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DETERMINATION OF ANTIOXIDANT AND TOTAL PHENOLIC CONTENT OF *PUERARIA LOBATA* AND EVALUATION OF NOVEL FOOD PRODUCTS CONTAINING KUDZU

By

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Kudzu (*Pueraria lobata*) is an edible vine from the legume family native to China but growing prolifically throughout the southeastern United States. Legumes are abundant in the beneficial compounds phytoestrogens, specifically isoflavones. This research analyzed Mississippi-grown kudzu roots, leaves, and flowers for antioxidant activity and total phenolic content and evaluated consumer acceptability of food products containing kudzu. Results indicated kudzu flowers, roots, and leaves contained phenolic compounds and the antioxidant activity amounts in flowers, roots, and leaves were 77.9%, 75.7%, and 56.5%, respectively. The dip products that were developed and evaluated were mayonnaise- and sour cream-based dips that contained either dried kudzu leaves or dried spinach and other seasonings. Healthier versions of dip products were developed using light mayonnaise and light sour cream and omitting salt. Consumer sensory panels evaluated appearance, aroma, flavor, texture, and overall acceptability of the dip samples using a 9-point hedonic scale. A randomized complete block design with three replications was used to determine if differences ($p<0.05$) in consumer acceptability

existed among treatments. On average, the regular spinach dip was moderately liked and preferred ($p<0.05$) over the other dips. The regular kudzu dip was moderately liked and preferred ($p<0.05$) over the healthier kudzu and spinach dips. Cluster analysis partitioned consumers into five groups based on preference and acceptability of vegetable dips. Results indicated 39% of panelists rated the dips at like very much and did not differ ($p>0.05$) in their liking of dips, 42% preferred ($p<0.05$) the regular spinach dip and 50% liked all vegetable dips. A kudzu blossom jelly product was developed using kudzu flower liquid, sugar, pectin, and lemon juice. Consumer sensory panels evaluated the prepared jelly product, a purchased kudzu jelly, and a purchased scuppernong jelly. A randomized complete block design with three replications was used to determine if differences ($p<0.05$) in consumer acceptability existed among jellies. The purchased kudzu jelly was preferred ($p<0.05$) for overall acceptability (like moderately) compared to the prepared kudzu and scuppernong jelly, which were rated similarly between like slightly and like moderately.

Key words: sensory analysis, novel food products, antioxidant activity, phenolics, kudzu

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CHAPTER I

INTRODUCTION

Pueraria lobata (Willd.) (kudzu) was introduced to the United States in 1876 and now grows more prolifically in the southern region of the United States than anywhere else in the world (Shurtleff & Aoyagi, 1985). It is estimated to be growing on greater than six million acres in the southern part of the United States (Parks, Tanner, & Prokop, 2002) with Mississippi, Alabama, and Georgia having the greatest concentration of kudzu growth (Blaustein, 2001; Forseth, Jr. & Innis, 2004). In comparison, cotton is cultivated on approximately 10 million acres annually in the same region (Parks et al., 2002). Kudzu is classified as a weed by the United States Department of Agriculture (USDA) and billions of dollars are spent each year trying to eradicate it. In 1960, the United States government began an attempt to control the growth of kudzu. These attempts continue today (Baldwin, 2003). Possibly, a more cost-effective, environmentally-friendly way to control the growth of kudzu in the United States would be to harvest and develop this weed into commercially acceptable and useful consumer products.

All parts of the kudzu plant contain valuable components that are suitable for commercial application and production. An excellent cooking starch is produced from the rhizome (root) along with strong absorbent cellulose fibers suitable for paper-making (Baldwin, 2003; Shurtleff & Aoyagi, 1985). The kudzu vine contains strong, fine fibers

that are suitable for textiles and coarse fibers suitable for structural support of flooring and paper-making. At least 30 valuable chemicals and potential drugs have been identified in kudzu plant components and further research is needed to fully elucidate its composition. Volatile chemicals are indicated by the sweet grape-like scent of the flowers. The roots and other components of kudzu contain volatile compounds as well. The chemicals in the flowers are similar to those in the roots, and it is predicted that all parts of the kudzu plant contain similar chemicals (Parks et al., 2002).

As a member of the legume family, all parts of the kudzu plant are edible. The root and the refined starch obtained from the root have been incorporated into numerous food products (Baldwin, 2003; Shurtleff & Aoyagi, 1985). The leaves are a readily harvested renewable food with culinary properties similar to spinach, collards and other greens. The flowers of the kudzu vine have been used for many years as an ingredient in making jelly (Hoots & Baldwin, 1996; Baldwin, 2003); however, there are no documented studies or previously performed research available that reports consumer testing or evaluation of kudzu jelly or other food products containing kudzu.

The objectives of this research were to: 1) determine radical scavenging activity and total phenolic content of Mississippi-grown kudzu roots, leaves and flowers, 2) develop a kudzu blossom jelly and a dried-kudzu leaf dip product, 3) conduct consumer acceptability sensory testing of the jelly and dip products, and 4) report nutritional aspects of the food products.

CHAPTER II

LITERATURE REVIEW

History of Kudzu in the United States

Pueraria lobata (kudzu) was introduced in the United States at the 1876 Centennial Exposition in Philadelphia, Pennsylvania. It was presented as an ornamental vine that provided shade (Blaustein, 2001; Forseth, Jr. & Innis, 2004). Kudzu was introduced in the South in 1883 after the New Orleans Exposition to be used as an ornamental vine to provide shade for southern porches (Blaustein, 2001). In the early 1900s, kudzu began to be used as a high-quality feed for cattle. The Alabama Agricultural Experiment Station at Auburn University began to study kudzu as a fodder in 1917 (Mitich, 2000).

In 1920, the Central Georgia Railroad initiated a program to provide free kudzu plants, along with instructions on how to grow kudzu for use as animal feed (which the railroad would later haul). By 1934, approximately 4,048 hectare (ha) (10,000 acres) of kudzu had been planted in the South (Shurtleff & Aoyagi, 1985). In the 1930's and 1940's, kudzu was promoted by the Soil Erosion Service, and later, the Soil Conservation Service, to be used for erosion control on over-cultivated Southern farmlands. Decades of extensive planting of cotton, corn, and tobacco crops had depleted the land and caused or worsened soil erosion. Farms were abandoned as people moved from rural to urban

areas (Blaustein, 2001; Forseth, Jr. & Innis, 2004; Shurtleff & Aoyagi, 1985). Kudzu from a variety of stock began to be extensively planted. The Soil Conservation Service offered payments as high as eight dollars per acre to farmers to plant kudzu for "conservation." The Soil Conservation Service nurseries had more than 73 million seedlings growing in the 1940s. Thousands of workers were employed, mostly in Mississippi, Alabama, and Georgia, by the Civilian Conservation Corp to plant kudzu along highways and in erosion-prone areas. By 1946, 1.2 million ha (2.5 million acres) of kudzu were estimated to be growing in the South (Blaustein, 2001; Forseth, Jr. & Innis, 2004; Shurtleff & Aoyagi, 1985). Kudzu was allowed to grow uncontrolled, over numerous acres, until it dominated forests (especially pine forests) and edge habitats.

Several publications during the late 1930s to the mid 1940s discussed practical uses for kudzu. These articles were intended for farmers and agriculture professionals. Publications entitled "Kudzu as a Farm Crop" (McKee & Stephens, 1943) and "The Production and Utilization of Kudzu" (O'Brien & Skelton, 1946) were placed in prominent agriculture publications. There were many uses for kudzu discussed in these publications during this period. Suggestions included using kudzu as a pasture crop for grazing, soil improvement, making hay, as a shade plant, and as a method of control for various types of erosion problems (McKee & Stephens, 1943; Miles & Gross, 1939; O'Brien & Skelton, 1946). As early as 1939, some of these authors, and others, recognized the possibility of kudzu becoming a "pest." Miles and Gross (1939) wrote, "It is true it is an aggressive plant, but this (experiment) station (in Mississippi) keeps it confined to individual plots without any serious trouble. It is easily killed by grazing or

frequent and thorough plowing." McKee and Stephens (1943) stated, "Kudzu is sometimes a nuisance when growing in places where not wanted; it is not hard to kill, but may take some work to kill." Bailey (1939) admitted there was a "prevalent belief" that kudzu may become a "serious pest if planted near cultivated cropland." He further wrote that "experience has shown that this belief is unfounded," and the growth habit of kudzu makes it simple to control. O'Brien and Skelton (1946) later discussed that "numerous reports give evidence that kudzu can be easily controlled or confined to a definite area such as a terrace, by clean cultivation of adjacent areas."

In 1953, the United States Department of Agriculture (USDA) removed kudzu from the list of permissible cover plants under the Agricultural Conservation Program. In 1970, it was officially listed as a weed by USDA and in 1997 the United States Congress listed it as a "noxious weed" under the Federal Noxious Weed Law (Blaustein, 2001). Today, kudzu is reported to be growing in 32 states (United States Department of Agriculture Natural Resources Conservation Service, 2009) and most of kudzu's growth is uncontrolled and considered a nuisance.

The United States National Park System is one of many organizations in the South that has been highly impacted by kudzu overgrowth. For example, the Vicksburg National Military Park located in Mississippi was the site of an historic Civil War battle and is a revered National Park consisting of 760 ha (1,877 acres). Kudzu has covered or threatened 80 ha (198 acres), threatened tree growth, the natural ecosystems (especially native grasses), and the historic bluffs that made the Vicksburg fortress arrangement possible in the Civil War (Blaustein, 2001). Due to the opportunistic growth habit of

kudzu, it is an aggressive competitor in the eastern pine and mixed pine forest habitats (Forseth, Jr. & Innis, 2004). According to Mitich (2000) "a single hectare of kudzu (2.47 acres) left uncontrolled for 100 years, would expand to 5,250 hectare (12,968 acres)." This is why the lumber industry and the United States Forest Service are heavily involved in kudzu control and eradication (Shurtleff & Aoyagi, 1985).

The introduction and spread of invasive species (plants and animals), intentional and unintentional, is emerging as among the most dramatic ways humans are transforming the planet. Non-native plants and animals are now recognized as a major factor in human-caused global change. The combined effect of the spread of non-natives and the extinction of rare species is causing ecological communities to become increasingly similar. Mounting ecological and economic impacts of non-native species are becoming apparent. Economic costs of invasive species in the United States have been estimated at $137 billion annually (Vander Zanden, 2005).

According to Lowney (2002), scientists' claims about kudzu occurred during three periods: 1917 – 1953 was an enthusiastic period when agronomists promoted the planting of kudzu as a solution to the problem of erosion; 1954 – 1984 was a period of disenchantment when forestry scientists redefined kudzu as a problem demanding eradication; and 1985 – present is a period of tempered enthusiasm when some scientists are searching for new uses for kudzu, while others continue to search for means of eradication. The status of kudzu as a highly prized "Southern savior" to an "ignoble pest" (Lowney, 2002) and the more recent view as a potentially useful plant may be leading to an enlightenment period for kudzu.

The Kudzu Plant

Pueraria lobata (kudzu) is a perennial vine from the pea, or legume (Fabaceae) family (Miller, 2007). There are several species of kudzu that grow in the United States, of which *Pueraria lobata* is the most common (Baldwin, 2003; Miller, 2007). Kudzu is native to China and grows primarily on the border of China and Vietnam. It is also found in Korea and in the Yoshino Valley in Japan (Parks et al., 2002). Within the United States, kudzu is classified as a noxious weed in Florida, Illinois, Kansas, Kentucky, Mississippi, Missouri, Pennsylvania, Texas, Washington, and West Virginia. Kudzu is banned or quarantined in several other states and reported to be invasive in 22 states (Bergman & Swearingen, 2005).

Kudzu is botanically classified as a twining, trailing, mat-forming, semi-woody deciduous, perennial vine, 10 to 30 m (35 to 100 ft) long (Foote & Jones, Jr., 1989; Miller, 2007). The fast-growing vine can grow up to 30 cm, or approximately one foot, a day (Mitich, 2000). The perennial vines take three years to reach maturity and seven to ten years may be needed to develop a successful crop similar to that of soybean in the southeast United States (Parks et al., 2002). The leaves are alternate, pinnately compound three-leaflet leaves with each leaflet measuring 8 to18 cm (3 to 7 in) long and 6 to 20 cm (2.5 to 8 in) wide. The individual flowers are small slender clusters (racemes), 5 to 30 cm (2 to 12 in) long, of pea-like flowers in pairs from raised nodes spiraling up the stalk, opening from the base to the top. The petals are generally lavender to wine colored with yellow centers and the flowers usually bloom from June to September (Miller, 2007). Flowers are produced on plants exposed to full sunlight and

only appear on vertically growing vines. Kudzu plants may not flower in the northern-most regions of the estimated growth range (Mitich, 2000).

The spread of kudzu in the United States is usually through running vines that root at the nodes through contact with the soil to form new plants (Bergmann & Swearingen, 2005; Miller, 2007; Mitich, 2000). The plants, which are established at the nodes during the previous growing season, begin new growth in the spring. The roots enlarge and form new crowns. Each crown produces three to five vines and these spread rapidly allowing kudzu to spread quickly, especially throughout many areas of the rural South. After the second growing season, the vine that connects the crowns of the new vines to the old vine dies and each new crown produces new vines the next year (Bodner & Hymowitz, 2002). The vines produce seeds shortly after flowering. The seed pods are brown and hairy, approximately 0.2 cm (.06 in) in size, and mature in the fall (Bergmann & Swearingen, 2005; Miller, 2007; Mitich, 2000). The seeds have a hard shell that is difficult to penetrate which is one of the contributing factors to the poor germination rate of kudzu (Baldwin, 2003). Usually, only one or two kidney-shaped seeds are produced per cluster of pods and it may take several years for germination to occur (Bergmann & Swearingen, 2005; Mitich, 2000). The seeds may be dispersed by wind, animals, or water (Miller, 2007). The seed-produced kudzu seedlings are delicate and compete poorly with aggressive weeds (Mitich, 2000). The seed pods and leaves are shed from the vines at the same time in the fall (Baldwin, 2003).

The root system of kudzu spreads horizontally from semiwoody tuberous roots. In the southeastern region of the United States, root depths of 2.5 m (8.2 ft) have been

reported with an average root depth of 1 m (3.28 ft). The roots swell in the fall and winter due to the storage of energy in the form of starch which is needed for growth in the spring. The roots vary in size and store large amounts of starch, water, and nitrogen (Forseth, Jr. & Innis, 2004). Kudzu has no significant biological enemies (diseases or insects) and thrives in the temperate climate of the southeastern United States where the growing season is long and the winters are generally mild. There are typically adequate amounts of rainfall, although kudzu can withstand dry weather due to its large taproot (Baldwin, 2003; Mitich, 2000). Kudzu can fix atmospheric nitrogen on poor soil sites where other vegetation cannot grow (leguminous nitrogen fixer). The soil nitrogen builds up quickly and allows the maintenance of large leaf canopy areas and thus high photosynthetic rates (Forseth, Jr. & Innis, 2004). This is one of the adaptive characteristics of kudzu that makes it so prolific.

Overall, the characteristics that make kudzu so competitive and prolific are the ability to root easily when stems come in contact with the soil, high photosynthetic rates, the ability to fix nitrogen, preferential allocation to stem elongation and leaf area, a large taproot, lack of serious predators, and the ability to grow in difficult areas with poor soil (Forseth, Jr. & Innis, 2004).

Kudzu Roots

The kudzu plant produces a large semi-woody tuberous root that may reach depths of 1 to 5 m (3 to 16 ft) (Miller, 2007). In Japan, the roots of the wild kudzu vine are difficult and expensive to harvest. The roots are typically dug in the mountainous

areas of Japan during the coldest part of the year from early December through late March, (Shurtleff & Aoyagi, 1985).

Research conducted in 1991 at Vanderbilt University investigated the best time for harvesting kudzu roots of various ages to maximize the amount of starch that is extracted. The authors collected kudzu roots from northwestern Alabama following the first frost and continued from October to December. They established that the greatest starch content occurred in the three- to four-year-old roots that were harvested in December. The root age (years) was determined on the basis of the anatomical structure of the tap root. This was accomplished by looking at the ring formation and the average diameter of the root branch (Achremowicz, Tanner, & Prokop, 1994).

Kudzu Root Starch

Kudzu powder (kuzu-ko in Japanese) is a starch-like extract of the kudzu root. In Japan, the root may grow to a length of 2.13 m (7 ft) or more and weigh up to 200 kg (440 lbs). The powder made from these roots is sold in the form of crumbled white chunks (Shurtleff & Aoyagi, 1985). The yield of starch is approximately 15% to 34% of the fresh roots (Hung & Morita, 2007; Suzuki, Hizukuri, & Takeda, 1981). In China, the diced and dried root is primarily used in traditional Chinese medicine as a key ingredient in kudzu root tea (Hung & Morita, 2007).

The starch produced from the kudzu root appears to be the most widely used part of the plant. It is valuable as a specialty food starch that improves the functionality of natural gelling agents, emulsifiers, and industrial products. It has a fine granule size and

is the most absorbent and stable of all natural starches. The starch produces a clear, colorless, flavorless, soft transparent gel that does not develop cloudiness or a paper-like starch residue. It can be used to gel medicines and foods, including high protease-containing foods such as pineapple and kiwi (Parks et al., 2002). Most recipes using the root of the kudzu plant process the root into a powder. The exception is kudzu root tea, which is made with young tender roots or dried mature roots (Baldwin, 1999).

Most of the kudzu powder sold in the West is imported from Japan. The Japanese place great importance in producing pure white kudzu powder. The 60 to 90 days required to create such a product raises the price. American producers could abbreviate the process by minimizing the repeated washing that occurs in the Japanese process. This would decrease the production costs and increase yield. The final powder is off-white to beige in color, has greater gel strength, and is thought to have greater medicinal value than the more refined white kudzu powder (Shurtleff & Aoyagi, 1985).

The following sequence was described as the typical commercial method of preparing kudzu powder in Japan. In early December, the wild roots are dug from the mountains. The roots are cut into 91.5 cm (3-foot) lengths, bundled, and transported to the processing facility. The firm, fibrous kudzu roots are cleaned and sized. The ideal size is 5-8 cm (2 to 3 inches) in diameter. The roots are cut crosswise into 3.8 cm-thick (1½-inch) discs and dropped into a box below the saw, where they are pulverized by a rapidly spinning roller covered with tiny teeth; cold mountain water is pumped in with the fibrous mash to form a slurry. The crude fiber is filtered by pumping the slurry over a series of shallow, vibrating 92-by-184 cm (3-by-6-foot) trays mounted at an angle and

lined with a 40-mesh silk screen. The resulting solution is pumped into a 100-mesh screen tray. The root fibers slide off the 40-mesh trays and are again mixed with cold water prior to filtration through four more 40-mesh trays. After each filtration step, the starch washed out of the fibers is pumped over to the 100-mesh tray. After the final filtering, the clean fiber is removed for future sale as fodder, fertilizer or as a hemp substitute. The starch solution is passed through gradually smaller mesh screens to a final mesh of 300. The resulting solution is centrifuged to condense the volume. The concentrated solution is pumped into a 15-foot-diameter, 3-foot-deep concrete tank and allowed to settle for 30 minutes. Protein, ash, tannins, and other impurities sink to the bottom and the white starch remains dissolved and suspended in the water. This process continues many times until in the final repetition the starch is allowed to settle for 48 hours. The slightly lower quality starch that has been removed from the upper and lower surfaces of the pure kudzu starch is collected, further refined and sold as a second grade kudzu powder. The pure central portion of the kudzu starch is filtered through a 300-mesh screen into a 92 cm deep (3-foot) 184-by-92 cm (6-by-3-foot) cement tank lined with a clean white cloth. After 15 hours, the pure-white claylike starch is cut into large blocks and dried for 45 to 90 days in a drying room at the natural temperatures of the region. After drying, each cake of dried kudzu starch contains 16.5% moisture (Shurtleff & Aoyagi, 1985).

Culinary Uses for Kudzu

Powdered starch that is obtained from kudzu roots has been described as the world's finest cooking starch and has been used for centuries in Japanese haute cuisine (Mitch, 2000). Kudzu starch can be used as a gluten-free thickening agent and as a coating for frying foods (Parks et al., 2002). Although all components of the kudzu plant are edible and safe to consume, the kudzu plant is usually not considered a mainstream food choice in the American diet. However, Asian countries such as China and Japan, have been using kudzu as a food source for many years. The uses of kudzu in cooking are numerous, especially in Japan. Historically, menu items from Japan's finest temple restaurants may have included sesame tofu, hot broth, and fresh kudzu noodles in molasses sauce. For dessert, azuki bean paste coated with gelled kudzu, or gelled kudzu sprinkled with a layer of kinako (roasted soy flour) may be served. All of these menu items contain kudzu (Shurtleff & Aoyagi, 1985). One of the best-known American books to discuss historical and culinary uses of kudzu is *The Book of Kudzu* by Shurtleff and Aoyagi (1985). The authors include numerous recipes for sauces, noodles, beverages, gelled salads, and desserts that use various parts of the kudzu plant.

The cooked leaves of the kudzu plant are somewhat similar to turnip, collard, and other greens and can be mixed with various greens to add texture and flavor (Parks et al., 2002). A popular recipe is deep-fat fried 3-inch kudzu leaves prepared with a tempura batter containing kudzu powder. Young leaves are covered with tiny hairs that wilt when cooked. Raw young leaves can be unappetizing to some people due to their texture

(Baldwin, 1999). Shores (1996) also described the leaves as being similar to turnip and collard greens but reported that the leaves are better if eaten after a light frost.

Kudzu flowers, or blossoms, are usually available for approximately six weeks each year, typically in late July through early September. The flowers must be harvested by hand and processed within a short time of harvesting since the flowers do not store well under normal refrigeration. Flash freezing shortly after harvest can decrease the degradation of the volatile floral scent (Parks et al., 2002). Along with being an ingredient in cooking, the blossoms can be used as a decorative, edible flower for use as a garnish and in specialty baking. Baldwin (1999) presents recipes for crystallized kudzu blossoms, kudzu blossom jelly, wine, vinegar, and ice cream. Kudzu blossom jelly appears to be a popular use for the blossoms in the United States and is sold in specialty shops in the South. Numerous websites and some specialty cookbooks contain recipes for kudzu jelly. A typical recipe lists ingredients as kudzu blossoms, water, lemon juice, pectin, and sugar (Hoots & Baldwin, 1996). Jellies produced using kudzu flowers have been described as having a distinctive, mild, and pleasant taste. There are reported to be color differences in the finished jelly product if the blossoms are used fresh versus if the blossoms have been frozen prior to processing (Baldwin, 2003; Hoots & Baldwin, 1996).

The popularity of edible flowers has increased since the late 1980's. Many fine dining restaurants use edible flowers to garnish food. Flowers can be used as ingredients in salads and main dishes, to garnish soups and entrees, sprinkled on desserts, and frozen in ice cubes and floated in drinks (Kelley, Behe, Biernbaum, & Poff, 2001a). Flowers may be marketed fresh, dried, candied, or in prepackaged salads (Edible Flowers, 2005).

Specialty growers will seasonally grow as many as 50 different edible flowers, including nasturtiums, pansies, and violas, for the hotel and restaurant market (Kelley, Behe, Biernbaum, & Poff, 2001b). Edible flowers are usually grown in conjunction with cut flowers, herbs, and specialty lettuces to complement them and create opportunities for value-added products (Gegner, 2004). An edible flower that is visually appealing to consumers may encourage them to taste the flower. If the flower is not appetizing, it may reduce visual appeal of the meal (Kelley et al., 2001b).

Edible flowers add visual appeal to food presentations and may provide some nutritional benefits. For example, the vitamin and beta-carotene concentration of viola on a weight basis is higher than that of oranges (Kelley et al., 2001a). Roses (especially rose hips) are very high in vitamin C. Marigolds and nasturtiums contain vitamin C and dandelion blossoms contain vitamins A and C (Gegner, 2004). However, flowers contain more than 95% water and therefore provide insignificant nutrient and caloric values, especially since a garnish may include only one or two flowers. Visual appeal, taste and the possibility of nutritional benefit enhance the potential marketability of edible flowers (Kelley et al., 2001a).

Identifying, marketing, and producing niche or novel crops may be one strategy that small-scale growers can use to sustain an income and build a customer base (Kelley, Behe, Biernbaum, & Poff, 2002). Organic growers have an advantage because the flowers (usually imported) available from commercial florists are often grown with heavy applications of pesticides (Gegner, 2004). Other outlets for niche crops are restaurants where chefs desire both raw and value-added products (Kelley et al., 2002). Value-added

products that feature edible flowers offer additional marketing opportunities. Minced flowers make a colorful and flavorful addition to herbal butters, cheese spreads, jellies, and jams. Dried flowers can be used in tea or wine to add flavor. Fresh flowers can be included in cooking oils, vinegars, salad dressings, and marinades (Edible Flowers, 2005). Not only is it necessary for growers to market niche products that have broad appeal, but it is also necessary to protect market share by continuously researching consumer acceptance of new products. Professional chefs are continually looking for new and novel products that will appeal to their customers' tastes (Kelley et al., 2002).

Nutritional Content of Kudzu

Although the roots, leaves, shoots, and flowers of kudzu have been used in salads, soups, sautéed dishes, and casseroles, there is limited information about the nutritional content of kudzu products. Baldwin (2003) reported the nutritional composition of kudzu powder which contained all parts of the plant – vines, leaves, flowers, and roots (Table 1). These results indicated kudzu powder is a nutritious product low in fat and sodium, and a good source of dietary fiber, calcium, iron, potassium, and vitamin A. Duke (1983) reported the nutrient content of cooked kudzu leaves. Table 2 lists the nutritional components of cooked kudzu leaves (Duke, 1983), cooked turnip greens and cooked spinach (United States Department of Agriculture Agricultural Research Service, 2009). All three products were low in fat as expected. The kudzu leaves had 7.7 g fiber per 100 g, whereas the turnip greens and spinach had 3.5 g and 2.1 g fiber per 100 g, respectively.

Table 1. Nutritional information for kudzu powder containing vines, leaves, flowers, and roots

Component	Kudzu powder (100 g)
Energy (kcal)	345
Protein (g)	21.9
Carbohydrate (g)	61.1
Fiber (g)	48.4
Total Fat (g)	1.5
Saturated Fat (g)	0.1
Potassium (mg)	1,950.0
Calcium (mg)	1,700.0
Iron (mg)	11.6
Sodium (mg)	9.0
Vitamin C (mg)	3.1
Vitamin A (IU)	14,600.0

Source: Baldwin, 2003

Table 2. Nutritional information for cooked kudzu leaves, turnip greens, and spinach

Component	Kudzu Leaves (100 g)	Turnip Greens (100 g)	Spinach (100 g)
Energy (kcal)	36	20	23
Protein (g)	0.4	1.1	3.0
Fat (g)	0.1	0.2	0.8
Carbohydrate (g)	9.7	4.4	2.7
Fiber (g)	7.7	3.5	2.1

Sources: Duke (1983) for kudzu leaves; United States Department of Agriculture Agricultural Research Service (2009) for turnip greens and spinach

Medicinal Uses for Kudzu

Overall, the medicinal qualities of kudzu seem to be more widely documented than culinary or nutritional qualities. Kudzu has been used for medicinal purposes in China for more than two millennia and was one of the first medicinal plants used in

traditional Chinese medicine (Keung & Vallee, 1998). Ko or Ko-shu, as it was called in China, was highly valued. The dried root (Ko-Ke^n) was an ingredient in many medicinal preparations and widely used in kudzu root tea which has been used to treat fever, dysentery, neck pain, eye pain, and insect and snake bites (Shurtleff & Aoyagi, 1985). An herbal tea made from kudzu root and/or kudzu flowers was used for treatment of drunkenness and the craving for alcohol (Baldwin, 2003; Keung & Vallee, 1998). This tea was used in China, Japan, and Korea for treatment or prevention of hangovers (Baldwin, 2003). Traditional Chinese medical practices currently use the kudzu varieties of *Pueraria lobata* and *Pueraria thomsonii*, which are known as Ge, for prevention and treatment of diseases. The root of Ge (Ge Gen) is usually mixed in a composite formula to treat various medical conditions including fever, headache, neck and back pain, polydipsia (excessive thirst) in diabetes, and hypertensive neck pain (Gao & Keung, 2002). Kudzu was thought to be a more powerful healing agent than ginseng (*Panax* sp.) which has also been widely used for medicinal purposes for thousands of years (Mitich, 2000; Shurtleff & Aoagi, 1985).

Although kudzu has been used in China for more than a thousand years as an amethystic (anti-alcohol intoxication) agent and as an antidipsotropic (anti-alcohol abuse) agent, there has been scientific research to evaluate its efficacy and biochemical mechanism of action in the past few decades. An experimental animal study using golden Syrian hamsters, which exhibit a high preference for alcohol in large quantities, reported significant reductions in alcohol consumption when the hamsters were administered a kudzu extract containing the isoflavone compound daidzin. A less potent

effect was shown by the intake of daidzein, another isoflavone compound in kudzu (Keung & Vallee, 1998). Other studies using human subjects reported reduction of alcohol consumption with kudzu treatment (Lukas et al., 2005; Penetar et al., 2006). It was postulated that physiological and biochemical activities of daidzin from kudzu suppress alcohol intake "by modulating activity of the central reward pathways through inhibiting the catabolism of monoamine neurotransmitters" (Keung & Vallee, 1998). In a report from the First Annual Meeting by the Complementary Medicine Research Group of the Italian Society for Alcohol Studies, kudzu was endorsed as a probable useful adjunct in reducing alcohol intake for alcohol-dependent patients (Abenavoli et al., 2008).

Kudzu and Isoflavones

In addition to the isoflavone compounds daidzin and daidzein, kudzu also contains puerarin, genistein, and formononetin, among others. All have purported antioxidant properties (Jun et al., 2003). The health benefits of antioxidants are numerous and enhance overall health and help prevent chronic disease. In the past several years, the United States Food and Drug Administration has approved the health claim that dietary isoflavone supplements can reduce the risk of cardiovascular disease, thus increasing the interest and need for research in developing diets or dietary supplements rich in isoflavones (Gao & Keung, 2002). An enormous market for this class of chemicals has been created, particularly in soybean-producing countries such as the United States and Australia. Many health foods, drinks, and food supplements are

now marketed (and available over the counter) based on the content and promoted beneficial effects of isoflavones. These products have encouraged much public interest and debate and initiated intensive research on the true efficacy, indications, safety, and mechanisms of action of the commercial products. Of interest is the estimation that most North American diets contain little isoflavone intake; the usual intake is approximately 1 to 3 mg per day. By contrast, people in Asian countries consume 25 to 100 mg per day primarily from soy products and legumes (Wong, 2002).

The prevalence of epidemiological data suggests that isoflavones play a preventive role in many diseases. Wong (2002) related the potential benefits of isoflavones in kudzu to other sources of the same isoflavones, especially from soy. Daidzein and daidzin are also phytoestrogens that are present in soy and kudzu, as well as other legumes. Hung and Morita (2007) reported that daidzein, daidzin, genistein, genistin, and puerarin existed in all kudzu plant parts (root, leaf, and seed), whereas commercial kudzu starch from Japan contained only low levels of diadzein. Daidzein and daidzin have considerable importance in promoting overall health and are beneficial to women's health in preventing menopausal symptoms. However, the unique isoflavone, puerarin, found in kudzu, may have special therapeutic benefits. Gao and Keung (2002) reported that puerarin injections are widely used in hospitals in China for the treatment of heart disease. Clinical applications using puerarin that is extracted from kudzu are widespread in China for treatment of cardiovascular disease, myocardial infarction, hypertension, and angina pectoris (Chai, Zhao, & Gao, 2002). Guerra et al. (2000) concluded that puerarin acted as an enzyme inhibitor of oxygen radical production

and protected the heart muscle from injury. Further research by Sun et al. (2007) also indicated that puerarin exhibited a vascular relaxing action beneficial for treating cardiovascular diseases such as hypertension.

Isoflavone compounds present in legumes and vegetables exhibit a wide spectrum of biochemical activities that can help cells in the body with preventing hormone-dependent diseases such as diseases of the prostate and uterus, breast cancer and osteoporosis (Wong, 2002). Effective dose ranges and guidelines to determine dietary isoflavone intakes that provide pharmokinetic properties in different types of foods need to be established (Wong, 2002; Gao & Keung, 2002). Natural products that have potentially active ingredients with pharmacological activities also need further study (Wong, 2002). Jun et al. (2003) concluded that the antioxidant activity of isoflavone compounds from the kudzu root has been underestimated and should be further investigated.

Phytoestrogens are compounds that have estrogenic-like activities and are found in a wide variety of plants. Isoflavones are a class of phytoestrogens (Higdon, 2007). Recent epidemiological studies have reported evidence of protective roles of isoflavones against the development of numerous chronic diseases including several cancers, cardiovascular disease and osteoporosis (Choi & Ji, 2005). Epidemiological studies have suggested that isoflavones may play a protective role in many hormone-dependent diseases and symptoms. The discovery that dietary isoflavones such as diadzein, genistein and others, can bind to estrogen receptors has raised the possibility that the phytoestrogens may exert beneficial effect by modulating estrogenic activity (Wong,

2002). Natural products and preparations for food and nutritional supplements have gained interest in the past several years. Fruits, vegetables, grains, seeds, and tubers have been studied to determine which plants or plant parts have high amounts of phytoestrogens. High amounts of isoflavones, with beneficial estrogenic effects, have been found in soybeans, soy products, and red clover (Kroyer, 2004).

The plant family most abundant in phytoestrogens is the Fabaceae, or the legume family. Nutritional properties of the Fabaceae have been extensively studied and legumes have been shown to exert many physiologically beneficial effects in animals and humans. Kudzu is a member of the legume family and all parts of the plant have been studied, specifically investigating the sources of isoflavone secondary metabolites, genistein and diadzein. Kudzu roots are good sources for these isoflavones that can be commercially extracted (Geng, Zongdao, & Yimin, 2007; Kaufman, Duke, Brielmann, Boik, & Hoyt, 1997; Mazur, Duke, Wahala, Rasku, & Adlercreutz, 1998).

Phytochemicals and Health

Phytochemicals in fruits and vegetables have generated a great deal of attention mainly due to their role in potentially preventing diseases caused as a result of oxidative stress (Chun et al., 2005; Higdon, 2007; Kaur & Kapoor, 2001). Oxygen is toxic to organisms when the exposure is greater than that of normal air. The main cause of this toxicity is the intracellular reduction of oxygen into highly reactive chemical species, or free radicals. Free radicals are a natural by-product of aerobic metabolism (2-3% of oxygen consumed by the cell is converted to free radicals). These findings led to the

belief that aging and age-related degenerative disease might be due to the long-term effects of oxidative damage that can be modified by genetic and environmental factors (Wickens, 2001).

Oxidative stress, which releases free oxygen radicals (free radicals) in the body and causes oxidative damage to large biomolecules such as proteins, deoxyribonucleic acid, and lipids, has been implicated in a number of disorders including cardiovascular disease, cataracts, some types of cancer, and auto-immune disorders (Chun et al., 2005; Higdon, 2007; Kaur & Kapoor, 2001). Free radicals are unstable, highly reactive and energized molecules with unpaired electrons. These free radicals try to capture electrons to gain stability, causing another electron to become a free radical. Antioxidants neutralize or stabilize free radicals by donating one electron, ending the electron-stealing reaction. The antioxidants do not become free radicals because they are stable in either form (Higdon, 2007; Kaur & Kapoor, 2001). Excessive free radical production and lipid peroxidation *in vivo* are known to cause many kinds of pathological processes and diseases such as atherosclerosis, cancer, and chronic inflammation (Chu, Chang, & Hsu, 2000). Lipid oxidation is a primary mechanism of quality deterioration of food products, especially foods high in fat. Antioxidants are added to foods during processing to improve food quality and stability (Yu et al., 2002).

Epidemiological studies have consistently shown significant positive associations among intake of fruits and vegetables and reduced rates of heart disease mortality, some types of cancers, degenerative diseases, and aging (Arts & Hollman, 2005; Higdon, 2007; Kaur & Kapoor, 2001). Unfortunately, there have been few studies that have evaluated

the total antioxidant content of food that is commonly consumed in the United States (Chun et al., 2005). Phenols are the most abundant antioxidants in the diet. Their total dietary intake can be as high as 1 gram/day, which is higher than that of all other classes of phytochemicals and known dietary antioxidants. This is purported to be approximately 10 times higher than the intake of vitamin C, and 100 times higher than the intakes of vitamin E and carotenoids. The primary dietary sources are fruits and plant-derived beverages such as tea, coffee, red wine, and fruit juices. Vegetables, cereals, chocolate, and legumes are also known contributors to total phenol intake (Scalbert, Johnson, & Saltmarsh, 2005).

Phenols are derived from the common intermediate amino acid, phenylalanine, or its close precursor shikimic acid, through the shikimic acid pathway in plants. Phenols can be divided into at least ten different classes on the basis of their general chemical structure. The common characteristic is at least one aromatic ring structure with one or more hydroxyl groups. A large variety of plant phenols exist, including benzoic acids, cinnamic acids, and flavonoids. There may be many compounds present within each family of plant phenols. More than 6,000 different flavonoids occurring in plants have been described (Arts & Hollman, 2005; Hanson, 2005).

Flavonoids, the largest subclass of phenols are found in high amounts in foods characterized by a high skin-volume ratio such as cherry tomatoes and grapes. The accumulation of plant flavonoids is positively related to the amount of sunlight received (Chun, Chung, & Song, 2007; Mullie, Clarys, Deriemaeker, & Hebbelinck, 2007). Flavonoid intake estimates in humans vary from 20 to 1,000 mg/day. This variation can

be attributed to widely differing intake levels of specific food sources of flavonoids, as well as the use of specific food composition tables which are often incomplete with respect to the flavonoid content of a particular food item (Mullie et al., 2007). Chun et al. (2007) conducted one of the first studies to estimate flavonoid intake among U.S. adults by expanding the USDA Flavonoid Database. This study described total and individual flavonoid intake among U.S. adults and sociodemographic subgroups and documented the contribution of specific foods to total and individual intake. Estimated mean total flavonoid intake was 189.7 mg/day, primarily from flavan-3-ols (83.5%). The greatest daily mean intakes of flavonoids were from tea (157 mg), citrus fruit juice (8 mg), wine (4 mg) and citrus fruits (3 mg). This study is one of the first to generate baseline data of flavonoid intake of U.S. adults and subgroups and is being followed up by an assessment of the total antioxidant intake (Chun et al., 2007).

Various hypotheses have been proposed to explain the beneficial effects of increased consumption of vegetables and fruits. A popular hypothesis is that fruits and vegetables contain compounds that have protective effects, independent of known nutrients and micronutrients (Arts & Hollman, 2005). Phenols have been reported to have physiological effects in animal and *in vitro* systems. These include trapping and scavenging free radicals, regulating nitric oxide, decreasing leukocyte immobilization, inducing apoptosis, inhibiting cell proliferation, and angiogenesis and exhibiting phytoestrogenic activity (Arts & Hollman, 2005). However, the evidence derived from *in vitro* or animal studies are performed with doses much higher than those that humans would be exposed to through diet. The major difficulty of elucidating health benefits of

phenols is the large number of phenolic compounds in food yielding differing biological activities. Major differences in bioavailability have been established and the influence of structural factors is better understood. It is thought that the active compounds may not be the native phenols in a food but the metabolites of the phenols. The native phenols are most often tested in *in vitro* studies (Scalbert et al., 2005). Individual antioxidants may act through multiple mechanisms in a single system or by a different single mechanism depending on the reaction system. Antioxidants may also respond in a different manner to different radical or oxidant sources (Prior, Wu, & Schaich, 2005).

When the Dietary Reference Intakes were established, the Institute of Medicine used biomarkers of oxidative stress to define dietary antioxidants. The Institute of Medicine's definition of dietary antioxidants includes "the ability to substantially decrease the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiologic function in humans." Sufficient scientific agreement does not exist regarding the validity of these biomarkers as a reflection of the action and efficacy of dietary antioxidants. In many studies, the difficulty of demonstrating an antioxidant effect unless oxidative stress is first markedly elevated, for example in smokers or other inflammatory conditions, is the main difficulty (Blumberg, 2004). Phenols have been shown to consistently improve the status of different oxidative stress biomarkers. Uncertainty persists regarding the relevance of the biomarkers as predictors of disease risk and the appropriateness of the different methods (Scalbert et al., 2005). No single assay of antioxidant capacity will accurately reflect all of the radical sources or all antioxidants in a mixed or complex biological system (Prior et al., 2005). The

question remains as to whether the animal and *in vitro* data are relevant for human disease outcomes, where exposure to phenols is at relatively low concentrations, depending on bioavailability and metabolism (Arts & Hollman, 2005).

Free radical scavenging is the accepted mechanism of antioxidants for inhibition of lipid oxidation. The laboratory method of scavenging the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radicals can be used to evaluate the antioxidant activity of specific compounds or extracts (Chu, Chang, & Hsu, 2000). Because it measures the most common natural antioxidants but is not interfered with by glucose, the DPPH method is convenient for antioxidant assays of biological materials. As a stable free radical, DPPH accepts an electron or hydrogen radical to become a stable molecule (Blois, 1958; Kroyer, 2004). The DPPH compound turns a deep violet color when dissolved in ethanol. When the DPPH/ethanol solution is mixed with an antioxidant which can donate a hydrogen atom to the free radical DPPH, the DPPH is reduced and the violet color is decreased or lost, depending on the amount of reduction. Because of its odd electron, dissolved DPPH exhibits a strong absorption band at 517 nm on an ultraviolet visible (UV/VIS) spectrophotometer (Blois, 1958).

Plant Volatile Organic Compounds

Plants are capable of synthesizing thousands of primary and secondary metabolites with diverse biological properties and functions. Plant volatile organic compounds generated from both primary and secondary metabolites are generally low molecular weight lipophilic compounds (Goff & Klee, 2006). The number of volatile

chemicals identified in various plants exceeds 1,000 and is likely to grow as more plants and more sensitive methods for detection are perfected. Plant volatiles serve multiple functions in both floral and vegetative organs, and these roles are not always related to volatility. Most plant volatiles are restricted to specific lineages and are involved in species-specific ecological interactions. To humans, pollinator-attracting floral scents have been a source of olfactory pleasure, and large numbers of aromatic plants are used as flavorings, preservatives, and herbal remedies (Pichersky, Noel, & Dudareva, 2006).

Fruit and vegetable flavors depend on taste (the balance between sweetness and sourness or acidity and the presence of astringency) and aroma (concentrations of odor-active volatile compounds). Although taste and aroma are well integrated in their combination to the overall flavor, aroma is often considered to play a dominant role in flavor (Kader, 2008). Many volatiles are produced in plant tissues at specific developmental stages, for example, during flowering, ripening, or maturation. Volatile emissions have evolved to facilitate seed production and dispersal. Although a single fruit or vegetable synthesizes more than one hundred volatiles, only a small subset generates the "flavor fingerprint" that helps animals and humans recognize appropriate foods and avoid poor or dangerous food choices (Goff & Klee, 2006).

Vegetables produce most of the volatiles sensed as flavors only after their cells are disrupted. This disruption mixes substrates with the enzymes responsible for generating flavor volatiles. The development of flavors and the availability of nutrients are promoted by cell lysis in vegetables, whereas in fruits, ripening produces most of the perceived volatile compounds (Goff & Klee, 2006). In fruit and vegetable production, it

is essential that good flavor quality be emphasized by selecting the best tasting genotypes, using optimal cultural practices to maximize flavor, harvesting at the maturity/ripeness stage that will optimize eating quality at the time of consumption, and using postharvest handling procedures that will maintain optimal flavor and nutritional quality between harvest and consumption (Kader, 2008).

Phenolic compounds are considered secondary metabolites that are synthesized by plants during normal development and in response to stress conditions, such as infections, wounding, and UV radiation. The compounds are a diversified group of phytochemicals derived from phenylalanine and tyrosine. Plant phenolics include simple phenols, phenolic acids, coumarins, flavonoids, stilbenes, tannins, lignans and lignins (Naczk and Shahidi, 2004). In plants, phenolics act as antifeedants, attractants for pollinators, antioxidants, contributors to plant pigmentation, and protective agents against UV light. Phenolics are responsible for the majority of the oxygen capacity in most plant-derived products, such as wine (Singleton, Orthofer, & Lamuela-Raventos, 1999). With a few exceptions, such as carotene, the antioxidants in foods are phenols. In food, phenolics may contribute to bitterness, color, flavor, odor, astringency, browning reactions, protein constituents, and oxidative stability of food products. The health-protecting capacity and anti-nutritional properties of different plant phenolics are of great importance to producers, processors, and consumers (Naczk & Shahidi, 2004; Singleton et al., 1999).

Although there are several methods for measuring phenolic content of foods, the Folin-Ciocalteu reagent procedure is widely used. The Folin-Ciocalteu reagent is the recommended reagent to use for total phenol and other oxidative substrate measurements

because it is simple, convenient, requires only common laboratory equipment and has a large body of comparable literature. Under proper conditions, the assay gives predictable reactions with the types of phenols occurring in nature (Singleton et al., 1999). Variations of this method continue to be widely used to determine phenolic substances in foods and other materials. It has been considered the most appropriate method for estimating total phenol content in complex plant products for many years. It has been utilized to determine the concentration of phenolic substances in grapes and wines as related to flavor and storage changes. Comparison standards and blanks are recommended to be included within each group of samples in the determination of phenols by the Folin-Ciocalteu spectrophotometric method. Gallic acid has been widely used as the comparison standard and the results are reported in milligrams gallic acid equivalents (GAE). Gallic acid is inexpensive, soluble in water, and stable in the dry form. A stock solution is usually made by dissolving gallic acid in a small amount of ethanol and diluting with deionized distilled water (Singleton et al., 1999; Singleton & Rossi, Jr., 1965).

Sensory Analysis

Sensory appeal is the principal determinant of food selection and ingestion. A large majority of consumers rank taste as more influential on their food selection and ingestion choices than other factors such as price, safety, or nutrition (Mattes & Cowart, 2008). Perception of flavor is often described as a combination of taste, smell, appearance, texture, temperature, mouth feel, and past experiences (Goff & Klee, 2006).

These are typically perceived in the order of appearance, aroma, consistency/texture, and flavor (aromatics, chemical feeling, taste) (Meilgaard, Civille, & Carr, 2007). This indicates the multiple distinct sensory inputs required to process and generate an overall sensation. Integration of this sensory information in the brain ultimately results in a flavor preference or aversion with a strong influence on subsequent perception and behavior (Goff & Klee, 2006). Acceptance and preference of sensory properties of foods are among the most important criteria for determining food choice and how people react to a food. Sensory evaluation encompasses use of all the senses as they come in contact with the food being evaluated (McWilliams, 2005).

Two general classes of consumer testing methods exist: acceptance and preference. Acceptance testing presents consumers with individual samples for a hedonic response without direct comparison to other samples, typically using a type of scale to quantify overall acceptability. This type of testing measures the degree to which a product is liked or disliked and gives interval or ratio data. Preference can be inferred from relative acceptance scores with higher scores being preferred. Preference testing requires the selection of one sample that is preferred over another and produces ordinal data that permits identification of sample preference within the test set. The preference test forces a choice of one item over another but does not indicate whether the samples are liked or disliked (Hein, Jaeger, Carr, & Delahunty, 2008; Meilgaard et al., 2007).

Hedonics refers to the pleasantness or appeal of stimuli such as from the consumption of a food or beverage. From a dietetics perspective, the most important point is that the affective interpretation of different forms and intensities of sensory

stimulation is not immutable. It can change with physiological status including hunger, pathological conditions, and dietary experience. Dietary experience provides an avenue for dietary interventions. One of the most powerful but complex influences on palatability is frequency of exposure. Familiarity enhances acceptability. Another powerful influence is culture. Foods seasoned according to the flavor principles of an individual's culture are generally well accepted, whereas other foods are not as well liked despite the lack of a clear biological basis (Mattes & Cowart, 2008).

The interpretation of hedonic data entails some fundamental considerations. In hedonic testing the key outcome is to capture the impressions of potential consumers, whose responses may be based on an entirely different set of influences such as cost, convenience, and availability. Hedonic data may also be especially susceptible to modification by cognitive factors. An example of this would be if brand name information was provided with the test samples. Another example of cognitive factors that could modify responses would be health beliefs. For example, ice cream may be regarded as more pleasant than cottage cheese, but cottage cheese may be preferred for health reasons (Mattes & Cowart, 2008)

Hedonic data can be analyzed via agglomerative hierarchical clustering, which is a statistical technique used to group panelists (consumers) together based on patterns for a certain response. This type of grouping of panelists is useful because panelists vary in their liking and preference of food items/products. It is also useful for analyzing hedonically scaled consumer data when descriptive analysis data are not available. Prior to this method of analysis, basic statistical analysis of consumer data is performed.

Panelists are then grouped into clusters based on preference and overall liking of the food product. After the cluster analysis is performed, the data within each cluster are analyzed with a mean separation technique. This allows for further explanation of the panelists/consumers liking and preference of the food product (Schilling & Coggins, 2007).

Nutritional Analysis

The USDA has kept records of food composition for more than 115 years. The pioneer in this field was W. O. Atwater. The first food composition table (the Atwater table) was published in 1892 and contained data of 178 food items determined by five proximate analysis components. The components were water, protein, fat, total carbohydrates, and ash. Calories labeled as fuel and refuse were also identified. There have been many updates to these tables as food, agricultural, and manufacturing practices have changed (Haytowitz, Pehrsson, & Holden 2008). As the understanding of food and nutrition expands, the analytical methods used to determine food components have become increasingly sophisticated. Newer methods provide a more precise separation of macro- and micronutrients (Food and Agriculture Organization, 2003). Today, the food composition data for the United States is provided by the Nutrient Data Laboratory (NDL), the Agricultural Research Service (ARS), and the USDA. The USDA generates the National Nutrient Database for Standard Reference (NDSR). The NDSR has been updated many times and presently contains 7,291 food items for up to 140 nutrients and other components. They are constantly updating and developing special interest

databases for emerging bioactive compounds and for meeting the increasing informational needs of health professionals, researchers, and nutrition policy makers (Haytowitz et al., 2008). The data maintained by the NDSR are provided by ARS-contracted food analysis labs, the food industry and the scientific literature. The quality of the food composition data is determined by the analytical methods used, the sampling design, the number of samples, and the reproducibility of the data (Pennington, et al., 2007).

The Food and Drug Administration (FDA) started overseeing nutrition labeling of food items in the 1970's. Scientific findings reported in the 1980's, regarding diet and health, increased the interest in providing credible information on food labels. In 1990, Congress passed the Nutrition Labeling and Education Act (NLEA). It directed the FDA to develop regulations for reporting the levels of certain nutrients on labels of packaged foods. This action has encouraged manufacturers to produce foods with healthier nutrient profiles and serves as a motivator for consumers to make healthier choices (Taylor & Wilkening, 2008a). Nutrition labeling is a public health policy that has an important foundation. It is continually being evaluated and updated as new information is provided by scientists and manufacturers (Taylor & Wilkening, 2008b).

Jelly and Preserves

Jams, jellies, and preserves have historically been important methods of preserving fruit. The process of preserving fruit with sugar was well-known in 17th-century Europe and the colonists in what is now the southern United States. The art of

making preserves and jellies did not become prevalent in the South until the beginning of the 19th century. Cookbooks published during the antebellum period contain detailed entries for preparation of preserves and jellies from the large variety of fruit that grew in the surrounding area. The classic proportions of a pound of sugar and a pound of fruit or a pint of juice were usually described. Calves feet and isinglass (a collagen-like substance from fish bladders) were used to enhance the gelling of low-pectin fruits or in jellies made with blossoms, teas, or coffee. If these were not available, currant or quince jelly was added to enhance gelling. The receptacle of choice for cooked jelly was stoneware containers which were usually sealed with brandy-soaked paper (Cashion, 2007).

The post-Civil War era can best be described as a subsistence period. Cookbooks published after the Civil War included recipes utilizing native fruit that grew uncultivated such as crabapple, muscadine, persimmon, pawpaw, and roselle. The Great Depression left the national economy in poor condition. During this era, the Ball Brothers Company, a manufacturer of glass jars for preserving and canning, developed a small, efficient canning unit that was used to establish community canning centers, helping to defray the labor and expense of canning in individual households. During World War II many more of the centers were established and subsidized by a number of emergency relief agencies. By 1946, there were 3,600 community canning centers in the United States, primarily located in the South. The consolidation of the canning industry in the 1950s and 1960s closed most small commercial canning and preserving companies, along with the community canning centers. Preserves and jellies made by large manufacturers are

available today in supermarkets throughout the South at prices most southerners can afford. Many southerners still make preserves and jellies at home in an attempt to preserve the taste and memory of "who and what they came from" (Cashion, 2007).

In 2004, the International Jelly and Preserve Association determined that approximately 1 billion pounds of fruit spreads were produced annually in the US. Per capita consumption is approximately 2.2 pounds annually. The market for preserve products has been stable for more than 20 years, following significant growth in the years since World War II. In the overall fruit spread category, jelly contributes 20% of sales. The entire category of fruit spread product supermarket sales was $671 million in 2003, with jelly sales contributing $135 million of that amount (International Jelly and Preserve Association, 2004).

Jelly and preserves are characterized by the concentration of the fruit components and the addition of sugars to produce a preserved product of low pH, high solids, and low water activity. Standards of identity have been established to require specific amounts of the fruit ingredient. Only the products of specific fruits are defined in the regulations. The standards for fruit jellies in the Code of Federal Regulations (21CFR150) dictate that 45 parts by weight of the fruit component and 55 parts of the sweetener solids (45:55) must be in the product. The finished percent soluble solids content of a jelly should not be less than 65%. In many products, the pectin must be added in order to produce the proper "set" at the proper pH level. The pectin set is a function of temperature, soluble solids, pectin type and concentration, and pH.

Jellies are usually made by cooking fruit juice and sugar. When the recommended solids level has been reached (measured by a refractometer), pectin is added. The mixture is adjusted to a pH of 2.8 to 3.2, usually by adding citric acid, to ensure gel set and inhibit microbial growth. At this point, the hot product can be packed into sterilized jars (Food and Drug Administration, 2008; Rushing, 1995). They are typically packed at high temperatures in sterilized jars with adequately heated lids to destroy mold spores (Rushing, 1995).

Dips, Dried Vegetables, and Spinach

Dips and dip mixes are an innovative way to introduce or enhance flavors and introduce new flavor combinations. In 2008, sales of dips and dip mixes showed a 2.2% dollar gain at $316 million. Packaging and marketing are keys to selling these products. One area of interest to snack developers is putting seasonings in bags and letting the consumers have control over the flavor combinations. Other trends in the dip category are focused on healthier formulations. Dairy-based dips are ideal for delivery of functional ingredients such as probiotics. Packaging in convenience-size dip cups provides portion control. Reduced-fat and lower-sodium versions of traditional dips are also being marketed (Snack Food Association, 2009).

Most Americans occasionally consume savory snacks. Although occasional use of these foods is not detrimental, heavy consumption of full-fat snacks is associated with poor diet quality and increased risk of chronic disease. One area of future research could be to clarify whether diet quality improves when consumers substitute non-fat for full-fat

snacks (Neuhauser et al., 2000). Healthier snacks led the way in snack sales in 2006. According to a State of the Snack Food Industry Report (2006), a large percentage (78%) of consumers cited they were trying to eat healthier, and 63% were trying to eat snacks with more nutritional value. Consumers are trying to figure out how to have overall wellness by focusing on the foods they eat. The opportunity exists to continue to create healthier versions of indulgent snacks. Baked cheese puffs, low-fat crackers, light ice cream, and baked potato chips, showed an increase in sales in 2006. Consumers aged 55 and older were found to be especially concerned about eating healthier and are important targets for healthy snack products, along with families with young children given growing concerns about childhood obesity (Snack Food Association, 2007).

Healthy snacks can be developed by using dried vegetables and fruits. Drying (dehydration) is one of the oldest methods of food preservation. Drying preserves foods by removing enough moisture from the food item to prevent spoilage and decay. The main purpose of drying is to extend the shelf life of foods by reducing water activity. Drying inhibits microbial growth and enzyme activity, but the processing temperature is usually insufficient to cause the inactivation of the enzymes. The reduction in water activity retards the growth of bacteria, yeasts, and molds, and lowers enzymatic and chemical reaction rates (Fellows, 2000; Kendall, DiPersio & Sofos, 2004; Perera, 2005). Water content of properly dried food varies from 5 to 25 percent, depending on the food. Successful drying depends on sufficient heat to draw out moisture without cooking the food, dry air to absorb moisture, and adequate air circulation to remove moisture (Kendall et al., 2004). The reduction of weight and bulk of dried food products reduces

transport and storage costs. For some types of food, dehydration provides convenient food products, or more easily handled ingredients for food processors. Some disadvantages to drying include deterioration in the nutritional value and eating quality of the food (Fellows, 2000; Perara, 2005).

There is usually a decrease in the quality of the dried food product because most conventional techniques use high temperatures during the drying process. The drying process may also introduce undesirable changes in appearance, flavor, and color. The goal of dehydration technologies is the production of dried products with little or no loss in their sensory characteristics together with the advantages of added convenience (Nijhuis, Torringa, Yuksel, Leguijt, & Kloek, 1998).

Thermostatically controlled electric dehydrators are recommended for home food drying. The best dehydrators have controlled heat settings and fans that blow warm air over the food. These types of dehydrators are convenient, inexpensive, easy to use, and useful for drying large or small batches of food. Most dehydrators are efficiently designed to dry foods quickly at approximately 140°F (Harrison & Andress, 2000; Kendall et al., 2004).

Drying, like all methods of food preservation, can result in loss of nutrients. The calorie content of the product does not change but is concentrated into a smaller mass as moisture is removed. There is no change in the fiber content of the product. Vitamin A is retained under controlled heat methods. Vitamin C is destroyed during blanching and drying of vegetables. Thiamin, riboflavin, niacin and some minerals are lost during blanching, but some may be retained if the rehydration water is consumed. Iron is not

destroyed during drying. For optimal retention of nutrients in dried foods, storage in a cool, dry, and dark place is ideal, and the product should be used within a year (Kendall et al., 2004; Perera, 2005).

Spinach is a popular ingredient in dip products. It is typically used in dips in dried form, or used thawed and drained from the frozen state. Spinach is often used in creamy-style dips. According to data compiled by the USDA-ERS, per capita use of all spinach totaled 2.35 pounds during 2000-02 compared with 1.69 pounds during the 1990s and 1.57 pounds in the 1980s. During 2000-02, consumption of spinach in the United States totaled 671 million pounds, 66% more than in 1990-92 (Lucier, Allshouse & Lin, 2004).

Spinacia oleracea L. (spinach) is a popular salad green and plate vegetable. Epidemiological data are accumulating on the health benefits of the carotenoids lutein and zeaxanthin present in leafy green vegetables, including spinach (Hasler, 2002).

Economic and Research Possibilities Utilizing Kudzu

The potential for research utilizing kudzu is controversial but possibly very rewarding. The economic benefits for using kudzu as a cash crop are mostly unrealized. Kudzu root starch is in demand in many countries, can be expensive to purchase where available and therefore, sizable profits are possible. Japan has limited land available for farming. An example of the possibility, in regards to kudzu production and economic benefits, includes the reported purchase of 65 ha (160 acres) in Alabama, by a Japanese company, for growing kudzu for kudzu root starch production (Mitich, 2000). Premium

prices are paid in the United States for kudzu root powder, which is usually shipped from China. There is also a substantial market for this product in China, Japan, and Korea (Baldwin, 2003).

Terrill et al., (2003) reported that kudzu holds promise as a low-impact forage for livestock production. They reported that with no fertilizer or lime inputs, a well-established stand of kudzu produced adequate yields and forage quality whether cut or left uncut. Digestibility of the leaf material remained high throughout the growing season and was therefore well suited for use as a protein bank or as an emergency feed supply during periods of drought. It was concluded that if managed as a forage crop, kudzu could make an important contribution to grazing systems in the South by providing nutritious feed during the late summer, early autumn period when the quality of perennial summer grasses is low.

The potential yields possible in the southeastern United States can be estimated as approximately 2,000 to 3,000 pounds of roots and 3,000 to 5,000 pounds of vines per acre. It is predicted that this could be increased with the use of modern agricultural methods. The vines can be harvested twice during the growing season. Vines and leaves can be cultivated in the greenhouse using aqua-culture, and kudzu plants are easily propagated and maintained through tissue-culture methods (Parks et al., 2002).

It is estimated that kudzu could produce a valuable commodity in as little as seven years. Although kudzu requires large acreage for production, 100,000 acres under cultivation can produce a crop suitable for use in kudzu products. Kudzu root powder produced from this acreage, for example, could be utilized in natural drugs and

supplements and in the culinary and food production industries (Parks et al., 2002). Additionally, leaves and flowers from the kudzu plant could be incorporated into novel food products.

CHAPTER III

MATERIALS AND METHODS

The research conducted was divided into three sections by plant part: roots, leaves and flowers. The materials and methods for each plant part will be presented separately. Kudzu plant parts were harvested in Choctaw, Desoto, Montgomery, Oktibbeha, or Webster counties in Mississippi.

Roots

The research conducted for this section was to determine and compare free-radical scavenging activity and total phenolic content of Mississippi-collected kudzu roots to a purchased, processed kudzu root starch manufactured in Japan.

Plant Materials

The roots of *Pueraria lobata* (kudzu) were harvested with a shovel in Choctaw County, Mississippi, on February 8, 2009. The roots were scrubbed, rinsed and air-dried in a single layer under normal room-temperature conditions from February 10, 2009 to March 13, 2009, as described by Shurtleff and Aoyagi (1985). The whole dried roots were chopped into small pieces by hand using pruners. The root pieces were placed in a container and weighed on a balance scale (Edlund Scale Model E80, Burlington, VT) to obtain a total weight measurement. Organic kuzu (kudzu) root starch was purchased

from Eden Foods® (Clinton, MI) via the Internet. The starch was labeled as a product of Japan and had been processed and refined to white starch particles.

Preparation of Kudzu Root and Starch Samples

The chopped Mississippi kudzu root pieces were taken to the Nutrition Laboratory in the James W. Scales Building, Mississippi State University, to be dried and ground. The samples were placed in a muffle furnace at 64°C for 72 hours. The technician attempted to grind the samples after 48 hours, but the samples were not sufficiently dried and required further drying. The samples were ground in a Thomas-Wiley Lab Mill (Thomas Scientific Model 4, Swedesboro, NJ), using a 2-millimeter screen. The Wiley Mill was cleaned prior to grinding the samples. The resulting ground powder was weighed. The powder was a light brown color and was stored in a desiccator in a laboratory (room 258) in the Herzer Building, Mississippi State University. The kudzu root starch obtained via the Internet (Eden Foods®, Clinton, MI) was stored in a cabinet in room 258, Herzer Building and used as purchased.

To prepare the kudzu root extracts for analysis, 0.1, 0.5, 1.0, and 5.0 grams (g) of the ground Mississippi kudzu root were placed in 50 milliliter (ml) flasks with 10.0 ml of methanol and gently swirled 5 times. For the purchased kudzu starch, 5.0 g of the starch were placed in a 50 ml flask with 10.0 ml of methanol and gently swirled 5 times. The difference in dilution between the two starch treatments was due to the purity and fine granule size of the highly processed purchased starch compared to the unprocessed, unrefined Mississippi roots. After 1 hour, each sample (flask) was gently hand swirled

and dumped into a glass funnel lined with Whatman No. 1 filter paper. The samples were allowed to filter through the paper for 15 minutes and the filtrates (extracts) were collected in 50 ml beakers. The resulting extracts were used to determine radical scavenging activity and total phenolics. This preparation procedure was based on the work by Lee et al., (2003).

Determination of Radical Scavenging Activity

The hydrogen donating, or radical scavenging ability, was evaluated by the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging method (Blois, 1958) using the stable radical DPPH (Sigma-Aldrich Co., St. Louis, MO) which is reduced in the presence of antioxidant active substances. All chemicals used in the experiments were purchased from Sigma-Aldrich Co., St. Louis, MO. The DPPH reagent used for analysis was prepared by weighing 0.0025g DPPH and placing it into a calibrated 100-ml volumetric flask. Ethanol (100%) was added to the bottom of the meniscus at the 100-ml calibration line, capped, and inverted 5 times to mix (Lee et al., 2003). The reagent solution was kept refrigerated (42ºC) in the 100-ml flask with a tight-fitting glass stopper and wrapped in aluminum foil for protection from light. The reagent was used as soon as possible after mixing (Blois, 1958).

One ml of four different dilutions (0.1, 0.5,1.0, and 5.0 g) of the Mississippi kudzu root extract and one ml of the 5.0 g dilution of the purchased kudzu root starch extract were transferred to test tubes using a pipette (Eppendorf Corp., Hamburg, Germany). To these, 1 ml of the DPPH reagent was added. All samples were done in

triplicate, including the control. The control contained the DPPH reagent and no antioxidant. The samples were incubated in the dark for 30 minutes at ambient temperature (~23°C). Before the samples were analyzed, the absorbance at 517 nm was zeroed against a blank of 100% ethanol. The samples were poured into10 mm, 1.5 ml capacity cuvettes (Fisher Brand® Semi-micro Style, Pittsburg, PA), placed in a UV/VIS spectrophotometer (Shamadzu Scientific Instruments Model UV-1201, Columbia, MD), read at 517nm, and the absorbance values were recorded (Blois, 1958; Huang, Wang, Eaves, Shikany, & Pace, 2009; Lee et al., 2003). The radical DPPH scavenging capacity was estimated from the difference in absorbance with or without antioxidants and expressed as the percentage DPPH inhibited. The antioxidant capacity can largely be attributed to the phenolic compounds found in plants. These include phenolic acids, flavonoids, and anthocyanins (Huang et al., 2009).

The following calculation was used for the determination of antioxidant activity (expressed as the inhibition of the DPPH radicals by the sample) (Blois, 1958; Lee et al., 2003).

$$\% \text{ Antioxidant activity} = \frac{\text{Absorbance of control} - \text{Mean absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Determination of Total Phenolic Content

The level of total phenols was determined using Folin-Ciocalteau colorimetric reaction method. Total phenols were quantified in the Mississippi kudzu roots and the purchased kudzu root starch by using gallic acid as a reference standard as described by Gutfinger (1981), Singleton and Rossi, Jr. (1965), and Waterhouse (2002). The gallic

acid stock solution was prepared by dissolving 0.50 g dry gallic acid with 10 ml 100% ethanol and swirled gently in a 100-ml volumetric flask. This solution was diluted to volume (100 ml) with deionized distilled water, capped, and carefully inverted 10 times (Singleton et al., 1999; Singleton & Rossi, Jr., 1965). The gallic acid solution was covered, labeled, and stored at 4°C when not in use (Waterhouse, 2002).

A 20% sodium carbonate (Na_2CO_3) buffer solution was prepared by dissolving 20 g anhydrous sodium carbonate in 100 ml deionized distilled water (Singleton et al., 1999). The sodium carbonate solution was covered, labeled, and stored at ambient temperature (Waterhouse, 2002). The Folin-Ciocalteu reagent was prepared by diluting Folin-Ciocalteu reagent with an equal volume of deionized distilled water and stored in a capped brown bottle, labeled, and stored in the refrigerator (4 ºC) when not in use (Food and Agriculture Organization/International Atomic Energy Agency, 2000).

The procedure began with 20µl of sample (extract) being added by pipette to 1.58 ml of deionized distilled water. Folin-Ciocalteu reagent (100 µl) was added and mixed. After 1 minute and before 8 minutes, 300 µl of the 20% sodium carbonate solution was added and the samples were placed in the dark. After 2 hours at ambient temperature (~23ºC) in the dark, the color generated was evaluated based on absorbance using a UV/VIS spectrophotometer at a wavelength of 760 nm with samples placed in 10 mm, 1.5 ml capacity cuvettes. An ethanol blank (100%) was used to zero the spectrophotometer. Absorbance values were read and recorded for all samples. All determinations were performed in triplicate for each sample and each concentration (Gutfinger, 1981; Lee et al., 2003; Singleton & Rossi, Jr., 1965; Waterhouse, 2002).

A standard curve of gallic acid was used to calculate the total phenolic content in the extracts, which were expressed as gallic acid equivalents (mg GAE/g extract) (Li, Gao, Zhao, & Wang, 2007). The procedure for the gallic acid standard curve began by preparing the Folin-Ciocalteu reagent, the buffer (sodium carbonate) solution, and the gallic acid stock solution discussed previously in this section. The gallic acid stock solution was diluted to make the calibration curve concentrations, by mixing 0, 25, 50, 75, 125, and 250 µg/ml of the stock solution into 25 ml volumetric flasks. These were diluted to volume with deionized distilled water. These solutions had phenol concentrations of 0, 1, 2, 3, 5 and 10 mg/L gallic acid.

The steps for preparing the standard curve were as follows: Pipette 20 µl of each calibration curve concentration (in triplicate) into test tubes. Dilute each test tube with 1058 µl distilled water. Add 100 µl Folin-Ciocalteu reagent to each test tube. Swirl to mix and let set between 1 and 8 minutes at ambient temperature. After at least 1 minute add 300 µl sodium carbonate solution to each test tube and vortex at speed 3 for 5 seconds each. Leave the solutions at ambient temperature for 2 hours. Measure the absorbance at 765 nm in a spectrophotometer. Subtract the absorbance of the blank from all readings and create a calibration curve from the standards (Waterhouse, 2002).

This curve was used to determine the corresponding gallic acid concentration of the samples. A dilution factor was used to correctly determine concentrations. The regression formula ($y = ax + b$, where a = slope of the standard, x = sample absorbance, and b = intercept) was used for developing the equation. The following equation was used to calculate total phenolics in the kudzu samples using gallic acid as the standard:

total phenolics, GAE = gallic acid standard curve value multiplied by the mean absorbance of 3 replications per sample plus the regression intercept value (Gutfinger, 1981).

Leaves

The research conducted for this section was: 1) to determine and compare free-radical scavenging activity and total phenolic content and of Mississippi-collected kudzu leaves and purchased spinach leaves, 2) to develop a dip food product and 3) to determine consumer acceptability of the dip product using dried kudzu leaves and a dip prepared with dried spinach leaves.

Plant Materials

The kudzu leaves were harvested by hand picking from a known source from the wild. Similar leaves of 10-13 cm in width and 12-14 cm in length were selected as much as possible. The harvest date for all the leaves used in the analyses and the dips was within a 24-hour time period. The leaves were harvested on November 3 or 4, 2009, from three counties in Mississippi: Montgomery, Oktibbeha, and Webster. The leaves harvested from each location (county) were kept separated. They were transported and processed within 24 hours. The leaves were washed, spun in a salad spinner (Zyliss®, Lyss, Switzerland), wrapped in moist paper towels, placed in 1 gallon zip-top storage bags, and stored at 4°C in a refrigerator (General Electric®, Model 18, Louisville, KY) for 24 hours (Liu et al., 2007). The spinach leaves (Private Selection® Organic Baby Spinach, Kroger Co., Cincinnati, OH) were purchased on two different dates at the same

grocery store in Starkville, Mississippi, and handled and stored in the same manner as the kudzu leaves.

Three leaves were selected from each harvest site (county) from kudzu and three leaves from each of 3 batches of spinach were measured (pre-drying), and leaf width (cm) and length (cm) were recorded. Pre- and post-drying weights (g) (Edlund Scale Model E80, Burlington, VT) and water activity (a_w) measurements (a_w-Wert-Messer, Durotherm, Germany) of selected fresh (2.0 g samples) and dried leaves (1.5 g samples) were also recorded. Water activity was determined to measure the ratio of vapor pressure of the sample to the vapor pressure of pure water (McWilliams, 2005). The fresh leaves were wrapped in moist paper towels, placed in zip-top storage bags, and refrigerated at 4°C until water activity measurements were taken. The fresh and dried leaf samples were placed in the a_w-Wert-Messer Durotherm for 24 hours before measurements were recorded. All measurements of the fresh leaves were taken within 96 hours of harvest. The water activity levels of the dried leaf samples were measured and recorded following determinations of water activity for the fresh leaves.

The kudzu and spinach leaves were dried in an electric food dehydrator (American Harvest Gardenmaster®, Beaverton, OR) at 62.8°C (145°F) until dry (brittle texture). The leaves were placed on the mesh trays of the dehydrator with minimal overlap. The kudzu leaves were dried for 2 hours and the spinach leaves were dried for 4 hours. The leaves were allowed to cool for 30 minutes in the dehydrator. Once cool, they were crushed by hand, labeled, and stored in zip-top storage bags at ambient temperature in a dark place until used. The dried leaves were used for all analyses and in the dip

products. The moisture percentage recommended for dried leaves used in food products is less than ten percent in order to inhibit microbial growth (Andress & Harrison, 2006).

Preparation of Leaf Extracts

The dried kudzu and spinach leaves were prepared for analysis by grinding the hand-crushed leaf samples in a food processor for approximately 10 seconds (Cuisinart®, East Windsor, NJ) (Huang et al., 2009; Liu et al., 2007). Mesh size (US Standard Series, USA Standard Testing Sieve, Newark Wire Cloth Co., Newark, NJ) was measured to estimate the predominate mesh sizes used for this part of the study. The dried material was stored in plastic bottles with tightly fitted caps in room 258 in the Herzer Building, Mississippi State University, until used for analysis. Mesh sizes of the dried leaves (and blossoms) were measured using 2 g samples. Each sample was placed in the largest mesh size sieve (1.70 mm) and swirled by hand 5 times until the particles fell through the smaller size mesh sieves to the smallest mesh size (.063 mm) stacked on one another. The weight (g) of the samples caught in each sieve was documented.

To prepare the kudzu and spinach leaf extracts for testing, 0.1, 0.5 and 1.0 grams of the ground kudzu and spinach leaves were placed in 50 ml flasks with 10 ml of methanol and gently swirled 5 times. After 1 hour, the samples were filtered through glass funnels with Whatman No. 1 filter paper for 15 minutes and the filtrates (extracts) were collected in 50 ml beakers. The resulting extracts were used to determine total phenolic content and radical scavenging activity of the dried kudzu and spinach leaves.

Determination of Radical Scavenging Activity

The radical scavenging activity was evaluated by the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging method (Blois, 1958) using the stable radical DPPH. The procedure was similar to the procedure discussed in the roots section. One ml of the DPPH solution discussed in the roots section was transferred to test tubes (samples were done in triplicate). To these, 1.0 ml of each different dilution (0.1%, 0.5%, and 1.0%) of the kudzu leaf extract and the spinach extract were added. The samples were incubated in the dark for 30 minutes at ambient temperature (~23°C). The blank used to zero the spectrophotometer was 100% ethanol and the control was the DPPH solution. The samples were poured into cuvettes, placed in the spectrophotometer, read at 517nm, and the absorbance values were recorded (Blois 1958; Huang et al., 2009). The same calculation discussed in the roots section was used to determine percent antioxidant activity of the leaves (Blois, 1958; Lee et al., 2003).

Determination of Total Phenolic Content

Total phenols were quantified in the Mississippi kudzu leaves and the purchased spinach leaves by the Folin-Ciocalteu spectrophotometric method using gallic acid as a reference standard (Gutfinger, 1981; Singleton & Rossi, Jr., 1965; Waterhouse, 2002). This procedure was similar to the procedure discussed in the roots section. Three dilutions were used (0.1, 0.5, and 1.0 g) instead of four; 5.0 g dilution was not done since preliminary analysis indicated that 5.0 g was too concentrated.

One ml of each methanolic dilution of the kudzu and spinach leaf extracts was added to 2.0 ml of the Folin-Ciocalteu reagent (samples were done in triplicate). This mixture was allowed to stand for 5 minutes before 2.0 ml of sodium carbonate solution was added (Lee et al., 2003). The mixture was mixed and kept at ambient temperature (~23°C) for 1 hour. The absorbance values of the samples were measured at 760 nm against a reagent blank (Waterhouse, 2002).

Food Product Development

Kudzu and spinach leaf dip formulations (recipes) were developed based on spinach dip recipes and products available on the market (Andress & Harrison, 2006). Two formulations were developed and tested. One formulation contained regular sour cream and mayonnaise and added salt. The healthier formulation contained light sour cream and mayonnaise and no added salt. All kudzu and spinach leaf dip formulations contained the same amounts of spices/ herbs, sour cream, and mayonnaise, by weight. Each treatment contained the same amount of dried leaves by weight. The dried leaves were prepared in the manner discussed in the plant materials section. The dips were formulated to be similar to popular products on the market.

The regular formulation developed was tested by a preliminary sensory panel. All of the dried dip ingredients were weighed (Mettler® AE 200, Mettler Instruments Corp., Highstown, NJ) and mixed together with the weighed wet ingredients (Edlund® Model E80, Burlington, VT) 18 hours before serving to the panelists. The dry ingredients for each dip (herbs, spices, and leaves) were mixed with the wet ingredients

(sour cream and mayonnaise) by stirring vigorously, approximately 20-30 strokes with a rubber spatula, until well mixed. The mixed dips were placed in labeled plastic containers, covered, and refrigerated at 3.5°C (Revco®, Thermo Fisher Scientific, Inc. Houston, TX). The same procedure for stirring the dips was repeated prior to portioning the samples into the soufflé cups for sensory evaluation.

The panelists for the preliminary study were to determine the acceptability of three dried-leaf dip products, containing leaves from Webster, Montgomery, or Oktibbeha County. The only difference in the treatments was the harvest location. Three panels (n = 10 panelists) were conducted. The panel members were recruited from the faculty, staff, and students of the Department of Food Science, Nutrition, and Health Promotion, at Mississippi State University. All panelists had some sensory testing experience. Each panelist was presented with three chilled (4°C) dip samples labeled with one of four 3-digit random numbers. The samples were randomized to account for bias. Each sample weighed approximately 12-15 grams and was served in a clear 2-ounce soufflé cup with a clear lid (Dart Container Corp®., Mason, Michigan). Panelists were provided with tap water, unsalted tops crackers (Kroger®, Cincinnati OH), an expectorant cup, a napkin, a spoon, a pencil, and a score sheet (Appendix A). Panelists were asked to evaluate each dip sample based on the acceptability of appearance, aroma, flavor, texture, and overall acceptability using a 9-point hedonic scale. The scale consisted of the following ratings: 1-dislike extremely, 2-dislike very much, 3-dislike moderately, 4-dislike slightly, 5-neither like nor dislike, 6-like slightly, 7-like moderately, 8-like very much, and 9-like extremely.

Minor modifications to the formulation were made prior to preliminary panels 2 and 3, based on recommendations of the preliminary panel members. The goals of the preliminary panels were to determine if there was a significant difference between the kudzu dip samples based on the harvest location (county) of the leaves and to optimize the formulation prior to consumer sensory testing. Table 3 lists the ingredients, brands, and weights used in the regular kudzu and spinach leaf dip formula. Table 4 lists the ingredients, brands, and weights used in the healthier kudzu and spinach leaf dip formula.

Table 3. List of ingredients by brand name, weight, and amounts (g) used in regular dip formulation for 10-12 and 40-48 servings

Ingredient	Brand	10-12 servings	40-48 servings
Sour Cream	Daisy Brand®, Dallas, TX	100 g	400 g
Mayonnaise	Kroger®, Cincinnati, OH	75 g	300 g
Iodized salt	Morton International, Inc. ®, Chicago, IL	2 g	8 g
Granulated onion	Blend Pak Gourmet Spices & Seasonings®, Bloomfield, KY	2 g	8 g
Dill weed	Private Selection Inter-American Products®, Cincinnati, OH	1 g	4 g
Parsley Flakes	Badia Spices, Inc.®, Miami, FL	1 g	4 g
Dried Lemon Peel	McCormick Gourmet Collection®, McCormick & Co., Inc., Hunt Valley, MD	1 g	4 g
Ground Cayenne pepper	McCormick Gourmet Collection®, McCormick & Co., Inc., Hunt Valley, MD	.50 g	2 g
Granulated Garlic	Blend Pak Gourmet Spices & Seasonings®, Bloomfield, KY	.50 g	2 g
Dried kudzu or spinach leaves		2 g	8 g
Total weight		185 g	740 g

Table 4. List of ingredients by brand name, weight, and amounts (g) used in healthier dip formulation for 10-12 and 40-48 servings

Ingredient	Brand	10-12 servings	40-48 servings
Lite Sour Cream	Daisy Brand®, Dallas, TX	100 g	400 g
Lite Mayonnaise	Kroger®, Cincinnati, OH	75 g	300 g
Granulated onion	Blend Pak Gourmet Spices & Seasonings®, Bloomfield, KY	2 g	8 g
Dill weed	Private Selection Inter-American Products®, Cincinnati, OH	1 g	4 g
Parsley Flakes	Badia Spices, Inc.®, Miami, FL	1 g	4 g
Dried Lemon Peel	McCormick Gourmet Collection®, McCormick & Co., Inc., Hunt Valley, MD	1 g	4 g
Ground Cayenne pepper	McCormick Gourmet Collection®, McCormick & Co., Inc., Hunt Valley, MD	.50 g	2 g
Granulated Garlic	Blend Pak Gourmet Spices & Seasonings®, Bloomfield, KY	.50 g	2 g
Dried kudzu or spinach leaves		2 g	8 g
Total weight		185 g	740 g

Consumer Acceptability

Consumer acceptability of the dried-leaf dip products (kudzu and spinach) was evaluated by a panel of approximately 150 consumers. The kudzu dip formulation used for these panels was based on the results of the preliminary sensory panels. The four treatments consisted of a regular and a healthier treatment for each leaf type. Three panels (n = 50-55) were conducted. The dip ingredients were weighed on the same scales that were used for the preparation of the preliminary dip treatments. The ingredients were mixed together for 18-20 hours prior to serving to panelists. The dry ingredients

(herbs, spices, and leaves) were mixed with the wet ingredients (sour cream and mayonnaise) by stirring vigorously, approximately 20-30 strokes with a rubber spatula, until well mixed. The mixed dips were placed in plastic containers, labeled, covered, and refrigerated at 4°C until ready for portioning. The same procedure for stirring the dips was repeated prior to portioning the samples into the soufflé cups. Each sample weighed approximately 12-15 grams and was served in a 2-ounce clear soufflé cup with a clear lid (Dart Container Corp®., Mason, MI). Panelists were provided with water, low-salt crackers (Ritz Hint of Salt®, Kraft, Northfield, IL), an expectorant cup, a napkin, a spoon, a pencil, and a score sheet (Appendix A). Panelists were asked to evaluate each dip sample independently from other samples.

The panel members were recruited from the Mississippi State University campus. The three panels were conducted at the same sensory laboratory (Garrison Sensory Laboratory, Mississippi State University) at the same time of day (beginning at 10:00 a.m. and ending at approximately 12:00 p.m.). Conditions for the preparation of the samples and the running of the panels were identical for each panel. The white overhead light was used in each booth. Each panelist was presented with four chilled (4°C) dip samples labeled with one of four 3-digit random numbers. The samples were randomized to account for bias. Panelists were asked to evaluate each dip sample based on the acceptability of appearance, aroma, flavor, texture, and overall acceptability using a 9-point hedonic scale. The scale consisted of the following ratings: 1-dislike extremely, 2-dislike very much, 3-dislike moderately, 4-dislike slightly, 5-neither like nor dislike, 6-like slightly, 7-like moderately, 8-like very much, and 9-like extremely (Meilgaard et al.,

2007). The score sheet for the consumer panels included two general demographic information questions and several general questions regarding vegetable dip consumption (Appendix A).

Statistical Analysis

For the preliminary panels, analysis of variance was utilized to differentiate ($p<0.05$) between the acceptability of dried-leaf dip treatments prepared with leaves harvested from three different locations (counties) regarding appearance, aroma, flavor, texture, and overall acceptability (SPSS 17.0, SPSS Inc., Chicago IL). The results regarding overall acceptability were deemed the most important for evaluating the treatments and for determining which treatment(s) would be used in the consumer acceptability panels.

For the consumer panels, a randomized complete block design with three replications ($n = 50$-55) was used to differentiate ($p<0.05$) between the consumer acceptability of dried-leaf dip treatments (spinach or kudzu) regarding appearance, aroma, flavor, texture, and overall acceptability. When significant differences occurred among treatments, the Least Significant Difference (LSD) test was used to separate the treatment means. Consumers were clustered together based on their liking for dried-leaf dips by agglomerative hierarchical clustering. Dendrogram and dissimilarity plots were used to determine if /or how many clusters could be used to group consumers. Randomized complete block designs were used to differentiate ($p<0.05$) among dried-leaf dip treatments within each cluster. When significant differences occurred between

treatments within a cluster, the LSD test was performed to separate treatment means (Meilgaard et al., 2007; Schilling & Coggins, 2007). The statistical programs SPSS and SAS were used for analysis of the data (SAS v.9.2, SAS Inst. Inc., Cary, N.C.). The agglomerate hierarchical clustering analysis was performed by using the statistical program XLstat (XLstat, New York, NY).

Nutritional Analysis of Dip Products

Proximate analysis was performed and reported on all four kudzu and spinach dip treatments used for the consumer acceptability sensory panels by the Mississippi State Chemical Laboratory at Mississippi State University. All percentages were determined in triplicate. The moisture percentage was measured for each formulation using a drying oven (AOAC. 1995. Method 39.1.02). The protein procedure was AOAC 990.03, which determines nitrogen by combustion analysis (Model FP-528, LECO Corp., St. Joseph, MI). The nitrogen amount determined was multiplied by 6.25 to determine percentage protein. The fat content or ether extract content (%) was determined using a fat extractor (AOAC. 2000. Method 39.1.05, Model 1043, Soxtec HT Extraction Unit, Tecator, Hoganas, Sweden). The ash procedure was AOAC 942.05. Ash was determined using a muffle furnace at 600°C for 3 hours (Model Isotemp, Fisher Programmable Muffle Furnace, Fisher, Pittsburg, PA). The carbohydrate percentage was calculated by subtracting percentage moisture, protein, fat, and ash from 100% (Food and Agriculture Organization, 2003).

Flowers

The research conducted for this section was to determine free-radical scavenging activity and total phenolic content of dried kudzu flowers and to determine the consumer acceptability of kudzu blossom jelly. The kudzu jelly product produced for the consumer acceptability sensory testing was based on a jelly recipe that is prevalent in the popular literature (Baldwin, 1999; Hoots & Baldwin, 1996).

Plant Materials

The kudzu blossoms (flowers) were harvested by hand from a known source in Desoto County, Mississippi, on September 7, 2008. All blossoms were harvested from the same location on the same date. The blossoms were placed in a cooler and chilled during transport. After approximately two hours of transport, the blossoms were spread in a single layer on large metal sheet pans and frozen (Kelvinator®, Martinez, GA) at -20° C for 24 hours. After 24 hours, the frozen blossoms were placed in 1-gallon zip-top freezer bags and returned to the freezer for 31 days. The liquid to be used for each jelly preparation was made by placing 210.0 g (4 cups) of the frozen blossoms in a glass bowl and pouring 925.0 ml (4 cups) of water over the blossoms. The bowl was covered with plastic wrap and the blossoms were allowed to steep (Table 5). All mixtures were strained through a household mesh strainer into a glass freezer container (Pyrex Storage Plus® 2.6 L, Corning, Inc., Corning, NY), covered with a tight-fitting lid, labeled, and placed in a freezer (Kelvinator®, Martinez, GA) at -20° C for 387 days. When the liquid was ready to be used, the glass container was placed in a refrigerator (General Electric®

Model 18, Fairfield, CT) at 2.2°C for 28 hours until thawed. Once the liquid was thawed, the jelly preparation proceeded.

Table 5. Measurements, temperatures, and amount of time used to extract kudzu blossom (flower) liquid for jelly treatments from frozen blossoms

Treatment	Kudzu Blossoms (g)	Water (ml)	Water Temperature (°C)	Amount of time steeped (minutes)
1	210.0 g	925.0 ml	100 °C then 2.2 °C	480 minutes
2	210.0 g	925.0 ml	100 °C then ambient	60 minutes
3	a. 210.0 g b. 210.0 g	a. 925.0 ml b. 925.0 ml	a. 100 °C then ambient b. the liquid from a (at 60 °C) was poured over fresh blossoms	a. 60 minutes b. 60 minutes

For the determination of radical scavenging activity and total phenolics, dried blossoms were used. The blossoms were harvested on September 7, 2009, in Choctaw County, Mississippi. The flowers were placed in a single layer on a large metal sheet pan within 30 minutes of harvesting and frozen for 24 hours (Kelvinator®, Martinez, GA) at -20° C. After 24 hours, the blossoms were placed in a zip-top freezer bag and returned to the freezer for 43 days. The blossoms were placed in one layer on mesh shelves in the electric food dehydrator (American Harvest Gardenmaster Dehydrator®, Beaverton, OR) and dried for 2 hours and 45 minutes at 62.8 °C (145°F) until they had a dry brittle texture. The dried blossoms were allowed to cool in the food dehydrator for 30 minutes prior to placement in a labeled zip-top storage bag. The dried blossoms were stored in a

cool, dark location until analyses were performed. Pre-and post-drying weight measurements were documented.

Preparation of Blossom Extract for Chemical Analysis

The extracts for the determination of antioxidant activity and total phenolics in the dried kudzu blossoms were prepared by grinding the samples harvested in Choctaw County, Mississippi (Li et al., 2007; Liu et al., 2007). The flowers were ground and the mesh size measured using the same procedure as discussed in the leaf section. The powder was stored in the same manner and in the same location as the leaves.

To prepare the extracts for testing, 0.1, 0.5, and 1.0 g of ground kudzu blossoms were placed in 50 ml flasks with 10 ml methanol and swirled gently 5 times. After 1 hour, the samples were filtered through glass funnels with Whatman No. 1 filter paper for 15 minutes and the filtrates (extracts) were collected in 50 ml beakers. The resulting extracts were used to determine radical scavenging activity and total phenolic content of the dried kudzu blossoms, using the methods previously listed for roots.

Jelly Preparation

The kudzu blossom jelly treatments prepared for this study were based on the formula/recipe cited by Hoots and Baldwin (1996). The length of time the kudzu blossoms (flowers) were allowed to steep in the water was slightly different than the original recipe (Table 5). The ingredient list for the original recipe consisted of 4 cups kudzu blossoms, 4 cups boiling water, 1 tablespoon lemon juice, 1 package pectin, and 5 cups of sugar. Hoots and Baldwin's (1996) recipe allowed the blossoms to steep in the

water in the refrigerator for 6 hours or overnight. Following this step, the liquid was strained and used in the same basic manner as the blossom liquid was used in the present study. The only difference was the amount of time the jelly was processed in a boiling water bath canner. Hoots and Baldwin (1996) recommended processing for 5 minutes, and the present study processed the jelly for 10 minutes. The ingredients and amounts used for the preparation of the kudzu blossom jelly treatments are listed in Table 6 (Hoots & Baldwin, 1996).

Table 6. Ingredients, brand name, and amounts of ingredients used in preparation of kudzu blossom jelly

Ingredient	Brand	Amount
Granulated sugar	Kroger®, Cincinnati, OH	1165 g
Kudzu flower liquid (water, kudzu flowers)		925 ml
Fruit pectin, powdered (dextrose, citric acid, fruit pectin)	Sure Jell®, Kraft Foods Global, Inc., Northfield, IL	49 g
Lemon juice from concentrate (water, concentrated lemon juice, sodium benzoate, odium metabisulfate, sodium sulfite, lemon oil)	ReaLemon®, Mott's Inc., Stamford, CT	15 ml

Source: Hoots & Baldwin, 1996

On October 21, 2009, the kudzu blossom jelly treatments were prepared. The procedure began with the sterilization of the jars (Ball Mason Jars®, 8 ounces, Jarden Home Brands, Daleville, IN) to be used for safe storage of the jelly. The jars were submerged in water in a 4-quart enamel household boiling water bath canner, brought to

a boil (100°C), and boiled for 15 minutes. The jars remained in the hot water until they were filled with jelly. All of the ingredients used for preparation of the jelly were measured. The lids and rings for the jars were submerged in hot water (65°C). The thawed kudzu blossom liquid was measured and poured into a 4-quart stainless steel stockpot (All-Clad® Metalcrafters LLC, Canonsburg, PA). The lemon juice and pectin were measured and stirred into the kudzu blossom liquid with a wooden spoon. This mixture was heated to a rolling boil (96°C) while stirring constantly. The sugar was slowly stirred into the hot mixture. The mixture was heated to a second rolling boil (100°C). The mixture was boiled for 3 minutes, while being stirred constantly. The stock pot was removed from the heat and foam was removed from the surface of the jelly with a stainless steel spoon. The jars were removed from the enamel canner and placed on clean towels. The hot jelly was poured into the sterilized jars, leaving .64 cm (1/4 inch) headspace. The jar rims and threads were wiped with a moist paper towel that had been dipped in very hot water (96°C). The lids and rings were placed on the jars and tightened. The jars were submerged in the enamel canner. The water was returned to a boil (100°C) and the jars were processed for 10 minutes. The jars were removed from the canner and placed on a wire rack to cool. After 1 hour the rings were tightened and the jars labeled. After 8 hours the jars were stored at ambient temperature (20°C) in a dark place until the consumer sensory panels were conducted.

The pH values of the kudzu blossom extracts and the final jelly products (after sugar, lemon juice, and pectin were added and cooked) were determined and documented (in triplicate). The recommended pH range of the final product is 2.8-3.2 (Rushing,

1995). This pH range is recommended for adequate pectin set and for preservation and safety of the jelly product (Rushing, 1995). The pH was measured in triplicate with a pH meter (Accumet Research AR25 Dual Channel, Fisher Scientific, Pitsburg, PA) by placing the probe into 60 ml ambient temperature samples for approximately 1 minute.

Soluble solids content (Brix) of the kudzu blossom extracts and final jelly products were measured in triplicate by placing approximately 10 ml samples on the surface of the hand refractometer lens. The value was read and recorded. The refractometer used was determined by the percent soluble solids (Brix) present in the sample. Two refractometers were available. One refractometer measured 0 to 30 degrees Brix (T/C Hand Refractometer, American Optical Corp., # 10430, Keene, NH) and the other measured 35 to 80 degrees Brix (Hand Refractometer, American Optical Corp., # 10422, Keene, NH).

The other jelly treatments that were evaluated were Pontotoc Ridge Kudzu Bloom Jelly® (Pontotoc, MS), purchased from the Mississippi Gift Company, Greenwood, Mississippi, and Bully's Scuppernong Jelly® (Mississippi State University, MS), purchased from Mississippi State University's MAFES Sales Store, on the campus of Mississippi State University.

Proximate analysis of the two kudzu blossom jelly products used for consumer acceptability sensory testing was determined and reported using the services of the Mississippi State Chemical Laboratory at Mississippi State University. All percentages were determined in triplicate. The moisture percentage was measured for each formulation using a drying oven (AOAC. 1995. Method 39.1.02). The protein procedure

was AOAC 990.03, which determines nitrogen by combustion analysis (Model FP-528, LECO Corp., St. Joseph, MI). The nitrogen amount determined was multiplied by 6.25 to determine percentage protein. The fat content or ether extract content (%) was determined using a fat extractor (AOAC. 2000. Method 39.1.05, Model 1043, Soxtec HT Extraction Unit, Tecator, Hoganas, Sweden). The ash procedure was AOAC 942.05. Ash was determined using a muffle furnace at 600°C for 3 hours (Model Isotemp, Fisher Programmable Muffle Furnace, Fisher, Pittsburg, PA). The carbohydrate percentage was calculated by subtracting percentage moisture, protein, fat, and ash from 100% (Food and Agriculture Organization, 2003).

Determination of Radical Scavenging Activity

The radical scavenging activity of the kudzu blossom extracts was evaluated by the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging method (Blois, 1958) using the stable radical DPPH. The procedure was identical to the procedure discussed in the leaves section (Blois 1958; Huang et al., 2009). The same calculation discussed in the roots section was used to determine percent antioxidant activity of the blossoms (Blois, 1958; Lee et al., 2003).

Determination of Total Phenolic Content

Total phenols were quantified in the Mississippi kudzu blossoms (flowers) by the Folin-Ciocalteu spectrophotometric method using gallic acid as the reference standard. This procedure was similar to the procedure discussed in the roots section. Three dilutions were used (0.1, 0.5, and 1.0 g) instead of four (5.0 g dilution was not done).

One ml of each methanolic dilution of the kudzu blossom extracts was added to 2.0 ml of the Folin-Ciocalteu reagent (samples were done in triplicate). This mixture was allowed to stand for 5 minutes before 2.0 ml of sodium carbonate (Na_2CO_3) solution was added. The mixture was mixed and kept at ambient temperature (~23°C) for 1 hour. The absorbance values of the samples were measured at 760 nm against a reagent blank (Gutfinger, 1981; Lee et al., 2003; Singleton & Rossi, Jr., 1965; Waterhouse, 2002).

Consumer Acceptability

Consumer acceptability of one prepared kudzu blossom jelly treatment, a purchased Kudzu Bloom Jelly® product, and a purchased Bully's Scuppernong Jelly® product was evaluated by a panel of approximately 150 consumers. Three sensory panels (n = 50-55) were conducted. Each panel was identical in sample preparation and size. Each panel used the same sensory lab (Garrison Sensory Laboratory, Mississippi State University) and maintained the same conditions. The panels were conducted from 10:00 a.m. to approximately 12:00 p.m. Panel members were recruited from the faculty, staff, and students of Mississippi State University. Each sample weighed approximately 5.0 to 6.0 grams and was served in a 2-ounce clear soufflé cup with a clear lid (Dart Container Corp®., Mason, Michigan). Panelists were provided with water, unsalted tops crackers (Kroger®, Cincinnati OH), an expectorant cup, a napkin, a spoon, a pencil, and a score sheet (Appendix A). Panelists were asked to evaluate each jelly sample independently from other samples.

Each panelist was presented with three samples labeled with one of three 3-digit random numbers. Panelists were asked to evaluate each jelly sample based on the acceptability of appearance, aroma, flavor, texture, and overall acceptability, using a 9-point hedonic scale. The scale consisted of the following ratings: 1-dislike extremely, 2-dislike very much, 3-dislike moderately, 4-dislike slightly, 5-neither like nor dislike, 6-like slightly, 7-like moderately, 8-like very much, and 9-like extremely (Meilgaard et al., 2007).

Prior to the consumer panels, two of the three prepared kudzu blossom jelly treatments (1 and 2) were evaluated by 3 members of the research team. These evaluators selected treatment 2 to be used in the consumer sensory panels. The decision was made based on the evaluators' perception of the overall acceptability of the product and the similarity to the purchased Kudzu Bloom Jelly® product. Demographic information and other general questions regarding jelly consumption were included on the score sheet for the consumer panels (Appendix A).

Statistical Analysis

A randomized complete block design with three replications (n=50-55) was used to differentiate between the consumer acceptability ($p<0.05$) of jelly treatments (purchased kudzu, prepared kudzu, and scuppernong) regarding appearance, aroma, flavor, texture, and overall acceptability. When significant differences occurred among treatments, the Least Significant Difference (LSD) test was used to separate treatment means. Consumers were clustered together based on their liking for jelly by

agglomerative hierarchical clustering. Dendrogram and dissimilarity plots were used to determine how many clusters could be used to group consumers. Randomized complete block designs were utilized to differentiate ($p<0.05$) among jelly treatments within each cluster. When significant differences occurred between treatments within a cluster, the LSD test was performed to separate the treatment means (Meilgaard et al., 2007; Schilling & Coggins, 2007).

Institutional Review Board Approval

Approval from the Mississippi State University Institutional Review Board (IRB Study #08-197) for Research with Human Subjects was obtained prior to consumer testing (Appendix B). All subjects that participated in preliminary and consumer sensory testing of the dip and jelly products provided written, informed consent prior to evaluating all products (Appendix B). To comply with IRB regulations, a list of ingredients for all products tested was provided to participants of the preliminary and consumer panels before sensory testing was conducted to alert participants of potential allergens.

CHAPTER IV

RESULTS AND DISCUSSSION

The results and discussion will be presented by plant part (roots, leaves, and flowers) as in the previous chapter. Kudzu roots, starch, leaves, and blossoms were analyzed for radical scavenging activity and total phenolic content. Moisture content, water activity, leaf size, pH, and proximate analysis of food products incorporating kudzu were also determined. Jelly and dip products were developed and tested for consumer acceptability.

Roots

Prior to determining radical scavenging activity and total phenolic content, the roots were dried and moisture content was determined. The weight measurement of the Mississippi kudzu root sample after air-drying and prior to drying in muffle furnace was 906 g. The weight of the sample after drying and grinding was 698 g, indicating 23% yield. Geng et al., (2007) reported a moisture content of 12.4% in kudzu starch harvested from plants that were cultivated in southwest China and processed by wet extraction into a food grade starch as determined by the Starch Industry Association of China. The Mississippi kudzu roots were not processed into a food grade refined starch, therefore, higher moisture content would be expected.

Li, Yan, and Zou (1998) determined the water content of fresh kudzu roots of five wild germplasms grown in China and reported a range of 57.0 to 66.6%. The differences in moisture reported by Li et al. (1998) and the present study may be due to different drying procedures. They did not air dry their samples prior to heating and used a higher temperature for a shorter time compared to the present study, which may account for differences in moisture content between root samples. Additionally, their samples were harvested from mountainous areas of Hunan and Hubei provinces in China. The season of the year and climate/temperature conditions, including chilling hours, were not reported. Achremowicz et al., (1994) determined the best time for harvesting kudzu roots of various ages to maximize extractable starch. They harvested roots collected from the wild in northwestern Alabama during the months of September, October, and December. They determined that the best time to harvest the kudzu roots in northern Alabama in order to maximize the starch recoverable by extraction is December (Acremowicz et al., 1994). All of these factors may impact water and starch content of the roots. The Mississippi kudzu roots were harvested in February after several months of cold temperatures to allow for starch accumulation in the roots (tubers). Based on the work of Acremowicz et al. (1994), it is estimated that the starch content would continue to increase in the tubers as cold temperatures continue throughout the winter months.

Radical Scavenging Activity

The antioxidant activity of the Mississippi kudzu roots was 75.7% and the purchased kudzu starch was 59.2% DPPH radical scavenging activity (Table 7). The

Mississippi kudzu roots were expected to have more antioxidant activity than the processed kudzu starch which was a refined starch product. Shurtleff and Aoyagi (1985) discussed the method used for kudzu starch extraction used by the kudzu starch producers in Japan. The starch is extracted from the roots by cold water washing. The wet cellulose is separated from the white starch by grinding, followed by washing 3 times and allowing gravity to settle the starch. The starch is recovered and the fiber residue is separated by filtration through a cotton cloth. The starch is then dried in an oven. The Mississippi kudzu root samples contained all parts of the root, including the fiber. The amount of rinsing and filtering involved in the processing of kudzu starch would affect the amount of potentially beneficial compounds that remain in the starch.

Jun et al. (2003) reported 32.0% DPPH activity in the puerarin compound and 33.0% activity in the diadzin compound, which were isolated from dried kudzu root that had been ground to a powder. Liu et al. (2008) reported 6.1% DPPH radical scavenging activity of kudzu root, however; their root samples were purchased in a Beijing drug retail outlet in Xi'an, China, and the source of the root samples is not reported. The samples were freeze dried after grinding and stored at -20°C until analyzed but storage time is not reported (Liu, Qiu, Ding, & Yao, 2008). Overall, differences in sample preparations and the varying amounts of starch in the root samples make it difficult to make accurate comparisons in antioxidant activity of kudzu roots.

Table 7. Antioxidant activity of Mississippi kudzu root, purchased kudzu starch, kudzu leaves, purchased spinach, and kudzu blossoms (flowers)

Sample[1]	Absorbance[2]	Antioxidant Activity (%)
Mississippi kudzu root[3]	.038	75.7
Purchased kudzu starch[3]	.064	59.2
Mississippi kudzu leaves[4]	.063	56.5
Purchased spinach leaves[4]	.079	45.5
Mississippi kudzu blossoms[4]	.032	77.9
DPPH solution (control)	.145	

[1]Kudzu root, purchased starch, and kudzu leaves were repeated in duplicate; spinach leaves and kudzu blossoms were repeated in triplicate.
[2]Mean optical absorbance at 517 nm
[3]Dry weight basis
[4]Not dry weight

Total Phenolic Content

The phenolic compound content was determined as GAE using the linear equation based on the gallic acid standard curve determined in the study. The r^2 value of the gallic acid standard curve was 0.98. The regression equation used to determine the concentration values based on the gallic acid standard curve was: y = .00069 x (x = absorption of sample) + .010 x 10 (dilution factor).

Total phenolic content values of the Mississippi kudzu roots and the purchased kudzu starch are listed in Table 8. The total phenolics in Mississippi kudzu roots and purchased kudzu starch are 3.98 and 0.73 mg GAE/g, respectively (Table 8). Liu et al., (2008) determined the phenol content of 68 Chinese herbals used for medicine and food on the market in China. They determined the total phenolic content of kudzu root samples as 13.7 mg GAE/g dry weight. The difference may be due to the differences in

sample preparation, extraction methods, and moisture content. Liu et al. (2008) used freeze-dried plant materials and extracted samples for 24 hours. Although kudzu was not used in the study conducted in Thailand, Mongkilsilp, Pongbupakit, Sae-Lee, & Sitthithowon (2004) reported total phenol contents of 0.02 to 9.74 mg GAE/100 mg samples for five medicinal plants used in primary health care in Thailand.

Table 8. Total phenolic content of Mississippi kudzu root, purchased kudzu starch, kudzu leaves, purchased spinach, and kudzu blossoms (flowers)

Sample [1]	Absorbance[2]	Total Phenolics (mg GAE[3]/g)
Mississippi kudzu root[4]	.143	3.98
Purchased kudzu starch[4]	.031	0.73
Mississippi kudzu leaves[5]	.090	1.22
Purchased spinach leaves[5]	.015	0.13
Mississippi kudzu blossoms[5]	.091	1.24

[1]Each sample was repeated in triplicate. Samples were not in dry weight matter.
[2]Mean optical absorbance at 760 nm
[3]GAE = Gallic acid equivalent
[4]Dry weight basis
[5]Not dry weight

Leaves

The mean leaf width and length, pre-and post-drying weights, percent moisture content, and fresh and dry water activity values for the leaves are listed in Table 9. The leaf measurements of the kudzu leaves ranged from 10.0 to 13.2 cm in width and 12.5 to 14.0 cm in length. The spinach leaves were much smaller and averaged 3.8 cm in width and 5.7 cm in length. The pre-and post-drying measurements represented batches of leaves. The percent moisture content for the kudzu leaf batches ranged from 61 to 72% and was 88% for the spinach leaves. The percent moisture content was expected to be

higher in the spinach leaves due to the increased length of time needed to dry the leaves to a crisp stage. Also, the kudzu leaves felt drier and less pliable than the spinach leaves. The water activity values for all the fresh leaf samples were similar (Table 9). The water activity values for all of the dried leaf samples were also similar (Table 9). These results may be useful for establishing basic information about fresh and dried kudzu and spinach leaves, especially as it relates to new product development.

Yousif, Durance, Scaman, and Girard (2000) determined physical characteristics of oregano (*Lippia berlandieri* Schauer) from several drying methods. The final moisture content and water activity amounts of the plant material were 13.2% and 0.55, respectively, for microwave vacuum drying, 13.2% and 0.53, respectively, for air-dried using a commercial dryer, and 9.0% and 0.54, respectively, for freeze-drying under vacuum. Yousif, Scaman, Durance, and Girard (1999) also determined the physical properties of vacuum-microwave- and air-dried sweet basil (*Ocimum basilicum* L.). They stated that the air-dried samples had a moisture content of 7.8% and a water activity of 0.34. Conventional hot-air drying is reported to cause heat damage and adversely affect texture, color, flavor, and nutritional value of products. Freeze drying can be applied to circumvent heat damage with better texture outcomes but is very costly. Vacuum microwave drying generates very rapid low temperature drying (Lin, Durance, & Scaman, 1998). For the present study, the results from the air-dried commercial dryer samples of oregano and basil were used for comparison. The kudzu leaf samples and the spinach leaf sample had water activity levels of 0.40 to 0.43 which were lower than the amounts reported for the air-dried oregano samples (Yousif et al., 2000) and higher than

the amounts reported for the basil samples (Yousif et al., 1999). Water activity values are important when developing food products in regards to food safety. Low water activity in a product means there is less water available for chemical, biological, or physical changes in the product. The spinach and kudzu leaf values were similar to the values cited in these studies.

Table 9. Mean leaf width and length, pre- and post-drying weights, percent moisture content, and water activity of fresh and dry kudzu and spinach leaves

Leaf type and location (county) or source	Leaf width (cm)	Leaf length (cm)	Pre-drying weight (g)	Post-drying weight (g)	Moisture content (%)	Water activity: fresh	Water activity: dry
Kudzu-Montgomery	10.0	12.5	219.0	77.0	65	0.93 @ 24° C	0.40 @ 21° C
Kudzu-Oktibbeha	11.2	14.0	210.0	59.0	72	0.93 @ 25° C	0.43 @ 22° C
Kudzu-Webster	13.2	13.2	228.0	88.0	61	0.94 @ 25° C	0.40 @ 23° C
Spinach-purchased	3.8	5.7	346.0	41.0	88	0.94 @25° C	0.40 @ 22° C

Radical Scavenging Activity

The radical scavenging activity values of the kudzu and spinach leaves are listed in Table 7. The mean antioxidant activity percentage of the kudzu leaves was 56.5 % and spinach leaves was 45.5% (Table 7). Liu et al (2007) evaluated radical scavenging activity of lettuce grown in Colorado. Five red leaf lettuce cultivars ranged from 81.2 to 82.6% DPPH, and seven green leaf lettuce cultivars ranged from 74.4 to 81.4% DPPH. Huang et al. (2009) determined that collard, mustard greens, and kale had 40 to 50% DPPH remaining, whereas sweet potato greens had the lowest percent DPPH remaining

(highest antioxidant activity) of the samples evaluated at 10% remaining. Truong, McFeeters, Thompson, Dean, and Shofran (2007) evaluated the DPPH radical scavenging activity of sweet potato cultivars grown in the United States as 37.6 to 38.6%. Liu et al. (2008) reported the % DPPH scavenging capacity values for several leaves which included dandelion (*Taraxacum officinale*) (69.2%), mulberry (*Morus alba*) (35.4%), and bamboo (*Bambusa vulgaris*) (6.0%).

Total Phenolic Content

Total phenolic content values for the kudzu and spinach leaves are 1.22 mg GAE/g and 0.13 mg GAE/g, respectively (Table 8). Huang et al., (2009) evaluated the total phenolic content of indigenous vegetables in the southeast United States, including collard greens (*Brassica oleracea* var. *viridis*), mustard greens (*Brassica juncea*), and kale (*Brassica oleracea* var. *acephala*). They reported the total phenolic content of collard greens as 23.9, mustard greens as 26.0, and kale as 27.3 mg GAE/g dried sample. The highest total phenolic content of all the vegetables they evaluated was present in sweet potato greens (*Ipomea batatas*) at 53.5 mg GAE/g dried sample (Huang et al., 2009). Liu et al (2007) evaluated total phenolics of lettuce grown in Colorado. Romaine lettuce cultivars ranged from 18.4 to 56.4 mg GAE/g. In addition, they noted that lettuce grown in July possessed higher amounts of total phenolics than did lettuce grown in September. The spinach in the present research was obtained at the local grocery store and storage time, light exposure, and other factors such as season of harvest are unknown; however, the numbers are unexpectedly low.

Zhou and Yu (2006) determined the total phenolic content of commonly consumed vegetables grown in Colorado. They reported that kale, spinach, broccoli, and rhubarb are better dietary sources of phenolic compounds. Howard, Pandjaitan, Morelock, and Gil (2002) determined that spinach plants grown in different seasons can have differing amounts of total phenolic content. They reported that spinach grown over spring planting dates, which incur higher temperatures and greater light intensity, develop higher phenolic and antioxidant content than spinach grown over fall. They suggested that plant breeders can select for increased phenolic content to increase antioxidant capacity in spinach cultivars, or the crops can be grown in different seasons or under certain stress conditions to elevate levels of antioxidants.

Few articles discuss kudzu foliage and its potential uses. Lau et al. (2005) used high-performance liquid chromatography and mass spectrometry analysis to identify and quantify the flavonoids isolated from kudzu foliage that are responsible for reported antioxidant potential. They discovered that robinin accounted for 0.65% (dry mass) of kudzu biomass, and that due to its antioxidant properties, this flavonoid may be worth extracting prior to energy production using kudzu biomass (Lau et al, 2005). Terrill et al., (2003) investigated the effect of cutting date and frequency on yield and quality of kudzu grown in central Georgia as possible low-impact forage for livestock. A field experiment was undertaken to determine the effects of cutting date and frequency on yield using a 20-year-old stand with no fertilizer or lime applications. The cutting treatments included an uncut control, plots cut once (September), twice (July and September), and three times (June, July, and September). They determined that total

herbage and leaf dry matter production was highest for the three-harvest system and lowest in the control, but these results were reversed in the harvest made in the previous year.

Several factors can affect the study of plants and preparation of plant extracts. Any type stress can cause the metabolic state of the plant to change. This can be a problem before and after harvesting a plant part for analysis. As cells die, the cellular integrity of the plant is lost and as a result the enzymes come in contact with substrates it is not normally exposed to in the living state. This increases the oxidation process, which is a problem with phenolic compounds due to their tendency to oxidize. Leaf age and stage of development affect levels and nature of phenolics. It is important to define the stage of maturity of plant and leaf as close as possible before collecting leaf material for analysis (Food and Agriculture Organization/International Atomic Energy Agency, 2000). In this research the kudzu leaves were most likely older than the baby spinach leaves. This may have had an effect on the total phenolic content determinations.

Food Product Development

Four dip treatments were developed and evaluated using dried kudzu and dried spinach leaves. The dried leaves were measured to determine mesh or sieve size. The mesh size measurements of the kudzu and spinach leaves used in the dip products, and the mesh size measurements of the ground leaves used for analysis are listed in Table 10. The kudzu leaves from the three harvest locations were combined to make one leaf batch.

For the dip products, the mesh size contributing the largest percentage (72%) by weight of the kudzu leaves was 1.70 mm, compared to 41% of spinach leaves with that mesh size. The next highest mesh size percentage (9.3%) of kudzu leaves was .063 mm (the smallest). The remainder of the kudzu leaves used in the dip products were fairly equally distributed among the remaining sieve sizes. For the spinach leaves used in the dip products, approximately 7 to 15 percent were distributed in the remaining sieves. The spinach leaves used in the dips were more evenly distributed among the different sieves, indicating more uniform leaf size in the food product. The potential influence of differing mesh sizes could affect consumer acceptability scores, especially texture scores.

The ground leaves used for preparation of the extracts for analysis were more evenly distributed among the sieves. Twenty percent of the ground kudzu leaves were 1.70 mm, followed by 1.0 mm (23 %), 1.40 mm (16 %), and .063 mm (15 %). The ground spinach leaves were distributed beginning with the highest percentage (31 %) at .063 mm size, 28% were .710 mm size, followed by 1.0 mm size (15%). The mesh size of the ground leaves could influence the efficiency of extraction for analysis due to the larger surface area of the smaller particle size pieces.

Table 10. Mesh size measurements of dried kudzu and spinach leaves used in the dip products and ground for analysis

Sieve size (mm)	Kudzu - dip (g)	Spinach - dip (g)	Kudzu - ground (g)	Spinach - ground (g)
1.70	1.47	.91	.42	.16
1.40	.13	.30	.34	.15
1.00	.09	.34	.50	.32
0.710	.05	.32	.39	.59
0.600	.11	.17	.18	.22
0.063	.19	.16	.32	.65

Consumer Acceptability

There were no differences ($p > 0.05$) in overall acceptability between the dips made with leaves harvested from different locations (counties). Based on these results, the leaves from the three locations were combined into one batch of leaves. This batch of leaves was used to prepare the dip treatments for the consumer sensory panels.

Differences existed ($p<0.05$) among the dried-vegetable leaf dip treatments for flavor and overall acceptability (Table 11). On average, consumers preferred ($p<0.05$) the flavor and overall acceptability of the regular spinach dip product over the other dips. The flavor and overall acceptability of the regular kudzu dip product was preferred ($p<0.05$) over the healthier spinach and kudzu dips. Both the regular dip products were liked moderately by consumers. The hedonic responses of the consumers to the healthier versions of the spinach and kudzu dips were "like slightly" for both flavor and overall acceptability. There was no preference ($p>0.05$) of one healthier dip treatment over the other. The healthier versions for both leaf types were the least liked by the consumers. Consumers found no differences ($p>0.05$) in the acceptability of the four dip treatments

for appearance, aroma, and texture. Each of these attributes were scored as “like moderately” by consumers.

Agglomerative hierarchical clustering was performed to further determine attitudes of the consumers toward the dried-leaf dip products. A dissimilarity plot and a dendrogram were used to determine how many clusters should be used, and the cluster structure of the data. It was determined that panelists could be partitioned into five clusters, or groups, based on preference and acceptability of dried-leaf vegetable dips (Table 12).

Forty percent (cluster 1) of panelists rated the dips at like very much and did not differ ($p>0.05$) in their liking of dips. Cluster 2 (26% of the panelists) ranged from "like slightly" to "like moderately" in their preference and were partial to the regular spinach dip, followed by the regular kudzu dip. This segment of panelists liked the healthier version of both leaf dip treatment between slightly and moderately. The consumers in cluster 3 (15% of consumers) were similar in their ranking of preference of the dips. They preferred ($p<0.05$) the regular spinach, followed by the regular kudzu dip, but with lower hedonic ratings of “like slightly” and “dislike slightly”, respectively. The healthier versions of both leaf types were similarly least liked, with neither like nor dislike hedonic scores. The panelists in cluster 4 (10% of consumers) did not differ ($p>0.05$) in their liking of the dip treatments and scored all treatments between "dislike slightly" and "neither like nor dislike". Cluster 5 (9% of consumers) preferred ($p<0.05$) the regular kudzu leaf dip over all other treatments but the healthier spinach treatment. The regular spinach dip was the least preferred ($p<0.05$) of the dips for this group of consumers and

was rated as neither like not dislike. Overall, 41% of the panelists preferred ($p<0.05$) the regular spinach dip over the other treatments and 50% of panelists liked all vegetable dip treatments. These results are encouraging considering the purported decrease in sensory quality of dried products when conventional techniques are used (Nijhuis et al., 1998; Perera, 2005). These results could inspire further investigation of the uses of dried kudzu leaves and other leaves native to the south such as sweet potato leaves.

Table 11. Mean scores for consumer acceptability (n =162) of dried-leaf vegetable dip treatments[1]

Dip Treatment	Appearance	Aroma	Flavor	Texture	Overall Acceptability
Spinach-Regular	7.5[a]	7.2[a]	7.2[a]	7.3[a]	7.3[a]
Spinach-Healthier	7.4[a]	7.0[a]	6.6[c]	7.7[a]	6.7[c]
Kudzu-Regular	7.4[a]	7.3[a]	7.0[b]	6.7[a]	7.0[b]
Kudzu-Healthier	7.3[a]	7.0[a]	6.6[c]	7.5[a]	6.7[c]

[a-c]Mean scores within a column with different letters are significantly different ($p<0.05$)
[1]Scores were based on a 9-point Hedonic scale (1 = dislike extremely, 5 = neither like not dislike, 9 = like extremely)

Table 12. Mean scores for overall consumer acceptability of dried-leaf vegetable dip treatments according to different clusters of consumer segments using a hedonic scale[1]

Cluster	Consumers		Dip Treatments			
	n	%	Spinach-Regular	Spinach-Healthier	Kudzu-Regular	Kudzu-Healthier
1	64	40	8.2[a]	8.1[a]	8.1[a]	8.1[a]
2	42	26	7.8[a]	6.5[c]	7.2[b]	6.5[c]
3	25	15	6.9[a]	5.1[c]	5.8[b]	4.6[c]
4	16	10	4.4[a]	4.7[a]	3.8[a]	5.0[a]
5	15	9	4.9[c]	6.3[ab]	6.9[a]	6.1[b]

[a-c]Mean scores within a row with different letters are significantly different ($p<0.05$)
[1]Scores were based on a 9-point Hedonic scale (1 = dislike extremely, 5 = neither like not dislike, 9 = like extremely)

Information gathered from the questions included on the score sheet (Appendix A) for the vegetable dip products provided interesting demographic information. Of the five age groups, 33% of the panelists were 18 – 24 years of age and the remaining age group categories ranged from 16 to 19% of the total panelists. Sixty-two percent of the panelists were female and 38 % were male. The majority (92%) stated they liked dip products.

Nutritional Analysis

Proximate analysis was determined for the four dried-leaf vegetable dip treatments by the Mississippi State Chemical Laboratory and the results are reported in Table 13. The moisture percentage was 63.5% and 63.4% in the healthier spinach and kudzu dips, respectively. These percentages were higher than the moisture percentages of 47.1 for regular spinach and 47.6 for regular kudzu. These percentages may have been

higher in the healthier versions possibly due to the higher moisture content of the light sour cream and mayonnaise used to prepare these dips.

The protein content was 4.3% and 4.1% in the healthier spinach and kudzu dip treatments, respectively. This was more than the 2.8% for the regular spinach dip and 2.6% for the regular kudzu dip treatments. The healthier dip treatments had higher protein percentages possibly due to the replacement of some of the whole milk/cream with lower fat milk in the sour cream used in these dip treatments. This would increase the percentage of protein in these products. Fat content was 22.3% and 21.0% for the healthier spinach and kudzu dips, respectively. These percentages were lower, as expected, than the percentages for the regular dip treatments of 42.1% for spinach and 40.9 % for kudzu. The healthier dip versions used lower fat sour cream and mayonnaise.

Crude fiber percentages were similar for all four dip treatments at 0.9 to 1.3%. The kudzu leaf dips appeared to have slightly higher carbohydrate content than their respective spinach dips. This may be due to the high water content of spinach leaves. The ash percentages, indicating the mineral portion of the products, were 2.3% and 2.5% for the regular dip treatments and 1.6% and 1.7% for the healthier versions. The slightly higher percentages may be due to the higher amounts of dairy and eggs in the regular sour cream and mayonnaise.

The carbohydrate percentages for the healthier treatments of spinach and kudzu dips were greater than the regular treatments with values of 8.2% and 9.9%, respectively. The regular dips contained 5.0% and 7.1% carbohydrate. The percent fat content was greater in the regular dip treatments whereas the carbohydrate percentages were greater in

the healthier treatments. This is possibly due to the replacement of some of the fat in the lighter versions of sour cream and mayonnaise with lower calorie carbohydrate-containing ingredients such as modified corn starch. The protein percentages were also greater in the healthier formulations. This is possibly due to the use of lower fat milk in the sour cream, which has a higher protein percentage. A 30g sample (approximately 2 Tbsp or 1 oz) of the regular kudzu or spinach dip would provide 123 kcals and the same size serving of the healthier dips would provide 75 kcals.

Bodner and Hymowitz (2002) reported the amount of carbohydrate in fresh kudzu leaves as 10.2%. The carbohydrate results of the regular kudzu dip was 7.1% and the healthier version contained 9.9% (Table 14). Duke (1983) performed a nutrient analysis of kudzu leaves. One hundred grams of cooked kudzu leaves were reported to contain 0.4 g protein, 0.1 g fat, 9.7 g carbohydrate, and 7.7 g fiber. The total calories estimated were 41.3 kcals/100 g cooked leaves (Duke, 1983).

Table 13. Proximate analysis results of the regular and healthier treatments of kudzu and spinach leaf dip products

Proximate Analysis Determinations	Spinach: Regular Treatment (75 g)	Kudzu: Regular Treatment (75 g)	Spinach: Healthier Treatment (75 g)	Kudzu: Healthier Treatment (75 g)
Moisture (%)	47.6	47.1	63.5	63.4
Crude Protein (%)	2.8	2.6	4.3	4.1
Crude Fat (%)	42.1	40.9	22.3	21.0
Crude Fiber (%)	0.9	1.3	1.0	0.9
Ash (%)	2.5	2.3	1.7	1.6
Carbohydrates (%)	5.0	7.1	8.2	9.9
Energy (kcal)	410.1 Kcal/100g	406.9 Kcal/100g	250.7 Kcal/100g	245.0 Kcal/100g

Flowers

The weight of the kudzu blossoms used for analysis of antioxidant activity and total phenolics was 247 g prior to drying and 59 g after drying. This indicates 76% moisture content. The dried blossoms were ground prior to being analyzed. The largest percentage (48%) of the blossoms was 1.70 mm mesh size, the second (16.5%) and third (12.0) most frequent percentages were 1.40 mm, and 1.00 mm, respectively. The remainder of the sample was fairly equally retained in the .710 mm, .600 mm, and .063 mm sieves. The dried, ground blossoms were used to prepare the extracts used for the determination of antioxidant activity and total phenolics.

Radical Scavenging Activity

The radical scavenging activity (antioxidant activity) of the Mississippi kudzu blossoms is listed in Table 7. The kudzu blossoms had the highest percent antioxidant activity of all the plant samples analyzed at 77.9%. None of the studies discussed in the previous section determined radical scavenging activity using the same methods. Liu et al (2008) determined that pagoda tree flowers (*Styphnolobium japonicum*) contained 85.2% DPPH scavenging activity, and honeysuckle flowers (*Lonicera japonica*) contained 89.8%. The method discussed in Liu et al. (2008) was similar to the method used for this study.

In general, most phenolics and flavonoids possess some degree of antioxidant activity. Therefore, extracts with a higher phenolic content or flavonoid content would generally show higher antioxidant activity, and some correlations have been found among

these parameters (Li et al., 2007). Several articles review the methods used to evaluate antioxidant capacity and phenolics and discuss the need for standardized methods for evaluating foods and biological systems. Prior et al., (2005) and Waterhouse (2002) discussed the advantages and disadvantages of the Folin-Ciocalteu method. They found that many of the papers they evaluated varied in one or more of the conditions needed for reliable results including the proper ratio of alkali to Folin-Ciocalteu reagent, optimal reaction time and temperature for color development, monitoring of optical density at 765 nm, and use of gallic acid as the reference standard phenol. They also stated that lack of standardization of methods can lead to several orders of magnitude difference in detected phenols (Prior et al., 2005; Waterhouse, 2002).

The rationale and basis for developing standardized antioxidant capacity methods for the food, nutraceutical, and dietary supplement industries have been discussed by several authors (Prior et al., 2005). Cheng, Moore, and Yu (2006); Prior et al., (2005); Sanchez-Moreno (2002); and Sharma and Bhat (2009) all discussed the need for a standardized procedure for the DPPH antioxidant assay procedure. They discussed the requirements of a standard assay and the importance for comparing results of different laboratories and for validation of results. All have found it difficult to compare conclusions in the literature due to widely different protocols which differ in the concentration of DPPH, incubation time, reaction solvent, absorbance setting, and pH of the reaction mixture.

Total Phenolic Content

The total phenolic content of the Mississippi kudzu blossoms was 1.24 mg GAE/g of sample (Table 8). Kahkonen et al. (1999) determined the total phenolics content of numerous plant materials, including blue lupin (*Lupinus angustifolius*) with 4.7, purple loosestrife (*Lythrum salicaria*) with 42.1, and red clover (*Trifolium pratense*) with 7.8 mg/GAE/g dry weight. Kroyer (2003) also studied red clover as a source of isoflavones and other bioactive antioxidant compounds. He determined that a purchased red clover extract contained 152.5 mg/g GAE. Liu et al., (2008) reported total phenolic content of several flowers including pagoda tree flower, with 86.93, and honeysuckle flower containing 34.78 mg GAE/g dry weight. Velioglu, Mazza, Gao, and Oomah (1998) determined that echinacea flower (*Echinacea purpurea*) heads (a purple flower) contained total phenolics amounts of 5467 mg/100 g ferulic acid equivalents. Each of these articles used different methods of collecting plant materials, different extraction techniques and dilutions, and often different standards.

Food Product Development

The mean scores of pH and percent soluble solids (Brix) measurements of the kudzu blossom (flower) liquid used to make the jelly treatments are listed in Table 14. The data were collected to document information needed for the formulation of a jelly product, for the development of kudzu blossom jelly procedures, and because information of this type has not been found for this type of jelly. Data collected prior to the

preparation of the jelly were used to determine treatments that were prepared for this part of the study.

The mean values of the pH measurements of the blossom liquid treatments before additions were highest for treatment 2 (7.08), which allowed the blossoms to steep in water for the shortest amount of time, and lowest for treatment 3 (6.68), which allowed fresh blossoms to be steeped twice in the same liquid. The mean values for treatments 1 and 2 were 6.81 and 7.08, respectively, but the longer the flowers steeped, the lower the pH. The percent soluble solids (Brix) measurements prior to the additions of the other jelly ingredients were lowest for treatment 2 (0.50), which had the shortest steep time. Interestingly, the mean values for treatment 1 and treatment 3 were the same (1.00) (Table 14). Treatment 3 contained double the amount of blossoms in the same amount of liquid, but the blossoms in treatment 1 were allowed to steep for 8 hours versus 2 hours for treatment 3.

The mean values for the pH measurements of the treatments of the blossom liquid after the addition of the other jelly ingredients ranged from 2.96 to 3.30. Treatments 2 and 3 had pH levels in the recommended range of 2.8 to 3.2 (Rushing, 1995). The mean values for the percent soluble solids measurements of the treatments of the blossom liquid after the additions of the other jelly ingredients were all well above the recommendation of not less than 65% soluble solids content (Rushing, 1995). All of the treatments had a degrees Brix (percent soluble solids) value of ≥ 70.0. The treatment that allowed the blossoms to steep the longest amount of time (1) had the highest degrees Brix value, and the treatment with the shortest steep time had the lowest. Interestingly, the

treatment that was steeped in fresh blossoms twice (treatment 3) had degrees Brix measurement the same as treatment 1 (Table 14). The results described in Table 14 are important for future development of this recipe/formula. Baseline information can be used in the development of an expanded formula for larger scale manufacturing of this jelly. This information would be useful for formula development so that the optimal proportions of soluble solids, pectin type and concentration, and pH can be determined.

The mean pH and degrees Brix values were determined for the purchased scuppernong and kudzu bloom jelly treatments (Table 15). These products were tested as purchased. The mean values for the pH measurements were 2.76 for the scuppernong and 2.70 for the purchased kudzu bloom jelly. These values were slightly below the recommended pH range of 2.8 to 3.2 (Rushing, 1995). The degrees Brix values (percent soluble solids) were 69.3 for the scuppernong and 59.0 for the purchased kudzu bloom jelly. The kudzu bloom jelly had a percent soluble solids percentage above the recommended 65%, and the percent for the scuppernong jelly treatment was below the recommendation. The pH and soluble solids readings can affect the pectin set of the jelly product, protect against spoilage and maintain the safety of the product, and allow the product to be able to be properly labeled according to the standards of identity (Food and Drug Administration, 2008; Rushing, 1995).

The prepared kudzu jelly treatment chosen to be used for consumer sensory testing was treatment 2. This prepared kudzu jelly treatment was used for all of the consumer sensory panels. It was referred to as "kudzu jelly-prepared" or "prepared kudzu jelly".

Table 14. Mean scores of pH and degrees Brix (percent soluble solids) measurements of Mississippi kudzu blossom liquid before and after additions of jelly ingredients

Treatment[a]	pH before additions[bc]	Degrees Brix before additions[bc]	pH after additions[bc]	Degrees Brix after additions[bc]
1	6.81 ± 0.05	1.0 ± 0.00	3.30 ± 0.43	74.0 ± 0.58
2	7.08 ± 0.03	0.5 ± 0.00	2.96 ± 0.01	70.0 ± 0.00
3	6.68 ± 0.57	1.0 ± 0.00	3.11 ± 0.01	72.0 ± 0.58

[a]Each treatment included 3 replications
[b]Mean score ± Standard Deviation
[c]Additions included sugar, powdered fruit pectin, and lemon juice.

Table 15. Mean scores of pH and degrees Brix (percent soluble solids) measurements of purchased scuppernong and kudzu bloom jellies

Treatment[a]	pH[b]	Degrees Brix[b]
Scuppernong	2.76 ± 0.04	69.3 ± 1.15
Kudzu Bloom	2.70 ± 0.02	59.0 ± 0.58

[a]Each treatment included 3 replications
[b]Mean score ± Standard Deviation

Consumer Acceptability

On average, consumers preferred ($p<0.05$) the appearance of the purchased kudzu jelly over the prepared kudzu and scuppernong jelly. In addition, consumers preferred ($p<0.05$) the appearance of the prepared kudzu jelly over the scuppernong jelly. Consumers preferred ($p<0.05$) the aroma and flavor of the purchased kudzu jelly over the scuppernong jelly, but no other differences ($p>0.05$) existed among treatments. On average, consumers preferred ($p<0.05$) the texture of the prepared kudzu jelly over the

scuppernong jelly, but no other differences existed ($p>0.05$) among treatments. The purchased kudzu jelly was preferred ($p<0.05$) for overall acceptability (like moderately) compared to the prepared kudzu and scuppernong jelly, which were rated between like slightly and like moderately (Table 16).

Agglomerative hierarchical clustering was performed to further determine the attitudes of the consumers toward the jelly products. A dissimilarity plot and dendrogram were used to determine how many clusters should be used and the cluster structure of the data. It was determined that panelists could be partitioned into seven clusters, or groups, based on preference and acceptability of jelly products (Table 17). Twenty-seven percent of consumers in cluster 1 preferred ($p<0.05$) the purchased and prepared kudzu jelly treatments over the scuppernong jelly with hedonic scores for the kudzu jellies as "like moderately" and for the scuppernong as "like slightly". Twenty-six percent of consumers in cluster 2 scored all of the samples high (7.9 to 8.3), and they very much liked and preferred ($p<0.05$) the purchased kudzu jelly and moderately liked the prepared kudzu jelly. The consumers in clusters 3 (15% of the total) preferred ($p<0.05$) the moderately liked scuppernong and prepared kudzu jelly over the slightly liked purchased kudzu jelly. Cluster 4 (12 % of consumers) liked moderately and preferred ($p<0.05$) the scuppernong and purchased kudzu jelly samples over the disliked slightly prepared kudzu jelly. Consumers in cluster 5 (11% of the total) liked slightly and preferred ($p<0.05$) the two kudzu jellies over the scuppernong jelly, which was disliked slightly. Cluster 6 consumers (6% of the total) preferred ($p<0.05$) the prepared kudzu and scuppernong jellies, and disliked moderately the purchased kudzu jelly. The four percent of

consumers in cluster 7 did not differ ($p>0.05$) in their preference of the jelly products with their "neither like nor dislike" to "dislike slightly" mean hedonic ratings. Overall, the acceptability of the jelly products across all clusters in Table 17 were generally reflected by the overall acceptability trends stated in Table 16.

Table 16. Mean scores for consumer acceptability (n =160) of three jelly treatments[1]

Jelly Treatment	Appearance	Aroma	Flavor	Texture	Overall Acceptability
Kudzu-purchased	7.5[a]	6.4[a]	7.2[a]	7.5[ab]	7.2[a]
Kudzu-prepared	6.8[b]	6.2[ab]	6.9[ab]	8.3[a]	6.8[b]
Scuppernong	6.3[c]	6.1[b]	6.8[b]	6.6[b]	6.7[b]

[a-c]Mean scores within a column with different letters are significantly different ($p<0.05$)
[1]Scores were based on a 9-point Hedonic scale (1 = dislike extremely, 5 = neither like not dislike, 9 = like extremely)

Table 17. Mean scores for overall consumer acceptability of jelly treatments according to different clusters of consumer segments using a hedonic scale[1]

Cluster	Consumers		Jelly Treatments		
	n	%	Scuppernong	Kudzu-purchased	Kudzu-prepared
1	43	27	6.2[b]	7.9[a]	7.7[a]
2	41	26	8.1[ab]	8.3[a]	7.9[b]
3	24	15	7.5[a]	6.3[b]	7.0[a]
4	19	12	7.5[a]	7.7[a]	3.9[b]
5	17	11	4.0[b]	6.6[a]	6.4[a]
6	9	6	5.8[a]	3.0[b]	6.9[a]
7	7	4	5.1[a]	4.4[a]	3.9[a]

[a-b]Mean scores within a row with different letters are significantly different ($p<0.05$)
[1]Scores were based on a 9-point Hedonic scale (1 = dislike extremely, 5 = neither like not dislike, 9 = like extremely)

Information gathered from the questions included on the score sheet (Appendix A) for the jelly products provided interesting demographic information. Of the five age groups represented, 46% of the panelists were 18 – 24 years of age, with the remaining age groups being represented with ranges of 13 to 15% of the total panelists. Sixty percent of the panelists for the jelly panels were female and 40 % were male. The majority (90%) stated they liked jelly products.

Nutritional Analysis

Proximate analysis was determined for the purchased and the prepared kudzu jelly treatments used in the consumer sensory testing by the Mississippi State Chemical Laboratory (Table 18). The purchased scuppernong jelly treatment used in the consumer testing was not analyzed because the product contained a Nutrition Facts label. The proximate analysis results indicated that the moisture percentage was 42.1% in the purchased kudzu jelly as compared to the 26.2% for the prepared kudzu jelly. This moisture percentage would appear to be accurate when the degrees Brix amounts are taken into account. The degrees Brix amount for the prepared kudzu jelly was 70% and 59% for the purchased kudzu jelly. This would indicate that the percent soluble solids percentage was higher in the prepared as compared to the purchased kudzu jelly. This is also indicated in the carbohydrate percentage results listed in Table 18. The carbohydrate percentage was 73% for the prepared kudzu jelly and 57% for the purchased kudzu jelly. This difference in carbohydrate percentage and percent soluble solids possibly affected the level of perceived sweetness detected by consumers. This may have been the reason

individual consumers chose one kudzu jelly treatment over another. The sweetness of each jelly product can also be seen when evaluating kcal/g data. The prepared kudzu jelly provided 3.0 kcal/g, the purchased kudzu provided 2.4 kcal/g, and the scuppernong jelly provided 2.3 kcal/g. This further indicates that perceived sweetness may have been a factor in consumer decision making.

Percentages of the other proximate analysis determinations for the kudzu jelly samples were very small, as expected, as compared to the moisture and carbohydrate percentages. The protein, fat, fiber, and ash determinations for the prepared jelly totaled 1.0%, and the same determinations for the purchased kudzu jelly equaled 1.3%. The nutrition facts label for the scuppernong jelly listed the percentages of fat, sodium, and protein as 0%. These were negligible, as expected, due to the fact that the major ingredients in the jelly treatments are sugar and kudzu blossom water or scuppernong juice. The percent soluble solids (Brix) content of the kudzu blossom liquid used in the prepared kudzu jelly was 0.5%, which would be close to the expected amount in the purchased kudzu jelly product. The scuppernong jelly product would be expected to have higher percent soluble solids (Brix) content before the sugar or sweetener was added, therefore, possibly affecting the sweetness perceived by consumers. The soluble solids percentage of muscadine juice usually ranges between 13.0 to 14.0%, depending on the cultivar (Morris & Brady, 2007).

Table 18. Proximate analysis results of the prepared kudzu blossom jelly and the purchased kudzu bloom jelly treatments

Proximate Analysis Determinations	Prepared kudzu Blossom jelly treatment (42 g)	Purchased kudzu bloom jelly treatment (42 g)
Moisture (%)	26.2	42.1
Crude Protein (%)	.02	.09
Crude Fat (%)	.9	1.1
Crude Fiber (%)	.02	.03
Ash (%)	.01	.03
Carbohydrates (%)	72.9	56.7
Energy (kcal)	299.8 Kcal/100g	237.1 Kcal/100g

CHAPTER V

CONCLUSION

Kudzu (*Pueraria lobata*) is an edible leguminous vine. Even though the entire plant is edible, limited research has been conducted to develop acceptable food products using kudzu. The primary interest of many in the southern United States in reference to kudzu is in the eradication of the vine from the landscape. According to Lowney (2002), the more recent view of kudzu as a potentially useful plant may be leading to an enlightenment period for kudzu. This research analyzed the roots, leaves, and flowers of kudzu for antioxidant activity and evaluated consumer acceptability of two food products using Mississippi-grown kudzu leaves and flowers.

Antioxidants are important aspects of a healthy diet. Results from the laboratory analyses indicated that kudzu flowers, roots, and leaves contained antioxidant activity and phenolic compounds. The antioxidant activity amounts determined in the flowers, roots, and leaves were 77.9%, 75.7%, and 56.5%, respectively. It was concluded that this warrants further investigation of the use of these plant parts as sources of beneficial antioxidants. Jun et al. (2003) also concluded that the antioxidant activity of isoflavone compounds in the kudzu root has been underestimated and should be further investigated. Isoflavones are a group of phytoestrogens. The plant family most abundant in phytoestrogens is the legume family of which kudzu is a member.

The dip product that was developed and evaluated contained dried kudzu leaves, mayonnaise, sour cream, herbs, spices, and salt. A comparable dip product using dried spinach leaves instead of kudzu was also developed. Additionally, healthier versions of the dip products were developed using light mayonnaise and light sour cream and omitting salt. Consumers indicated they liked the regular spinach dip better than the others for overall acceptability; however, there were no differences for appearance, aroma, and texture among all the dip products. The healthier versions of kudzu and spinach dips were rated lower for flavor than the regular versions. Consumers indicated they liked the regular spinach dip best for flavor compared to regular kudzu dip, and the two healthier dips were rated lower but were not significantly different from each other. A dried-leaf mix could be developed using a combination of kudzu and spinach leaves and probably be more acceptable to consumers. Nutritional analysis of the kudzu and spinach leaf dip products indicated that the products were similar in nutrient content.

A kudzu blossom jelly product was developed using kudzu flower liquid, sugar, a commercial pectin product, and a commercial lemon juice product. This jelly was evaluated by consumer panels with a commercial purchased kudzu blossom jelly and a scuppernong muscadine jelly. The purchased kudzu jelly was preferred for overall acceptability (like moderately) compared to the prepared kudzu and scuppernong jelly, which were rated similarly between like slightly and like moderately. Kudzu blossom jelly is a novel food item; however, harvesting kudzu flowers can be difficult with unpredictable flowering seasons and the difficulty of hand-picking the small flowers.

This research demonstrated that dried kudzu leaves have the potential for use in food products and overall, consumer responses to the food products made with kudzu leaves and flowers were positive. There is potential for further food product development and sensory testing using kudzu, which hopefully will be of interest to other scientists in developing novel products. The underutilization of kudzu in the United States warrants expanded endeavors, especially providing valuable nutritional/health benefits and integration into complementary medicine. Environmentally, the possibility of its use as an alternative energy source is being investigated. Researchers are investigating the potential use of kudzu as a new source for bioethanol production (Gjerstad et al., 2006; Sage et al., 2009). Kudzu has potential as a positive economic commodity. The resource is plentiful and broad-scale eradication of kudzu is not possible in the foreseeable future. It is too well-established and it would be expensive and impractical to attempt complete eradication. With creative thinking and collaboration among scientists and professionals in many fields of study such as agriculture, horticulture, nutrition, sociology, culinary, and food science, the economic potential of kudzu is an important area to explore.

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APPENDIX A

SCORE SHEETS FOR PRELIMINARY AND CONSUMER SENSORY PANELS

Acceptability of Food Products Made with Kudzu
(Preliminary Panels)

Samples: Dip Products containing Kudzu **Date:**
_____

What is your gender? ____Female____Male

What is your age group? ___18 – 23 ___24 – 29 ___30 – 39 ___40 – 49 ___50 and older

Please taste each sample in the order presented on the score sheet. After tasting, if you do not wish to swallow the sample, you may expectorate in the cup and rinse with the water provided.
Rate each sample in each of the five categories listed.
Each column will need one check mark if you choose to evaluate all samples.

Appearance	147	552	631
Like extremely			
Like very much			
Like moderately			
Like slightly			
Neither like nor dislike			
Dislike slightly			
Dislike moderately			
Dislike very much			
Dislike extremely			

Aroma	147	552	631
Like extremely			
Like very much			
Like moderately			
Like slightly			
Neither like nor dislike			
Dislike slightly			
Dislike moderately			
Dislike very much			
Dislike extremely			

Flavor	147	552	631
Like extremely			
Like very much			
Like moderately			
Like slightly			
Neither like nor dislike			
Dislike slightly			
Dislike moderately			
Dislike very much			
Dislike extremely			
Texture	147	552	631
Like extremely			
Like very much			
Like moderately			
Like slightly			
Neither like nor dislike			
Dislike slightly			
Dislike moderately			
Dislike very much			
Dislike extremely			

Overall Acceptability	147	552	631
Like extremely			
Like very much			
Like moderately			
Like slightly			
Neither like nor dislike			
Dislike slightly			
Dislike moderately			
Dislike very much			
Dislike extremely			

Acceptability of Vegetable Dip Products made with Dried Leafy Green Vegetables (Consumer Panels)

Samples: Vegetable Dip **Date:**_____

What is your gender?

___Female ___Male

What is your age group?

___18–23 ___24–29 ___30–39 ___40–49 ___50 and older

Please evaluate each sample independently. After tasting, if you do not wish to swallow the sample, you may expectorate in the cup and rinse with the water provided.

Each column will need one check mark if you choose to evaluate all samples.

Sample 795	**Appearance**	**Aroma**	**Flavor**	**Texture**	**Overall Acceptability**
Like extremely					
Like very much					
Like moderately					
Like slightly					
Neither like nor dislike					
Dislike slightly					
Dislike moderately					
Dislike very much					
Dislike extremely					

Sample 245	**Appearance**	**Aroma**	**Flavor**	**Texture**	**Overall Acceptability**
Like extremely					
Like very much					
Like moderately					
Like slightly					
Neither like nor dislike					
Dislike slightly					
Dislike moderately					
Dislike very much					
Dislike extremely					

Sample 416	Appearance	Aroma	Flavor	Texture	Overall Acceptability
Like extremely					
Like very much					
Like moderately					
Like slightly					
Neither like nor dislike					
Dislike slightly					
Dislike moderately					
Dislike very much					
Dislike extremely					

Sample 333	Appearance	Aroma	Flavor	Texture	Overall Acceptability
Like extremely					
Like very much					
Like moderately					
Like slightly					
Neither like nor dislike					
Dislike slightly					
Dislike moderately					
Dislike very much					
Dislike extremely					

To help us gain more data about consumption of dips, please respond to the following questions:

1. In general, do you like dip products? ____ yes ____ no
2. If you like dip products, which of the following do you like? (check all that apply)
   - ___Bean Dip
   - ___Cheese Dip
   - ___Creamy-style Dip (such as Dill or Ranch)
   - ___Tomato-based Salsa Dip
   - ___Vegetable Dip (such as Artichoke, Avocado, Onion, Spinach)
   - ___Other, please list_____
3. In general, how often do you consume dip products?

   ___Never
   ___Once a month
   ___Twice a month
   ___Once a week
   ___2 – 4 times per week
   ___5 – 7 times per week

Acceptability of Jelly Products (Consumer Panels)

Samples: Jelly **Date:** _____

Please evaluate each sample independently. After tasting, if you do not wish to swallow the sample, you may expectorate in the cup and rinse with the water provided.

The crackers are provided for cleansing the palate between samples.

Each column will need one check mark if you choose to evaluate all samples.

Sample 951	**Appearance**	**Aroma**	**Flavor**	**Texture**	**Overall Acceptability**
Like extremely					
Like very much					
Like moderately					
Like slightly					
Neither like nor dislike					
Dislike slightly					
Dislike moderately					
Dislike very much					
Dislike extremely					

Sample 677	**Appearance**	**Aroma**	**Flavor**	**Texture**	**Overall Acceptability**
Like extremely					
Like very much					
Like moderately					
Like slightly					
Neither like nor dislike					
Dislike slightly					
Dislike moderately					
Dislike very much					
Dislike extremely					

Sample 383	Appearance	Aroma	Flavor	Texture	Overall Acceptability
Like extremely					
Like very much					
Like moderately					
Like slightly					
Neither like nor dislike					
Dislike slightly					
Dislike moderately					
Dislike very much					
Dislike extremely					

To help us gain more data about jelly consumption, please respond to the following questions:

1. What is your gender?

   ___Female ___Male

2. What is your age group?

   ___18–23 ___24–29 ___30–39 ___40–49 ___50 and older

3. In general, do you like jelly products? ____ yes ____ no

4. In general, how often do you consume jelly products?

   ___Never
   ___Once a month
   ___Twice a month
   ___Once a week
   ___2 – 4 times per week
   ___5 – 7 times per week

5. If you like jelly products, which of the following do you like? (check all that apply)

   ___ Apple jelly
   ___ Blueberry jelly
   ___ Grape jelly
   ___ Muscadine jelly
   ___ Plum jelly
   ___ Other, please list

_____

APPENDIX B

INFORMED CONSENT AND MISSISSIPPI STATE UNIVERSITY INSTITUTIONAL REVIEW BOARD APPROVAL

Informed Consent Form for Consumer and Descriptive Panels (You must be at least 18 years old to participate)

Title of Study: Developing and Evaluating Food Products using *Pueraria lobata* (Kudzu)

Study Site: Garrison Sensory Evaluation Laboratory, Mississippi State University

Researchers & University affiliation: Ms. Sandra Lynn Burney and Dr. Diane K. Tidwell, Mississippi State University

What is the purpose of this research project? To determine consumer acceptability and sensory properties of food products containing kudzu (*Pueraria lobata*).

How will the research be conducted? You will be provided with food samples made from kudzu leaves or flowers. Please taste them and record your responses on the provided score sheets.

Are there any risks or discomforts to me because of my participation? There are no anticipated risks or discomforts. A list of all ingredients will be provided to you to prevent a possible food allergy reaction. You may discontinue your participation at any point.

Does participation in this research provide any benefits to others or myself? Yes. Valuable information will be obtained that will help determine the possibility of further development of food products and/or research utilizing kudzu, or similar plant materials.

Will this information be kept confidential? Yes. Only the researchers who designed this study will have access to this information. Also, please note that these records will be held by a state entity and therefore are subject to disclosure if required by law.

Who do I contact with research questions? If you should have any questions about this research project, please feel free to contact Ms. Lynn Burney at 662-325-0368 or Dr. Diane Tidwell at 662-325-0239. For additional information regarding your rights as a research subject, please feel free to contact the MSU Regulatory Compliance Office at 662-325-3994.

What do I do if I am injured at a result of this research? In addition to reporting an injury to Dr. Diane Tidwell, 662-325-0239, and to the Regulatory Compliance Office, 662-325-3994, you may be able to obtain limited compensation from the State of Mississippi if the injury was caused by the negligent act of a state employee where the damage is a result of an act for which payment may be made under §11-46-1, et seq. Mississippi Code Annotated 1972. To obtain a claim form, contact the University Police Department at *MSU UNIVERSITY POLICE DEPARTMENT, Williams Building, Mississippi State, MS 39762, (662) 325-2121.*

What if I do not want to participate? Please understand that your **participation is voluntary,** your **refusal to participate will involve no penalty or loss** of benefits to which you are otherwise entitled, and you **may discontinue your participation** at any time without penalty or loss of benefits. Additionally, you may skip any portion of the taste evaluation process.

ALL INGREDIENTS INVOLVED IN MAKING THIS FOOD PRODUCT ARE APPROVED BY THE FOOD & DRUG ADMINISTRATION FOR CONSUMPTION ACCORDING TO THEIR REGULATIONS.

You will be provided a copy of this form for you records.

Participant Signature Date

Investigator Signature Date

Revised 08/09

MSU IRB
Approved: 10/7/09
Expires: 9/9/10

MISSISSIPPI STATE UNIVERSITY

Compliance Division
Administrative Offices
Animal Care and Use (IACUC)
Human Research Protection Program (IRB)
1207 Hwy 182 West
Starkville, MS 39759
(662) 325-3496 - fax

Safety Division
Biosafety (IBC)
Radiation Safety
Hazardous Waste
Chemical & Lab Safety
Fire & Life Safety
70 Morgan Avenue
Mississippi State, MS 39762
(662) 325-8776 - fax

http://www.orc.msstate.edu
compliance@research.msstate.edu
(662) 325-3294

October 7, 2009

Sandra Burney
P.O. Box 9805
Mississippi State, MS 39762

RE: IRB Study #08-197: Sensory Properties and Acceptability of Food Products Made with Kudzu

Dear Ms. Burney:

The above referenced project was reviewed and approved via administrative review on 10/7/2009 in accordance with 45 CFR 46.101(b)(6). Continuing review is not necessary for this project. However, any modification to the project must be reviewed and approved by the IRB prior to implementation. Any failure to adhere to the approved protocol could result in suspension or termination of your project. The IRB reserves the right, at anytime during the project period, to observe you and the additional researchers on this project.

Please note that the MSU IRB is in the process of seeking accreditation for our human subjects protection program. As a result of these efforts, you will likely notice many changes in the IRB's policies and procedures in the coming months. These changes will be posted online at http://www.orc.msstate.edu/human/aahrpp.php. The first of these changes is the implementation of an approval stamp for consent forms. The approval stamp will assist in ensuring the IRB approved version of the consent form is used in the actual conduct of research. You must use copies of the stamped consent form for obtaining consent from participants.

Please refer to your IRB number (#08-197) when contacting our office regarding this application.

Thank you for your cooperation and good luck to you in conducting this research project. If you have questions or concerns, please contact me at jmiller@research.msstate.edu or call 662-325-2238.

Sincerely,

[For use with electronic submissions]

Jonathan Miller
IRB Officer

cc: Diane Tidwell