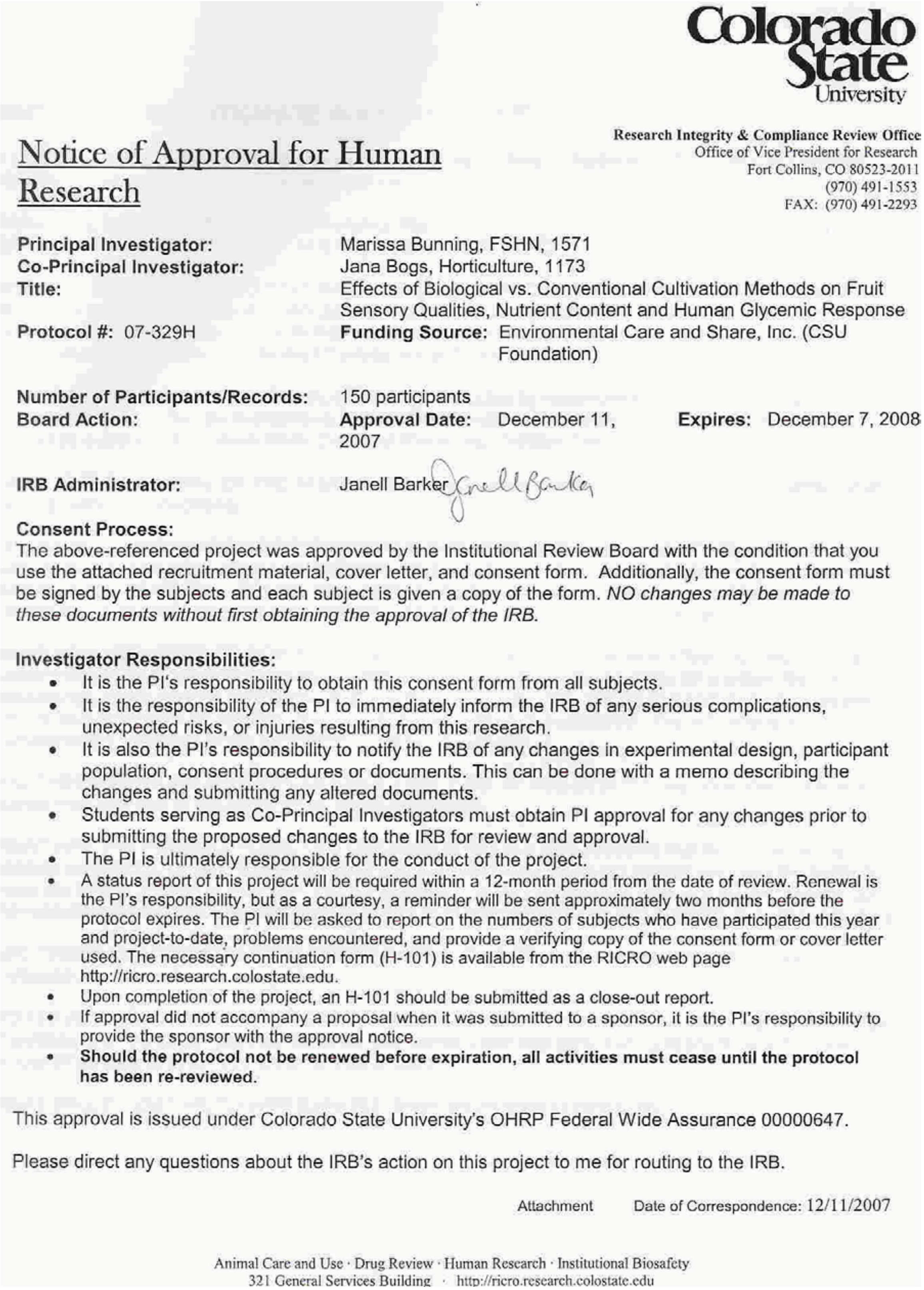
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Organic vs. Conv.	37.21	1	37.21	18.19784	2.49E-05	3.86504
Outside vs. Inside	65.61	1	65.61	32.08709	2.84E-08	3.86504
Interaction	42.25	1	42.25	20.66269	7.29E-06	3.86504
Within	809.72	396	2.04474			
Total	954.79	399				

ANOVA 'Gala' Soluble Solids

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Biological vs. Conv.	5.61125	1	5.61125	8.58815	0.0046436	3.986269
Dates Tested	2.201944	2	1.100972	1.685064	0.1933114	3.135918
Interaction	1.230833	2	0.615417	0.94191	0.3950599	3.135918
Within	43.1225	66	0.653371			
Total	52.16653	71				

ANOVA 'Gala' Penetrometer Readings over Time

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Biological vs. Conv.	21.125	1	21.125	4.613539	0.035396	3.986269
Dates Tested	1429.201	2	714.6006	156.0633	9.614E-26	3.135918
Interaction	34.11	2	17.055	3.724682	0.0293322	3.135918
Within	302.2083	66	4.578914			
Total	1786.644	71				



Colorado
State
University

Research Integrity & Compliance Review Office
Office of Vice President for Research
Fort Collins, CO 80523-2011
(970) 491-1553
FAX: (970) 491-2293

Notice of Approval for Human Research

Principal Investigator: Marissa Bunning, FSHN, 1571
Co-Principal Investigator: Jana Bogs, Horticulture, 1173
Title: Effects of Biological vs. Conventional Cultivation Methods on Fruit Sensory Qualities, Nutrient Content and Human Glycemic Response
Protocol #: 07-329H
Funding Source: Environmental Care and Share, Inc. (CSU Foundation)

Number of Participants/Records: 150 participants
Board Action: **Approval Date:** December 11, 2007 **Expires:** December 7, 2008

IRB Administrator: Janell Barker Janell Barker

Consent Process:
The above-referenced project was approved by the Institutional Review Board with the condition that you use the attached recruitment material, cover letter, and consent form. Additionally, the consent form must be signed by the subjects and each subject is given a copy of the form. *NO changes may be made to these documents without first obtaining the approval of the IRB.*

Investigator Responsibilities:

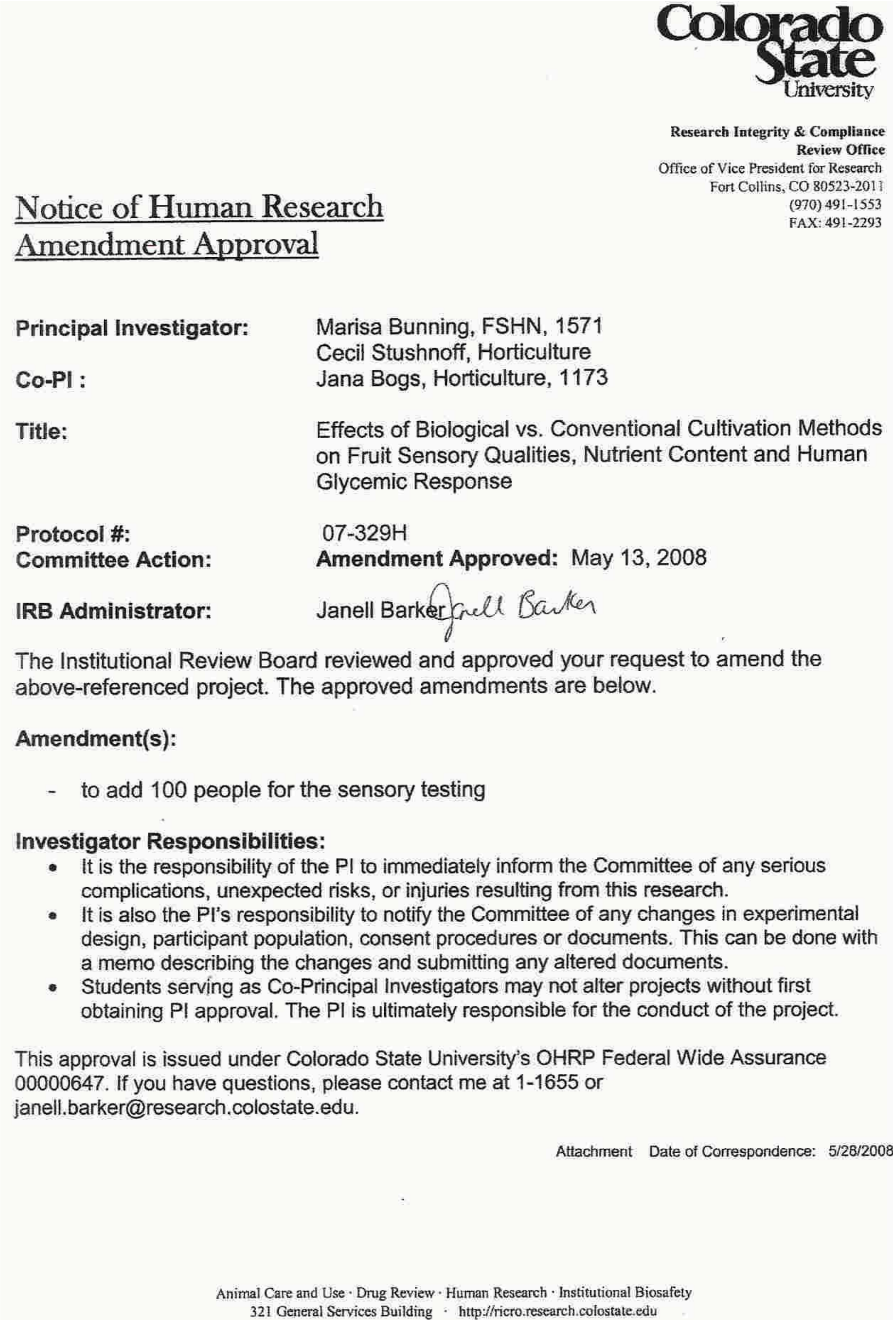
- It is the PI's responsibility to obtain this consent form from all subjects.
- It is the responsibility of the PI to immediately inform the IRB of any serious complications, unexpected risks, or injuries resulting from this research.
- It is also the PI's responsibility to notify the IRB of any changes in experimental design, participant population, consent procedures or documents. This can be done with a memo describing the changes and submitting any altered documents.
- Students serving as Co-Principal Investigators must obtain PI approval for any changes prior to submitting the proposed changes to the IRB for review and approval.
- The PI is ultimately responsible for the conduct of the project.
- A status report of this project will be required within a 12-month period from the date of review. Renewal is the PI's responsibility, but as a courtesy, a reminder will be sent approximately two months before the protocol expires. The PI will be asked to report on the numbers of subjects who have participated this year and project-to-date, problems encountered, and provide a verifying copy of the consent form or cover letter used. The necessary continuation form (H-101) is available from the RICRO web page http://ricro.research.colostate.edu.
- Upon completion of the project, an H-101 should be submitted as a close-out report.
- If approval did not accompany a proposal when it was submitted to a sponsor, it is the PI's responsibility to provide the sponsor with the approval notice.
- **Should the protocol not be renewed before expiration, all activities must cease until the protocol has been re-reviewed.**

This approval is issued under Colorado State University's OHRP Federal Wide Assurance 00000647.

Please direct any questions about the IRB's action on this project to me for routing to the IRB.

Attachment Date of Correspondence: 12/11/2007

Animal Care and Use · Drug Review · Human Research · Institutional Biosafety
321 General Services Building · http://ricro.research.colostate.edu



Colorado State University

Research Integrity & Compliance
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Notice of Human Research Amendment Approval

Principal Investigator: Marisa Bunning, FSHN, 1571
Cecil Stushnoff, Horticulture

Co-PI : Jana Bogs, Horticulture, 1173

Title: Effects of Biological vs. Conventional Cultivation Methods on Fruit Sensory Qualities, Nutrient Content and Human Glycemic Response

Protocol #: 07-329H
Committee Action: **Amendment Approved:** May 13, 2008

IRB Administrator: Janell Barker Janell Barker

The Institutional Review Board reviewed and approved your request to amend the above-referenced project. The approved amendments are below.

Amendment(s):

- to add 100 people for the sensory testing

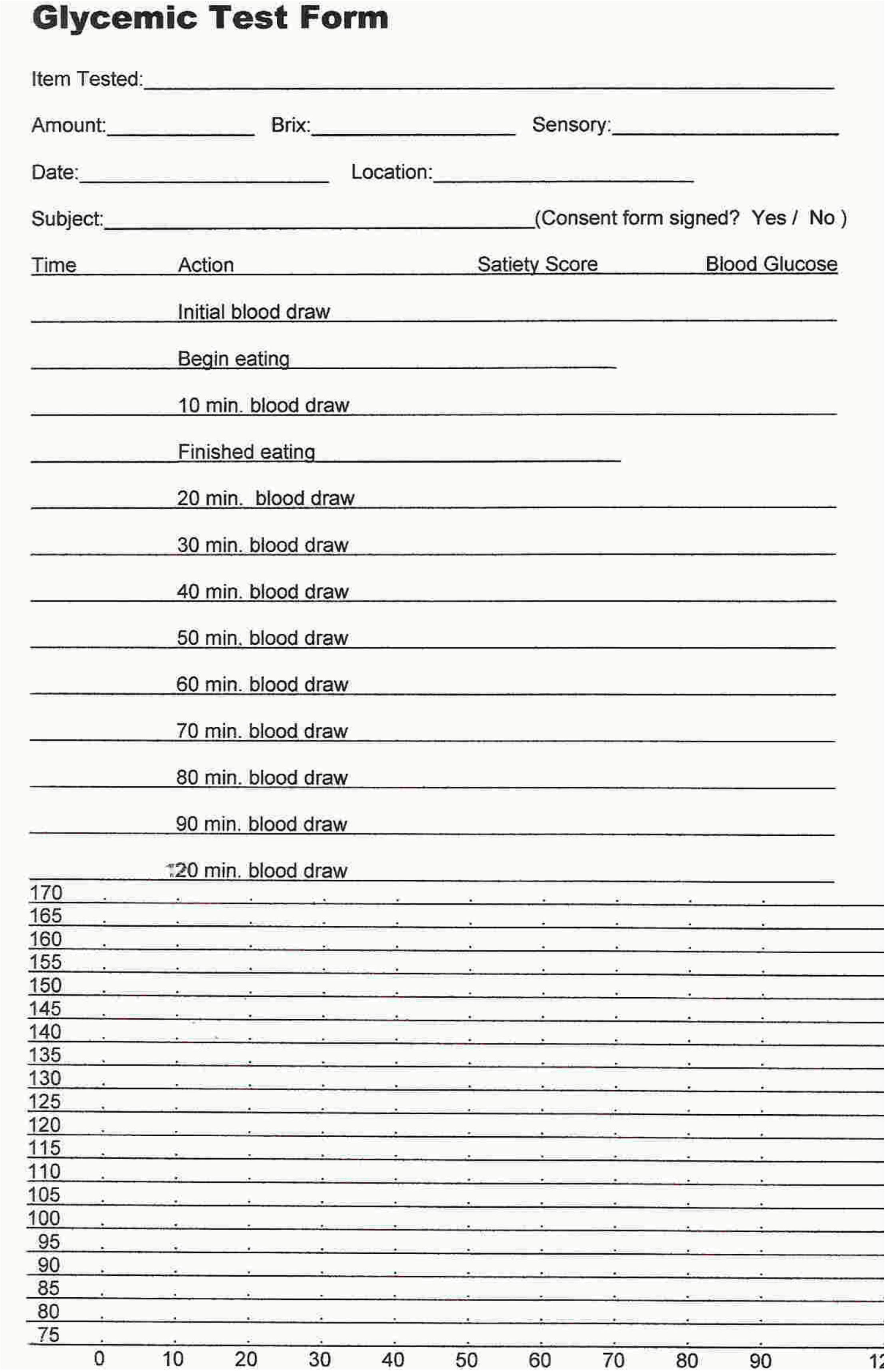
Investigator Responsibilities:

- It is the responsibility of the PI to immediately inform the Committee of any serious complications, unexpected risks, or injuries resulting from this research.
- It is also the PI's responsibility to notify the Committee of any changes in experimental design, participant population, consent procedures or documents. This can be done with a memo describing the changes and submitting any altered documents.
- Students serving as Co-Principal Investigators may not alter projects without first obtaining PI approval. The PI is ultimately responsible for the conduct of the project.

This approval is issued under Colorado State University's OHRP Federal Wide Assurance 00000647. If you have questions, please contact me at 1-1655 or janell.barker@research.colostate.edu.

Attachment   Date of Correspondence: 5/28/2008

Animal Care and Use · Drug Review · Human Research · Institutional Biosafety
321 General Services Building · http://ricro.research.colostate.edu



Glycemic Test Form

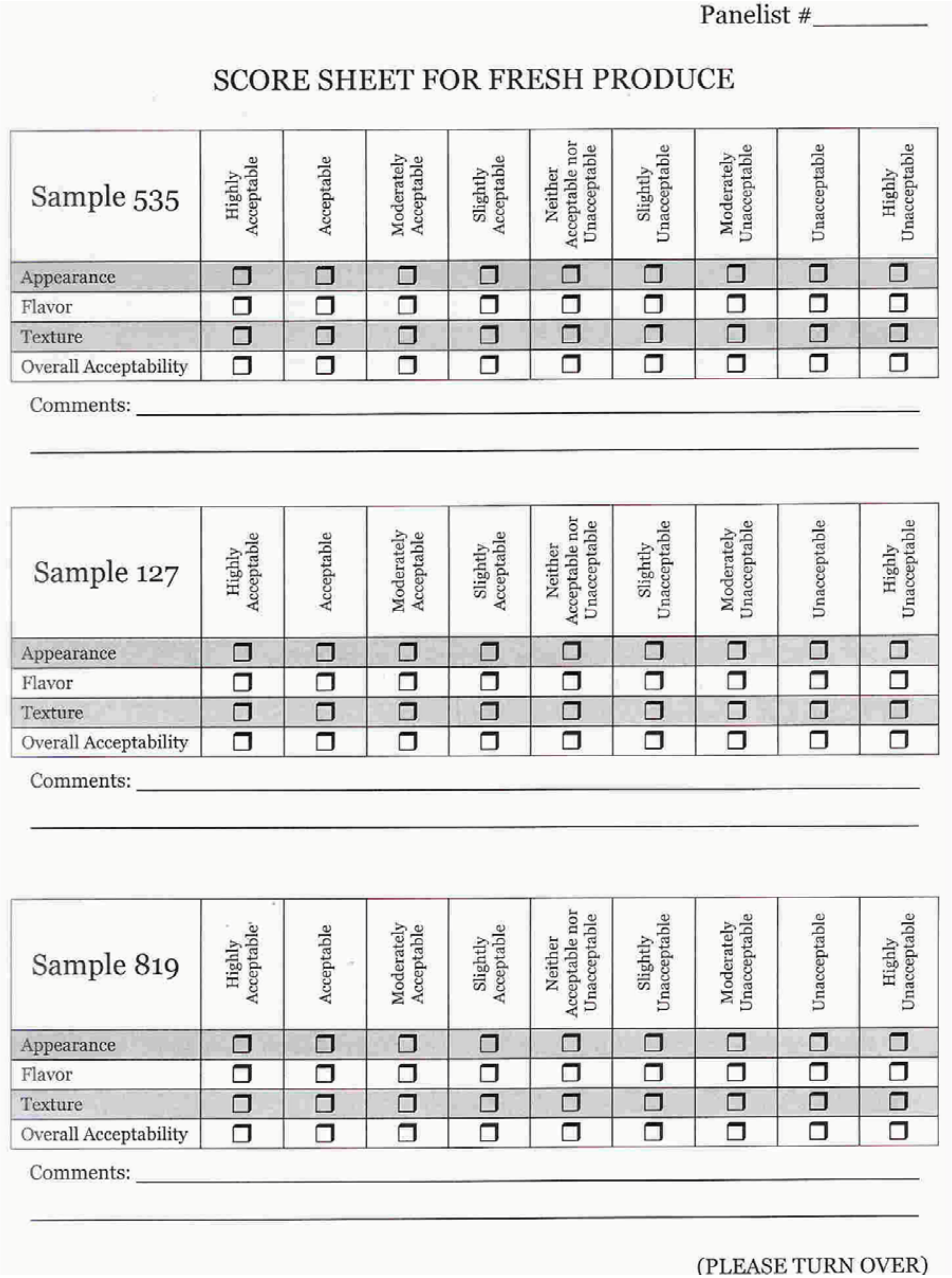
Item Tested:____

Amount:__________ Brix:______________ Sensory:______________

Date:________________ Location:________________

Subject:____________________________(Consent form signed? Yes / No)

Time	Action	Satiety Score	Blood Glucose
	Initial blood draw		
	Begin eating		
	10 min. blood draw		
	Finished eating		
	20 min. blood draw		
	30 min. blood draw		
	40 min. blood draw		
	50 min. blood draw		
	60 min. blood draw		
	70 min. blood draw		
	80 min. blood draw		
	90 min. blood draw		
	120 min. blood draw		



Panelist #________

SCORE SHEET FOR FRESH PRODUCE

Sample 535	Highly Acceptable	Acceptable	Moderately Acceptable	Slightly Acceptable	Neither Acceptable nor Unacceptable	Slightly Unacceptable	Moderately Unacceptable	Unacceptable	Highly Unacceptable
Appearance	☐	☐	☐	☐	☐	☐	☐	☐	☐
Flavor	☐	☐	☐	☐	☐	☐	☐	☐	☐
Texture	☐	☐	☐	☐	☐	☐	☐	☐	☐
Overall Acceptability	☐	☐	☐	☐	☐	☐	☐	☐	☐

Comments: ____

____

Sample 127	Highly Acceptable	Acceptable	Moderately Acceptable	Slightly Acceptable	Neither Acceptable nor Unacceptable	Slightly Unacceptable	Moderately Unacceptable	Unacceptable	Highly Unacceptable
Appearance	☐	☐	☐	☐	☐	☐	☐	☐	☐
Flavor	☐	☐	☐	☐	☐	☐	☐	☐	☐
Texture	☐	☐	☐	☐	☐	☐	☐	☐	☐
Overall Acceptability	☐	☐	☐	☐	☐	☐	☐	☐	☐

Comments: ____

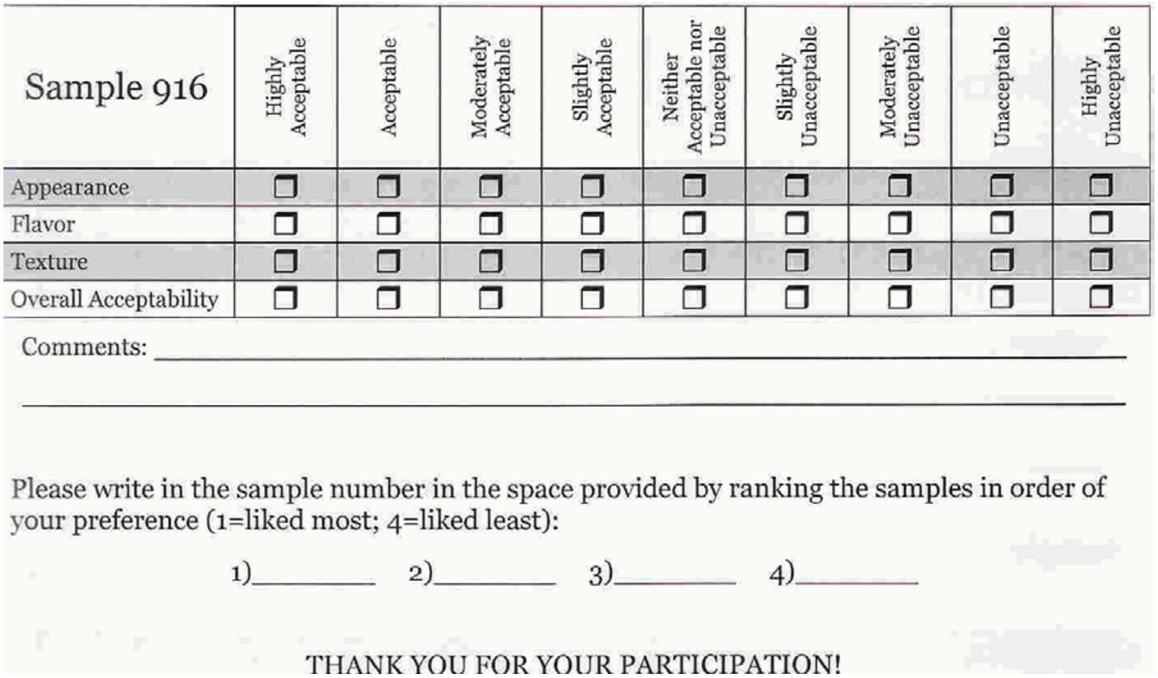
____

Sample 819	Highly Acceptable	Acceptable	Moderately Acceptable	Slightly Acceptable	Neither Acceptable nor Unacceptable	Slightly Unacceptable	Moderately Unacceptable	Unacceptable	Highly Unacceptable
Appearance	☐	☐	☐	☐	☐	☐	☐	☐	☐
Flavor	☐	☐	☐	☐	☐	☐	☐	☐	☐
Texture	☐	☐	☐	☐	☐	☐	☐	☐	☐
Overall Acceptability	☐	☐	☐	☐	☐	☐	☐	☐	☐

Comments: ____

____

(PLEASE TURN OVER)



Sample 916	Highly Acceptable	Acceptable	Moderately Acceptable	Slightly Acceptable	Neither Acceptable nor Unacceptable	Slightly Unacceptable	Moderately Unacceptable	Unacceptable	Highly Unacceptable
Appearance	☐	☐	☐	☐	☐	☐	☐	☐	☐
Flavor	☐	☐	☐	☐	☐	☐	☐	☐	☐
Texture	☐	☐	☐	☐	☐	☐	☐	☐	☐
Overall Acceptability	☐	☐	☐	☐	☐	☐	☐	☐	☐

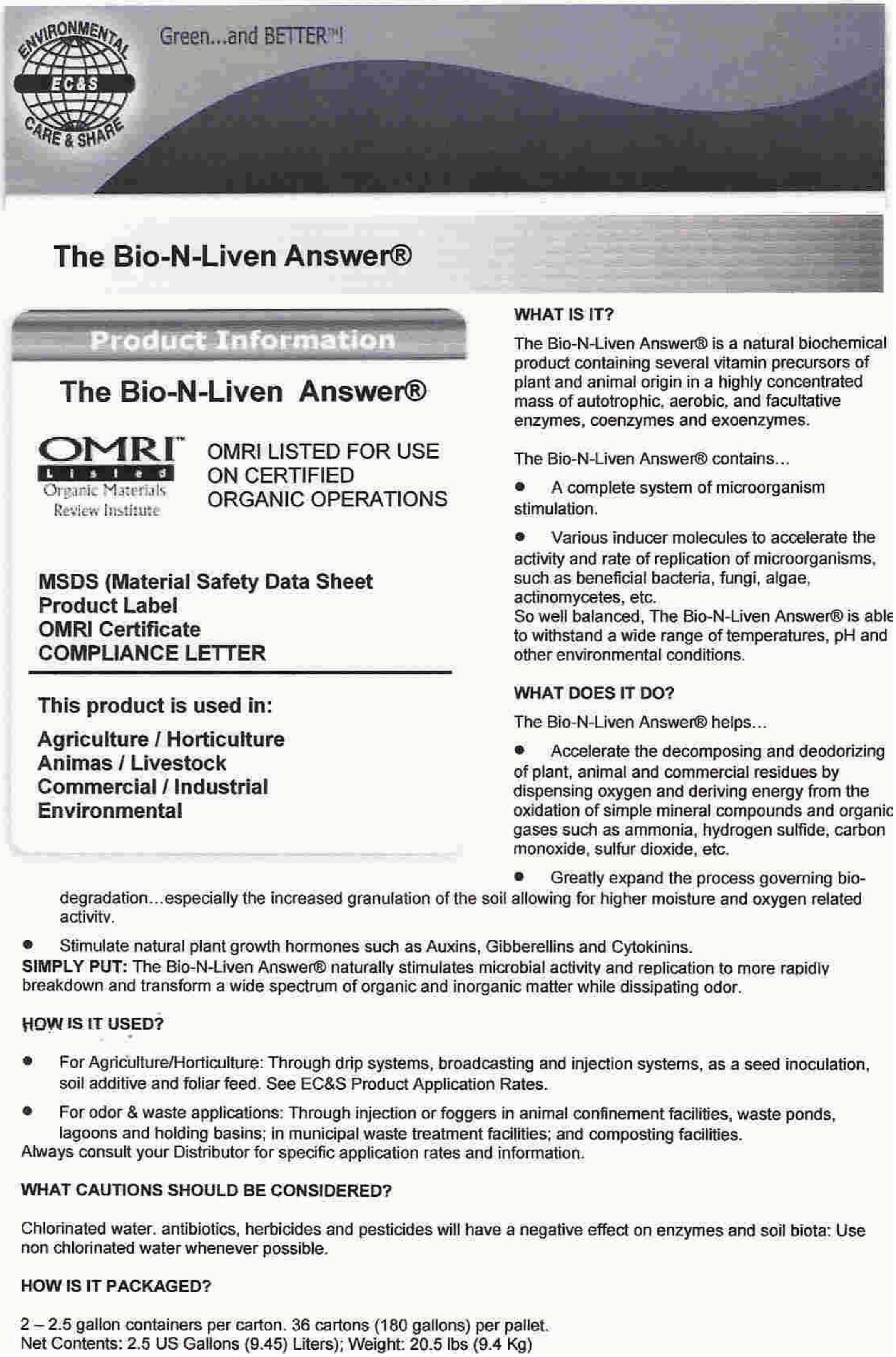
Comments: ______________________________

____

Please write in the sample number in the space provided by ranking the samples in order of your preference (1=liked most; 4=liked least):

1)________ 2)________ 3)________ 4)________

THANK YOU FOR YOUR PARTICIPATION!



ENVIRONMENTAL EC&S CARE & SHARE

Green...and BETTER™!

The Bio-N-Liven Answer®

Product Information

The Bio-N-Liven Answer®

OMRI™ Listed Organic Materials Review Institute

OMRI LISTED FOR USE ON CERTIFIED ORGANIC OPERATIONS

MSDS (Material Safety Data Sheet
Product Label
OMRI Certificate
COMPLIANCE LETTER

This product is used in:

Agriculture / Horticulture
Animas / Livestock
Commercial / Industrial
Environmental

WHAT IS IT?

The Bio-N-Liven Answer® is a natural biochemical product containing several vitamin precursors of plant and animal origin in a highly concentrated mass of autotrophic, aerobic, and facultative enzymes, coenzymes and exoenzymes.

The Bio-N-Liven Answer® contains...

- A complete system of microorganism stimulation.
- Various inducer molecules to accelerate the activity and rate of replication of microorganisms, such as beneficial bacteria, fungi, algae, actinomycetes, etc.

So well balanced, The Bio-N-Liven Answer® is able to withstand a wide range of temperatures, pH and other environmental conditions.

WHAT DOES IT DO?

The Bio-N-Liven Answer® helps...

- Accelerate the decomposing and deodorizing of plant, animal and commercial residues by dispensing oxygen and deriving energy from the oxidation of simple mineral compounds and organic gases such as ammonia, hydrogen sulfide, carbon monoxide, sulfur dioxide, etc.
- Greatly expand the process governing bio-degradation...especially the increased granulation of the soil allowing for higher moisture and oxygen related activity.
- Stimulate natural plant growth hormones such as Auxins, Gibberellins and Cytokinins.

SIMPLY PUT: The Bio-N-Liven Answer® naturally stimulates microbial activity and replication to more rapidly breakdown and transform a wide spectrum of organic and inorganic matter while dissipating odor.

HOW IS IT USED?

- For Agriculture/Horticulture: Through drip systems, broadcasting and injection systems, as a seed inoculation, soil additive and foliar feed. See EC&S Product Application Rates.
- For odor & waste applications: Through injection or foggers in animal confinement facilities, waste ponds, lagoons and holding basins; in municipal waste treatment facilities; and composting facilities.

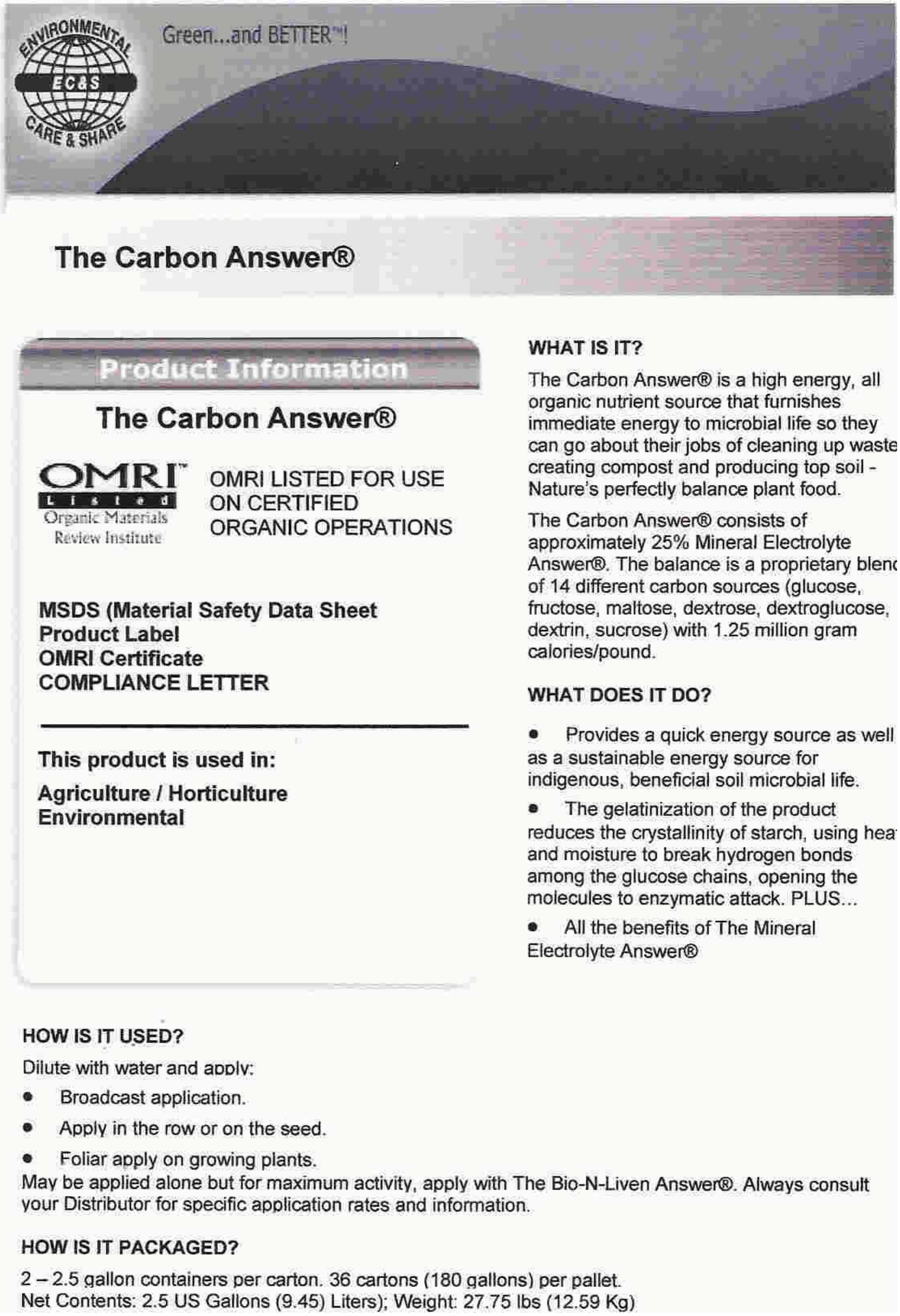
Always consult your Distributor for specific application rates and information.

WHAT CAUTIONS SHOULD BE CONSIDERED?

Chlorinated water. antibiotics, herbicides and pesticides will have a negative effect on enzymes and soil biota: Use non chlorinated water whenever possible.

HOW IS IT PACKAGED?

2 – 2.5 gallon containers per carton. 36 cartons (180 gallons) per pallet.
Net Contents: 2.5 US Gallons (9.45) Liters); Weight: 20.5 lbs (9.4 Kg)



The Carbon Answer®

Product Information

The Carbon Answer®

OMRI™ Listed Organic Materials Review Institute

OMRI LISTED FOR USE
ON CERTIFIED
ORGANIC OPERATIONS

MSDS (Material Safety Data Sheet
Product Label
OMRI Certificate
COMPLIANCE LETTER

This product is used in:

Agriculture / Horticulture
Environmental

WHAT IS IT?

The Carbon Answer® is a high energy, all organic nutrient source that furnishes immediate energy to microbial life so they can go about their jobs of cleaning up waste creating compost and producing top soil - Nature's perfectly balance plant food.

The Carbon Answer® consists of approximately 25% Mineral Electrolyte Answer®. The balance is a proprietary blen of 14 different carbon sources (glucose, fructose, maltose, dextrose, dextroglucose, dextrin, sucrose) with 1.25 million gram calories/pound.

WHAT DOES IT DO?

- Provides a quick energy source as well as a sustainable energy source for indigenous, beneficial soil microbial life.
- The gelatinization of the product reduces the crystallinity of starch, using hea and moisture to break hydrogen bonds among the glucose chains, opening the molecules to enzymatic attack. PLUS...
- All the benefits of The Mineral Electrolyte Answer®

HOW IS IT USED?

Dilute with water and apply:

- Broadcast application.
- Apply in the row or on the seed.
- Foliar apply on growing plants.

May be applied alone but for maximum activity, apply with The Bio-N-Liven Answer®. Always consult your Distributor for specific application rates and information.

HOW IS IT PACKAGED?

2 – 2.5 gallon containers per carton. 36 cartons (180 gallons) per pallet.
Net Contents: 2.5 US Gallons (9.45) Liters); Weight: 27.75 lbs (12.59 Kg)