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Red Sour Plum (*Ximenia caffra*) Seed: A Potential Non-conventional Energy and Protein Source for Livestock Feeds

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ABSTRACT

This study was carried out to determine the potential of *Ximenia caffra* seed as a feed ingredient. Organic matter, crude protein, ether extract, and ash constituted 93.5, 18.3, 48.5% and 2.0% of the seed mass, respectively. Phosphorus (345.5 mg 100 g^{-1} DM) and glutamic acid (2.34±0.18 g 100 g^{-1} DM) were the most abundant mineral and amino acid, respectively. Vitamin E and gross energy constituted 0.5 µg g^{-1} and 32.1±0.04 MJ kg^{-1}, respectively. The phytate-phosphate content of the seed was 0.04%. The NDF and ADF constituted 21.3 and 5.1% of the seed mass, respectively. *X. caffra* seed could be exploited as a dietary protein and energy source in feeds. © 2012 Friends Science Publishers

Key Words: *Ximenia caffra* seed; Protein; Energy; Minerals; Fiber; Amino acid profile

INTRODUCTION

In sub-Saharan Africa (SSA), the low dietary intake of animal protein results in a high prevalence of protein malnutrition. Soyabean, a major protein source in monogastric animal feeds, also serves as a human food ingredient. Consequently, animal production competes heavily with man (Thornton, 2010) for soyabean. The use of fishmeal as a protein source in feeds is limited by its high cost and the odour tainting it imparts to animal products if used in finisher diets. Similarly, cereal grains, the major energy sources in intensive animal production are in short supply for human consumption in the SSA region resulting in substandard and contaminated cereal grain being fed to livestock. Competition and drought-induced soyabean shortages, the limitations to the use of fishmeal and shortages in cereal grain necessitate a need to search for non-conventional livestock feed sources of protein and energy that are adapted to the climatic and edaphic environment of the SSA region and elsewhere.

Indigenous fruit bearing trees (IFBTs) are an essential component of Southern Africa's natural resources. IFBTs supply nutrients, construction timber, fuel and are also used in ethnomedical practice (Saka *et al.,* 1994). Oils derived from IFBTs have been and are exploited by human societies for culinary and industrial purposes and for the treatment of conditions as diverse as dandruff, muscle spasms and varicose veins (Mohammad & Mahmood, 2005). Some IFBTs provide browse for both domestic and wild animals, contributing to ruminant animal protein production (Katjiua & Ward, 2006). Communities in the sub-region also harvest honey and fruit from the IFBTs.

The large Sourplum, *Ximenia caffra* var. *caffra* (*X. caffra*) (family Olacaceae) is an IFBT widely distributed in southern Africa that withstands moderate frost and is drought resistant when mature (Lee, 1973). The flesh of *X. caffra* fruit immediately around the fruit stone has a high protein value and is rich in ascorbic acid at 27 mg 100 mg^{-1} (Roodt, 1998). Fresh *X. caffra* fruit juice and the dried fruit flesh are used to add flavour to porridge. Roodt (1998) reported that *X. caffra* fruit jelly is used as an ingredient when making tarts. The kernels have a high oil yield (48%) of which 62.8% of the oil yield is oleic acid (Chivandi *et al.,* 2008). The Khoi-san (indigenous inhabitants) of the Kalahari Desert use *X. caffra* seed oil for softening skin and rub it on chapped hands and feet.

While there are reports on the composition and uses of the *X. caffra* fruit pulp and juice, there is a dearth of data on the chemical nutrient composition of *X. caffra* seeds that are usually discarded following fruit pulp utilization. The seeds are potential sources of biomass and other nutrients. We have previously analyzed the fatty acid content of *X. caffra* seed oil (Chivandi *et al.,* 2008). In this follow up study, a preliminary evaluation of the potential of *X. caffra* seed as a non-conventional feed ingredient by determining its chemical nutrient composition has been undertaken.

MATERIALS AND METHODS

Seed source: Fresh ripe *X. caffra* fruit were collected from Zhombe District, Zimbabwe (latitude 14°45′S; longitude

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26°50′E). Zhombe district is characterized by low annual rainfall (mean 550 mm per annum) and a mean annual temperature of 26°C. The *X. caffra* fruit for chemical analyses were collected from 20 trees randomly selected from a sample of 100 identified *X. caffra* fruit trees that had ripe fruit. One hundred ripe fruit were picked from each of the 20 trees. Out of the 100 fruit picked from each tree, 20 were randomly selected. The fruit pulp was removed from the selected fruit and the fruit stones (containing the seeds) were dried in the shade and stored separately in dark sample bottles at 4^0C in the refrigerator until the time of assaying. Prior to assaying, the seeds were manually extracted from the fruit stones, and milled through a 1 mm screen to produce a composite meal from which the various assays were done. The seeds were imported into the Republic of South Africa (permit number P0039683) for the analyses.

Proximate determinations: The proximate, mineral, amino acid, fiber and phytate-phosphate determinations were done at the Agricultural Research Council's Irene Analytical Services Laboratories, South Africa. Dry matter was determined as outlined by the Official Methods of Analysis of Analytical Chemists (AOAC, 2005). The other proximate components organic matter, crude protein, ether extract, and ash were determined as outlined by AOAC (1995). The gross energy value of the seeds was determined using an MC-1000 Modular Calorimeter equipped with a PC and MC1000 software (Energy Instrumentation, Centurion, South Africa).

Calcium, magnesium and phosphorus determination: Prior to the determination of the mineral concentration in the seed samples, 0.5 g of the milled samples was digested in concentrated nitric acid and perchloric acid at 200°C to generate the digest solution (Zasoski & Burau, 1977). An aliquot of the digest solution was used for the inductively coupled plasma optical emission spectrometric (ICP-OES) determination of calcium, magnesium and phosphorus on a Varian Liberty 200 spectrometer (Varian, Perth, Australia) as described by Huang and Schulte (1985).

Amino acid assay: The concentration of each of the assayed amino acids was determined as described by Einarsson *et al.* (1983). Briefly, the assay involved acid hydrolysis with 6M HCl at 110°C for 24 h and pre-column fluorescence derivatization of amino acids with 9-flourenylmethyl chloroformate. The amino acids were extracted with pentane, and separated by gradient elution on a chromatograph. The chromatograph consisted of a SpectraSystem P4000 Quaternary HPLC (Rigas Labs S.A., Thessaloniki, Greece) equipped with a SpectraSystem FL3000 fluorescence detector and Rheodyne 7125 valve with 20 µL injection loop. The eluent varied with a concave curve from sodium citrate buffer (pH 2.95)-acetonitrile (70:30) to sodium citrate buffer (pH 4.5)-methanol-acetonitrile (14:6:70) and a flow-rate of 1.4 mL/min. An OmniSper 5 C18 150 x 4.6 analytical column and guard-column (Varian, Perth, Australia) were used for separation of the amino acids. Identification of the amino acids was done at an excitation wavelength of 264 nm and an emission wavelength of 340 nm. A PC equipped with TSP software was used for quantification. Quantification was performed by using an external calibration procedure.

Fiber determinations: Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined as described by Van Soest *et al.* (1991). In summary, NDF determination involved refluxing a 0.5 g sample for 1 h in 100 mL of neutral detergent solutions of sodium lauryl sulphate and ethylenediamine tetra acetic acid to which heat-stable alpha-amylase (20 350 IU/mL) (dietary fiber kit, Sigma-Aldrich) was added. After refluxing for 1 h, the mixture was filtered; the residue was dried and then weighed. ADF was determined by refluxing for 1 h, a 0.5 g sample in acid detergent solution (20 g cetyl-trimethyl ammonium bromide dissolved in 1 L N H_2SO_4). After refluxing, the mixture was filtered; the residue was dried and then weighed.

Phytate-phosphorus determination: Phytate-phosphate was determined colorimetrically as described by Wheeler and Ferrel (1971) using a Perkin Elmer Lambda25 UV/Vis Spectrometer (Perkin Elmer, California, USA) equipped with a PC and Lambda25 software. The standard curve was prepared using $Fe(NO_3)_3$ solution. In summary, samples were treated with 3% trichloroacetic acid followed by addition of ferric chloride (2 mg ferric iron per ml in 3% trichloroacetic acid) and the precipitate dissolved in 3.2N nitric acid. After addition of 1.5 M potassium thiocynate, the absorbance was read at 480 nm.

Vitamin E and squalene determination: Lipid extracts used in the assays were prepared using standard lipid extraction procedures (Bligh & Dyer, 1959). After evaporation to dryness, the lipids were re-dissolved in an equal volume of the respective running solvent; methanol:water (95:5) for vitamin E and hexane:propan-2-ol:water (98:2:0.02) for squalene prior to injection into the HPLC system. Assays for vitamin E were done as described by De Leenheer *et al.* (1985) and Gratzfeld-Huesgen *et al.* (1992) whereby, following dissolution of the lipid extracts into the running phase solvent, the sample was injected into the HPLC system (LKB Bromma 2150 HPLC; LKB, Bromma, Sweden). The mobile phase ran at 2 ml/min. Vitamin E was separated using methanol: water (95:5) and a C18, 15 cm × 4.6 mm ID, 5µm particle size column (Phenomenex, Torrance, USA); and detection at 290 nm by a Lambda-Max Model 481 LC spectrophotometer (Millipore Water Corporation, Ontario, Canada) with vitamin quantification using an HP 3390A integrator (Hewlett Packard, Bristol, United Kingdom). Squalene was assayed as described by Sulpice and Ferezou (1984). The sample was injected into an HPLC system (LKB Bromma 2150 HPLC). The mobile phase ran at 5 mL/min with squalene separation using hexane:propan-2-ol:water (98:2:0.02) and a silica gel C18, 25 cm x 4.6 mm ID, 5 µm particle size column; and detection at 215 nm by a Lambda-Max Model 481 LC spectrophotometer with squalene quantification using an HP 3390A integrator. Authentic

vitamin E and squalene standards (Sigma Aldrich, Germany) were used to identify and quantitate vitamin E and squalene, respectively.

Statistical analyses: All data are presented as mean ± standard deviation of three independent determinations for each parameter.

RESULTS AND DISCUSSION

Table I shows proximate, minerals, fiber, vitamin E and squalene content of *X. caffra* seed. While squalene was not detected, the vitamin E concentration was 0.5 µg g^{-1}. *X. caffra* had a gross energy (GE) content of 32.1 ± 0.04 MJ kg^{-1}. The phytate phosphate content of the seed was 0.42±0.00 g kg^{-1}. The amino acid profile of the seed is shown in Table II. The assayed 17 amino acids accounted for 81.4% of the crude protein (CP) content of *X. caffra* seed. Glutamic acid, the most abundant amino acid in *X. caffra* seed, constituted 12.8% of the CP content.

The energy content and potential of *X. caffra* seed as an oil source: Maize (*Zea mays*), a major dietary energy source in animal feeds has a reported GE value of 17 MJ kg^{-1} (Fagbenro, 1999) which is low in comparison to the 32.1 MJ kg^{-1} reported for *X. caffra* seed. The high GE value makes *X. caffra* seed a potential dietary energy source in animal feeds. The high ether extract (lipid) content of *X. caffra* seed (Table I) may have contributed to the observed high GE of the seed. On a unit mass basis, lipids are more energy dense when compared to carbohydrates. The high lipid content could therefore explain the very high GE value of *X. caffra* seed compared to maize which has much lower lipid content.

The *X. caffra* seed's oil yield at 48.5% is higher than the 15–25% and 35–40% reported for the traditional oil seed crops soybean (*Glycine max)* and cotton seed (*Gossypium hirstum)*, respectively. *X. caffra* seed, in view of its high oil yield of which 62.8% is oleic acid (Chivandi *et al.,* 2008) could therefore be exploited as a viable plant oil source for possible commercial exploitation. Oils with high oleic acid content have high oxidative stability which (high oxidative stability) is a vital characteristic of lubricants (Cahoon, 2003).

Protein content and amino acid profile: The CP content of *X. caffra* seed at 18.3% is similar to the 18.8% CP reported by FAO (1992) for undefatted sunflower seed. Sunflower seed and soyabean are key protein sources in animal feeds. *X. caffra* seed could be used to contribute to the protein content in feeds. Full-fat kernels of most groundnut (*Arachis hypogaea*) varieties contain about 44–56% lipid/oil and CP range of 23–30% (Savage & Keenan, 1994) but solvent extracted groundnut meal has a CP content of about 54%. While the amino acid content of full-fat *X. caffra* seed is lower than that of the FAO/WHO reference protein (FAO, 1992), extraction of oil from the seed (48% lipid) could potentially result in an increase in the CP and amino acid content of the residual meal to levels high enough for use as a protein concentrate in animal feeds. Although the essential amino acid concentration of full-fat *X. caffra* seed is low compared to that of soybean (Cerny *et al.,* 1971), deffating of *X. caffra* seed prior to use as a feed ingredient over and above increasing the concentration of amino acids would curb the likely problem of rancidity due to the seed's high oil content.

Table I: Proximate, mineral, fibre composition and vitamin E and squalene content of full-fat *X. caffra* seeds

Proximate component (g kg^{-1})	Mean ± SD
Dry matter (DM)	955.13±0.78
Organic matter (OM)	934.69±1.97
Crude protein (CP)	182.55±0.52
Ether extract (EE)	484.47±0.08
Ash	20.44±1.19
Mineral (mg 100g^{-1})	
Calcium (Ca^{2+})	17.85±0.74
Magnesium (Mg^{2+})	207.90±5.94
Phosphorus (P)	345.45±5.94
Fibre fraction (g kg^{-1})	
Neutral detergent fibre (NDF)	213.31±5.45
Acid detergent fibre (ADF)	51.17±1.70
Vitamin E and squalene (µg g^{-1})	
Vitamin E	0.53±0.12
Squalene	nd

Data presented as mean ± standard deviation, nd = not detected, n = 3 composite samples of seeds from the fruit of 20 trees

Table II: Amino acid profile of full-fat *X. caffra* seed

Amino acid (g 100-g^{-1})	Mean ± SD
Alanine	1.17±0.04
Arginine	1.85±0.16
Aspartic acid	1.21±0.11
Glutamic acid	2.34±0.18
Glycine	0.58±0.05
Histidine	0.47±0.07
Hydroxyproline	0.24±0.01
Isoleucine	0.62±0.02
Leucine	1.03±0.05
Lysine	1.03±0.09
Methionine	0.16±0.02
Phenylalanine	0.55±0.04
Proline	0.79±0.00
Serine	0.64±0.04
Threonine	0.73±0.08
Tyrosine	0.75±0.13
Valine	0.71±0.04
Total	**14.87**

Data presented as mean ± standard deviation, n = 3 composite samples of seeds from the fruit of 20 trees

Mineral and phytate-phosphate content: Soyabean *(Glycine max)*, a major protein source, and maize *(Zea mays)*, a major energy source constitute the major feed ingredients in commercial animal protein production. Soyabean has a mineral content of content of 7.48% (Hadjipanayiotou & Economides, 2001), which is higher than that of *X. caffra* seed (Table I). Although *X. caffra* seed's calcium content (17.85±0.74 mg 100 g^{-1}) is much less than the 48.3±12.9 mg 100 g^{-1} reported for maize by FAO (1992), the phosphorus and magnesium content of *X. caffra* seed (345.45±5.94 mg 100 g^{-1} and 207.90±5.94 mg 100-g^{-1},

respectively) is higher than that of maize. Thus, if *X. caffra* seed were to be used as a dietary ingredient (energy or protein source), it would be essential to add adequate amounts of the calcium to mineral premixes in order to cater for the lower calcium content of the seed. However, due to the high concentrations of magnesium and phosphorus in *X. caffra* seed, there might be a saving in these minerals' content in the mineral premix.

The phytate-phosphate content in *X. caffra* seed (0.04%) is much lower than in cereals and legume grains potentially making more phosphorus available for absorption from the gastrointestinal tract (GIT) and subsequent assimilation by the body. Phytate (phytic acid) naturally occurs as an organic complex found in plants where it forms insoluble salts with divalent and trivalent minerals (Sebastian *et al.,* 1998) making them unavailable for absorption from the GIT. Additionally, in the GIT phytic acid complexes with dietary proteins (making them refract hydrolysis by digestive enzymes) and it also forms complexes with digestive enzymes consequently reducing their capacity to digest protein, fat and carbohydrates (Sebastain *et al.,* 1998). Due to the low concentration of phytate-phosphorus in *X. caffra* seed, utilisation of the seed as a dietary ingredient is therefore, less likely to result in interference with digestive enzymes and complexing with minerals and other nutrients in the GIT.

Fiber content: The fiber content of shelled *X. caffra* seed (21.3% & 5.1% NDF & ADF, respectively) is relatively higher than the 10.8% (NDF) and 2.8% (ADF), respectively reported for maize (FAO, 1992). While high fiber content usually limits inclusion levels of potential energy and protein sources in feeds for monogastric animals (due to a low innate physiological capacity to digest highly fibrous feeds), respectively, the medium fiber content in *X. caffra* seed could be useful in providing the necessary bulk for the facilitation of normal gastrointestinal motility.

Squalene and Vitamin E content: Wild fruits and their seeds are valuable sources of vitamins necessary for the maintenance of good health (Saka *et al.,* 1994). Squalene, an isoprenoid antioxidant, found in large concentration in shark liver oils (Farvin *et al.,* 2004) and also found in some plants (e.g., Amaranth seeds & olives) (Vázquez *et al.,* 2007), is reported to protect cells and cell membranes against the harmful effects of free radicals. However, squalene was not detected in the *X. caffra* seed.

Plants and all other photosynthetic organisms synthesize vitamin E (Sattler *et al.,* 2004) thus the presence of the vitamin in *X. caffra* seed is not surprising. Ladan *et al.* (2010) reported that sunflower (*Helianthus annuus*) and Sesame (*Sesamun indicum*) seed, both commercial seed oil sources have a vitamin E concentration ranging between 1.4 mg g^{-1} and 41.1 mg g^{-1}, in their respective seed oils; concentrations that are much higher than the 0.5 µg g^{-1} vitamin E concentration reported for *X. caffra* seed oil, thus making *X. caffra* seed oil a relatively poor source of the vitamin. Vitamin E, *in vivo*, is reported to function as a recyclable chain reaction terminator of polyunsaturated fatty acid free radicals that are generated from endogenous oxidation of lipids (Kamal-Eldin & Appelqvist, 1996). The mopping up of free radicals by vitamin E protects the membranes and cells against oxidative damage from the reactive oxygen species. *X. caffra* seed, despite its low Vitamin E concentration, could contribute, if used as a dietary ingredient, to the systemic antioxidant pool.

CONCLUSION

X. caffra seed could be exploited both as an energy and protein source in feeds. There is a need to fully characterise the seed in terms of its anti-nutrient content in order to have a firm basis to explore the actual feeding value of *X. caffra* seed through *in vivo* feeding trials. The high ether extract content of *X. caffra* seed makes it a potential commercial oil source. Although *X. caffra* is widely distributed in the sub-Saharan Africa, for commercial exploitation of its seed meal as a feed resource (and possible commercial exploitation of its oil) it would be necessary to establish commercially viable tree plantations.

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