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POTENTIAL COMMERCIALIZATION OF OSAGE ORANGE (MACLURA POMIFERA) FRUIT GROWN ON SILVOPASTURES IN TEXAS AND OKLAHOMA

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ABSTRACT

This presentation explores the commercialization of Osage Orange fruit as a source of biofuel feedstocks and animal feed. It may be possible to harvest fruit from existing trees while at the same time planting new Osage Orange trees. The overall goal is to create silvopastures of Osage Orange trees with sufficient fruit volumes. The Osage Orange fruit must be processed to extract marketable biofuel feedstocks. Osage Orange is a tree species found naturally in Texas and Oklahoma. The Osage Orange is dioecious: all trees are either female or male. In a natural stand of trees, there is an equal distribution of male and female trees. A silvopasture can be started with the addition of female trees amongst the existing trees. There is sufficient information in the scientific literature detailing the chemical extraction of vegetable oil, carbohydrates and isoflavones from the Osage Orange fruit. The high flavonoids levels present a challenge in the separation of vegetable oil and carbohydrates. Isoflavonoids have been overlooked as a potential source of biomass for renewable energy. There is enough evidence to further explore developing renewable energy from cultivating Osage Orange (*Maclura pomifera*) in Oklahoma and Texas.

Keywords: osage orange, silvopasture, biofuel feedstocks, biomass, renewable energy, osajin, pomiferin, isoflavonoid

INTRODUCTION

Currently there are limitations to developing a large scale biofuel industry in Oklahoma and Texas. However much of the acreage that could be used for producing biofuel/biomass feedstocks are not suitable because of their persistent low yields due to high erosion potential, low water holding capacity, and extremes in weather (Redfean, 2011). One must assess the role of weather in sustaining the renewable energy crops in these two states. Farmers and ranchers recognize that the incoming supply of energy and moisture varies widely over both location and time. The climate extremes in Texas and Oklahoma are due to their position on the North America Continent (Bomar, 2008). These states have been plagued by long and persistent droughts which have caused economic havoc in the Agriculture Communities of both states. Why should Osage Orange (*Maclura pomifera*) be considering an alternative non-food feedstock for Bioenergy? There are potential feedstocks in the fruit: vegetable oil, carbohydrates, and phytochemicals (such as flavones) and remaining high protein biomass consumable as cattle feed (Clopton and Roberts, 1949). The Osage Orange (*Maclura pomifera*) is a tree that is part of the historic climax plant community in Texas and Oklahoma prior to the advent of European settlement and agriculture practices (Smith et al, 1981). The Osage Orange tree can be found in the Blackland Prairies, Post Oak Savannahs, Cross Timbers, Central Great Plains and Eastern parts of the Edwards Plateau of Texas, and also in the Central Great Plains, Cross Timbers and Central Irregular Plains of Oklahoma. Osage Orange trees can easily be spotted by the trained eye while traveling through these ecoregions. This a pioneer species forever invading exposed mineral soils, particularly over grazed pastures and abandoned crop fields(Burton, 1973). Other common names of the Osage Orange are d'arc, bodark, hedge, hedge apple, Osage apple, horse apple and bow wood. The USDA hardiness classification for Osage Orange is 5-9. Irrigation is not required as long as the tree receives 24-40 inches of rainfall per year. The tree grows fast and starts to bear fruit at 5-10 years of age, lives 150 years or more, and can reach a height of 9-12 meters. It is one of the most insect and disease resistant tree species in North America. The tree is hardy in drought, high heat, ice and high winds. It is dioecious, with different flowers on the male and female trees. If there is no male tree present during pollination, the female trees will produce a seedless fruit, which may lower the oil and protein content of the fruit. It produces an unpalatable, multiple globular fruit which is about the size of a large orange weighing about 1 pound, and 80% which is water (Burton, 1990).

In Texas, the author has observed that even after a freeze in November or December, the fallen fruit can subsequently experience several days and possibly weeks of high temperatures and low humidity. The fruit will soften and begin to dehydrate while turning brown at the surface. The fruit will stay intact until April at which time the spring rains begin to break down the outer surface, but the fruit remains on the grounds as a dehydrated ball with its seeds intact until the heavy rains in the spring. This would allow the fruit to be harvested from November to early April.

The Osage Orange fruit has three potential non-food feedstocks: vegetable oil, sugars/carbohydrates and phytochemicals. Seeds are 11% of the weight of the fruit and are composed of 5.9% water, 6.7% ash, 20.8% carbohydrate, 33.9 % protein, and 32% fat (Soloua et al., 2009). Researchers at the USDA, Bio-Oils Research Unit (Moser et al, 2011) were able to prepare biodiesel from the oil extracted from Osage Orange seeds. It had been reported that a fruit tree will yield 450 kg of fruit/tree, hence it equates to 49.2 kg of seeds and 16.2 kg of vegetable oil per tree. Assuming 100 producing female trees per hectare, one hectare would produce 1620 kg/ha or 1800 liters/ha. The fruit has been reported to contain about 15 % sugars and 7% other carbohydrates on a dry weight basis (Clopton and Roberts, 1949). If planted at a density of 100 trees/ha (10 meter centers), 1,073 liters/ha (115 gallons/ac) of ethanol could be produced annually (Seibert et al, 1986). In other studies, the fresh fruit could have as much as 46% pectin in its solids (Aliev, 1961). If the complex carbohydrate is indeed pectin, *Saccharomyces cervisiae* will not convert the galacturonic acid subunit to ethanol (van Leeuwenhoek, 2006). Certain anaerobic bacteria and yeast can convert galacturonic acid to ethanol by using anaerobic bacteria and yeasts (Edwards and Doran-Peterson, 2012). Very little has been discussed in the technical literature about the high levels of flavones and other phytochemicals present in the fruit as a potential renewable energy sources.

In past research on extracting phytochemicals from Osage Orange fruit for pharmaceutical research, antioxidant, antifungal, antibiotic and repellant products (Florian et al, 2006, Altuner et al, 2012, Wagner and Harris, 1952), the main solvents in extracting the phytochemicals have been listed in Table 1.

Water	Ethanol	Methanol	Dichloromethane	Acetone
Anthocyanins Lectins Polypetides Saponins Starches Tannins Terpenoids	Alkaloids Flavonols Polyacetylenes Polyphenols Sterols Tannins Terpenoids	Anthocyanins Flavonols Flavones Lactones Phenones Polyphenols Saponins Tannins Terpenoids	Terpenoids	Flavones

Table 1. Solvents used for active compound extraction of Phytochemicals (Cowan, 1999)

The two main phytochemicals extracted from Osage Orange discussed in the literature are pomiferin and osajin. It has been found in amounts of 10 – 15 % based on dry weight basis (Wagner and Harris, 1952). Phytochemicals (resins and pigments) were reported to be 21.67% of the dry weight (Compton and Roberts, 1949). The alcohol extract represents a mixture of phytochemicals that all have fairly high Heats of Combustion. The Heats of Combustion based on Hess' Law and bond energies are estimated for the following phytochemicals in Table 2.

Compund	Formula	Molecular Wt.	Heats of Combustion	
Phytochemical			MJ/kg	BTU/lb
Osajin	$C_{25}H_{25}O_5$	404	32.64	14,040
Iso-osajin	$C_{25}H_{25}O_5$	404	32.22	13,860
Pomiferin	$C_{25}H_{24}O_5$	420	31.10	13,380
Iso-pomiferin	$C_{25}H_{24}O_5$	420	30.54	13,140
Maclurin	$C_{13}H_{10}O_6$	262	23.85	10,260
Lupenyl acetate	$C_{32}H_{52}O_5$	468	41.00	17,640
Butyrospermol	$C_{30}H_{50}O$	410	44.70	13,140
Fuels				
Ethanol			25.00	10,755
Gasoline			46.00	19,790
Biodiesel			38.80	16,262
Diesel			48.10	13,140

Table 2. Comparative Heats of Combustion for Phytochemicals and Fuels

Unfortunately, there is very little information published on determining the levels of individual phytochemicals in the Osage orange fruit. Most of the research was conducted in and around the 1950s without the technology of GC–MS, NMR, and HPLC. The population of fruit tested was limited to one or two fruits per only one tree in one specific area or country. One of the objectives of this study was to look at several options to commercially process the fruit.

MATERIALS AND METHODS

In order to find a process that could be used to give a commercial plausible process, several trial and error experiments were performed by the author. Based on this initial work, the following is recommended:

1. Field dried fruit was preferred over fresh fruit.
2. Fruit must be blanched
3. Fruit should be macerated during steeping
4. Blanched, macerated fruits should be dried
5. Seeds should be separated from the dried fruit mass
6. Solvent extractions should be performed on separately on fruit mass and seeds.

Materials

Osage Orange fruit was collected from trees in Tarrant County, Texas. All the fruit was gathered from the ground. The first fruit was collected in December and fruit not used immediately was stored in a commercial refrigerator (34^0 to 40^0F). Fruit was collected in February which were the outer coat was changing to a brownish yellow and tissue was soft but not rotten. This unused fruit was refrigerated. Fruit collected in April and May had turned brown and in most cases was very hard. This fruit had moisture content of less than 5 % moisture on the wet wt. basis. This fruit was stored under dry conditions at ambient conditions. Solvents used were Fox Pure Reagent Hexanes, min. 99.9% C6 Hydrocarbons and Fox Pure Reagent Methanol, min. 99.9% purchased from Fox Scientific, Inc., Alvarado, TX. Tap water was used for water soluble extractions, tissue softening, and water blanching.

Methods

Moistures samples were determined by air drying for 24 to 48 hours, then dried in a vacuum oven at 80^0C for 24 hours. In the extraction trials, fruit was collected on May 5, 2013 from three different trees. All the fruit was put in a container, and randomly selected for study. The fruit was cut into pieces, about 1 inch cubes and the moisture was determined.

Water Extractions

In the first extraction, tap water was added to the weighed field dried fruit sample and brought to a temperature of 180^0F and held at this temperature for 2 hours, then allowed to steep in the blanch water for 8 hours to soften the fruit. During steeping the fruit suspension was agitated at irregular intervals. After steeping, the batch was pressed in a hand operated fruit press with a cotton filter cloth. In the second and third extraction, fresh tap water was added to the pressed solids at ambient temperature and steeped for 2 hours. During steeping the fruit suspension was agitated at irregular intervals. After steeping, the fruit mass was pressed as in first step. After the third extraction, remaining fruit mass was weighed and dried and the moisture content was determined.

Methanol Extractions

The remaining fruit solids were air dried and the moisture determined. Methanol was added to a sealable glass container with the remaining dried fruit solids. Sufficient quantity of ethanol was added to completely submerge all of the solids. The weight of ethanol was determined and recorded. The first methanol extraction was for 12 hours at ambient temperature. The container was shaken intermittently. The methanol solution was filtered from the fruit solids. This procedure was repeated four (4) times, but, the extraction time was shortened to two (2) hours on subsequent extractions. The methanol extraction solutions were distilled in a weighed round flask that was heated in a steam bath. The distillation was stopped when methanol no longer was condensing in the water cooled condenser. The flask was heated to 220^0 F in a drying oven until the extract stopped losing weight. The flask was cooled in a desiccator and weighed. The total methanol extract weight was determined.

Hexane Extractions

The ethanol extracted fruit solids (the solids were not dried to remove the methanol) were added to a sealable glass container. Sufficient quantity of hexane was added to completely submerge all of the solids. The weight of hexane was determined and recorded. All extractions were at ambient temperatures (70 -75^0 F) and the container was shaken intermittently. After 2 hours, the hexane solution was drained and filtered into a 1000 ml glass separation funnel and allowed to stand for 1 hour. The hexane solution separated to the top and the remaining ethanol solution separated to the bottom. The ethanol solution was drawn off, weighed and added to the methanol extraction solutions. The remaining hexane extraction solution was weighed. This procedure was repeated three (3) times. The hexane extraction solutions were combined and distilled in a weighed 1000ml round glass flask that was heated in a steam bath. The distillation was stopped when the hexane no longer was condensing in the water cooled condenser. The flask was heated to 250^0 F in a drying oven until the extract stopped losing weight. The flask was cooled in a desiccator and weighed. The total Hexane extract was determined.

RESULTS AND DISCUSSION

Water Extraction Results

The results of the water extractions are shown in Table 3. The amount of soluble solids in ground dried fruit was low. The hot water blanch did deactivate the enzymes because no gas formation was observed during the steeping in all three extractions. Agitation and pressing does break the fruit into smaller pieces. Based on results, it is recommended that the dried fruit be blanched and steeped for several hours under continuous agitation until the seeds easily separate from the fruit tissues. The suspended solids can be easily separated with a cloth plate or bag filter. The filtered solution can be reused in subsequent blanching and steeping operations.

Extraction	Water Added (grams)	Water Solution Removed (grams)
First	1,778	1082
Second	797	822
Third	765	772
Totals	3,350	2,676
The initial fruit sample was 571 grams (554 grams dry weight solids). Filtered Wet Solids - 598 grams: Moisture Content – 85% Non Water Extracted Solids – 508 grams: Soluble solids – 46 grams (calculated).		

Table 3. Water Extraction Summary

Methanol Extraction Results

Methanol was used for the phytochemical extractions of the dried fruit solids which are summarized in Table 4. The relative high level of phytochemicals in the fruit required at least five (5) extractions. It is recommended from observation that a countercurrent extraction process with heated alcohol be used to minimize the amount of alcohol used. Energy consumption is lowered by decreasing the alcohol distillation load.

Extraction	Methanol Added (grams)	Methanol Removed (grams)	Color
First	1,184	1,021	Very Dark Brown
Second	995	993	Very Dark Brown
Third	1,158	1,098	Dark Brown
Fourth	1,031	1,010	Clear Brown
Fifth	828	776	Clear Light Brown
Hexane Solution		170	Lite Yellow
Totals	5,196	5,067	
Alcohol Extractable fruit solids (phytochemicals) after distillation - 128 grams			

Table 4. Alcohol extractions

Hexane Extraction Results

Hexane extraction is the typical method used for removing vegetable oil from high oleiferous material. In these extractions, clear lite green/brown oil was produced. The hexane removed the oil quickly and was easily distilled to concentrate the oil. In the literature, the oil is found in the largest concentration in the seeds. The seeds can be separated by several methods such as sieves, gravity tables or air separators. If seeds were the only part of the fruit extracted with hexane, both equipment and energy use would be less. In Table 5, the hexane extracted solutions have been summarized. The amounts from this experiment were about half the values reported in the literature. It is possible there were not male trees in the area of the female trees to produce the maximum amount of seeds. Also, Tarrant County, Texas has been experiencing extreme drought for two (2) years. The seeds in the fruit were not ground; hence, the seed coat would prevent the soluble carbohydrates from going into solution.

Extarction	Hexane Added (grams)	Solution Recovered (grams)	Color
First (8 hours)	639	441	Dark Green
Second (2 hours)	932	873	Clear Green
Third (2 hours)	564	516	Clear Lite Green
Totals	2,135	1830	
Hexane Extractable Fruit Solids (Oil) after Distillation and Drying - 47 grams (8.5% dwb)			

Table 5. Hexane Extractions

Total Extraction and Energy Content Results

The total extraction results are covered in Table 6 for the purpose of creating a total final product energy content estimate. Based on the content weights from the extractions, the energy content has been calculated based on the yields of field dried fruit in Table 7. The amount of energy from the extracted products, the energy needed to both harvest and process have been calculated, and the net energy is positive.

Extraction	Weight Extracted (grams)	% of Solids
Water Soluble	46	8.3
Methanol Soluble	127	22.9
Hexane Soluble	47	8.5
Remaining Solids	334 (calculated)	60.3
Starting Solids	554	100.0

Table 6. Summary of Extractions

Extractions	Percentage	Weight/1000lb	Heat of Combustion (Btu/lb)	Heat Energy (Btu/1000lb)
Hexane/Oil	8.4	84	16,900	1,436,500
Alchol	22.9	229	13,380	3,064,020
Total Energy Extracted from 1000 lbs.				4,500,520
Energy Required to Harvest and Process 1000 lbs. (Huxel, 2013)				2,990,850
Net Energy Produced from 1000 lbs.				1,509,670

Table 7. Energy Content and Net Energy after Processing of 1000 Pounds of Dry Fruit Solids.

Economic Value Estimations

Though the numbers are based on many assumptions and estimates, the preliminary data suggests that the Osage Orange tree can be used as a non-food renewable energy source. The fruit produces more energy than the energy needed to harvest and produce the extractable fuels. In addition, the trees are sequestering carbon and tree pruning can be used as an energy source in the processing plant. Based on the energy produced, the final product has a market price shown in Table 8. Extending the amounts to price per pound, one would get an estimate of the Potential Value Added to an acre of land that has been turned from range land to either a silvopasture or tree crop farm. What has not been priced is the value of the remaining solids as an animal feed.

Female Trees/acre	lb/acre	$/lb	$/ac
One			
Hexane Extract	17	0.400[1]	6.80
Alcohol Extract	46	0.074[2]	3.40
Total			10.20
Silvopasture /20 trees			
Hexane Extract	340	0.400[1]	136.00
Alcohol Extract	916	0.074[2]	67.78
Total			203.78
Tree Cropping Farm/120 Trees			
Hexane Extract	2,040	0.400[1]	816.00
Alcohol Extract	5,496	0.074[2]	406.63
Total			1,222.63
Notes: 1. Crude Soybean Oil, Chicago, May 6, 2013 - $.483/lb. The oil produced is very similar to unrefined soybean oil - $0.40/lb. value. 2. Price determined at the price of $7.40/1,000,000 BTU based on Pomifera			

Table 8. Potential Value Added from a Silvopasture and a Tree Cropping Farm

CONCLUSIONS

In both Oklahoma and Texas, many livestock farms are located on land degraded by cultivation in the earl part of the 20th century, or on otherwise marginal land of inherently low productivity. The cultivation of Osage Orange trees, a native tree, on livestock farms has the potential of producing additional sustainable income without major capital expenditures and large annual input costs. The Osage Orange fruit has sufficient enough levels of vegetable oil and phytochemicals and at today's energy prices to make it a viable product for a renewable energy business. The Osage Orange trees will reduce water and wind erosion, increase habitat for wildlife, increase the retention of water, prevent soil cracking and carbon sequestration on silvopastures. What is most important is that it gives addition revenue to the livestock farmer to make their operation economically sustainable and the nation finds a new alternative source of biomass used for renewable energy.

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