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Amino Acid Evaluation and GC-MS Analysis of Bambaranut (*Vigna subterranea* (L) Verdc.)†

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Bambaranut (*Vigna subterranea* (L) Verdc.) has been neglected over the years in Kano state, Nigeria. This study evaluated the amino acid and fatty acid contents of Bambaranut. The amino acid profile of Bambaranut was determined using Technicon sequential Multi-sample Amino acid Analyzer (TSM) while the fatty acid composition was determined by GC-MS. The total amino acid content of Bambaranut was 88.37 g/100 g protein, with total essential and nonessential amino acids of 41.20 g/100 g protein (46.62%) and 47.17 g/100 g protein (53.37%), respectively. The total aromatic and sulphur containing amino acids were 7.99 g/100g protein (9.04%) and 2.68 g/100 g protein (3.03%), respectively. All the essential amino acids and aromatic and sulphur containing amino acids were present in good proportions and exceeded the WHO/FAO/UNICEF standard values except leucine, which was below the standard value for children. The GC-MS analysis indicated that the fatty acid present in Bambaranut oil included saturated and unsaturated fatty acids with varying chain lengths, numbers and positions of double bonds. Different fatty acids were detected including arachidic acid (eicosanoic acid). Vitamin C fatty acid conjugate (1-(+)-ascorbic acid-2,6-dihexadecanoate) was also detected indicating the presence of vitamin C in the plant. The presence of arachidic acid (eicosanoic acid) implied that Bambaranut oil may be rich in essential fatty acids. The presence of unsaturated fatty acids suggested that Bambaranut oil could play a significant role in cardiovascular fitness; while that of saturated fatty acids implied that the oil could be a source of energy. The results indicated that Bambaranut has excellent amino acid and fatty acid profiles and could be used as an ingredient in baby foods, as amino acid supplement, and in pharmaceutical industries.

Key words: Bambaranut; amino acid; fatty acid; GC-MS

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World demand for proteins is increasing from both conventional and new sources of protein. Although all proteins have nutritional values but the food industry requires that the protein should have good functional properties in order to be accepted as a food ingredient [1]. Protein plays an important role in biochemical, biophysical, and physiological processes. Proteins in the body come from both plant and animal sources [2]. Consumption of animal protein may lead to some health implications as it contains uric acid and saturated fatty acids; thus consumption of animal protein may lead to hyperuricaemia and cardiovascular complications. There is growing evidence that cereals and legumes play important roles in the prevention of chronic diseases [3]. Grain legumes serve as cheap sources of protein to a large proportion of the population in poor countries of the tropics. Bambaranut, an indigenous African legume, plays an important socio-economic role in the semi-arid regions of Africa. It is a rich source of protein and along with other local sources of protein could help to alleviate nutritional problems in these areas. As an under-utilized crop in the fast, Bambaranut has not received sustainable research input largely because most funding agencies are not willing to support research on crops of unproven potential and unknown commercial value [4]. Nevertheless, despite the uncertainties in research sponsors, in recent years there has been a growing awareness of the potential of Bambaranut to contribute to increased food production in Africa and the need to improve the crop [4].

One of the most disturbing and prevalent nutritional problems in the developing countries is protein-energy malnutrition [5]. The cause has been attributed to many factors including high cost of first-class proteins. The use of cereal-legume based food is

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therefore advocated as alternative protein and energy sources for infant and adult food products. Bambaranut could be an excellent source of protein, lipid, carbohydrate, and mineral elements and it also has nutritional potentials that can alleviate nutritional problems when properly explored [6, 7]. Fats are used locally and industrially in food processing and medicaments. Dietary fats or lipids are rich in fatty acids which possess alkyl chain length in which the carbon number ranges from one to more than 30 [7]. Saturated fatty acids usually has 12–18 carbons while unsaturated fatty acids possess 16–22 carbons [7]. Apparently, fatty acids can be categorized as essential and non-essential based on the synthetic ability and nutritional requirement. The present study was aimed to evaluate the amino acid and fatty acid contents of Nigerian Bambaranut.

MATERIALS AND METHOD

Sample Collection

The sample (Bambara groundnut) used in this research was collected from farmers in Madobi. The sample was dried and the seeds were removed from the hulls and brought to the laboratory. The seeds were ground into powder using mortar and pestle, and stored in a dry place.

Amino Acid Analysis

The amino acid profile in the sample was determined using the method described by Benitez [8]. The sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator, and loaded into the Technicon sequential Multi-Sample Amino Acid Analyzer (TSM). Crude protein was determined by the micro-Kjeldahl method [9].

Defatting the Sample

Defatting was carried out using Sohxlet apparatus as described by Nielson (2002). In the Sohxlet assembly that was connected to a reflux condenser, petroleum ether was employed to dissolve fat in the sample and wash it down into a flask. 200 g of powdered Bambaranut sample was carefully weighed into a folded filter paper and a small cotton wool placed on top. This was properly tied with a thread at both ends. The content was placed in the extraction thimble, and a small cotton wool was placed on top. The whole apparatus was then connected upon the addition of 300 mL of petroleum ether into the extraction flask. The extraction was carried out for 4 h using the heating mantle in order to maintain a continuous flow of water in the condenser. The defatted sample was then removed and air dried.

Hydrolysis of the Sample

100 mg of the defatted sample was weighed into a glass ampoule. 7 cm^3 of 6N HCl was added and oxygen was expelled by passing nitrogen into the ampoule to avoid possible oxidation of some amino acids such as methionine and cystine during hydrolysis. The glass ampoule was then sealed using Bunsen burner flame and put in an oven preset at 105°C ± 5°C for 22 h. The ampoule was allowed to cool before it was open at the tip and the content was filtered to remove humus. It should be noted that tryptophan was destroyed by 6N HCl during hydrolysis.

The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residue was dissolved with 5cm^3 of acetate buffer (pH 2.0) and stored in a plastic bottle and kept in the freezer.

Determination of Amino Acid Profile

Loading of the Hydrolysate into TSM Amino Acid Analyzer

The amount loaded was 10 µL. This was dispensed into the cartridge of the analyzer. The TSM analyzer is designed to separate and analyze free acidic, neutral, and basic amino acids of hydrolysate. The period of an analysis lasted for 76 minutes.

Method of Calculating Amino Acid Values from the Chromatogram Peaks

The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half-height of the peak on the chart was found and the width of the peak of the half-height was accurately measured and recorded. Approximate area corresponding to each peak was then obtained by multiplying the height with the width at half-height.

The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the following formula.

$$NE = \frac{\text{Area of norleucine peak}}{\text{Area of each amino acid}}$$

A constant S was calculated for each amino acid in the standard mixture where

$$S_{std} = NE_{std} \times \text{Molecular weight} \times \mu MAA_{std}$$

Finally, the amount for each amino acid present in the sample was calculated in g/16gN or g/100g protein using the following formula:

$$\text{Concentration (g/100g protein)} = NH \times W@NH/2 \times S_{std} \times C$$

$$\text{Where, } C = \frac{\text{Dilution} \times 16}{\text{Sample wt. (g)} \times N\% \times 10 \times \text{vol. loaded}} \div NH \times W \text{ (nleu)}$$

Wherein, NH = Net height
W = Width @ half height
nleu = Norleucine

Determination of Quality of Dietary Protein

The essential amino acid score was calculated using the formula: amino acid score = amount of amino acid per test protein (g/100 g)/amount of amino acid per protein in reference pattern (g/100 g) [10], while amino acid score for essential and non-essential amino acid was calculated based on whole hen's egg [11]. Percentage total essential amino acid (TEAA) in total amino acid (TAA) (%TEAA/TAA), percentage total non-essential amino acid (TNAA) in total amino acid (TAA) (%TNAA/TAA), total sulphur amino acid (TSAA), percentage cystine in TSAA (%Cys/TSAA), total aromatic amino acid (TArAA), and leucine/isoleucine ratios were calculated. The predicted protein efficiency ratio (P-PER) was determined using the equation P-PER= - 0.468 + 0.454 (Leu) – 0.105 (Tyr) [12].

Determination of Fatty Acid Composition

Oil Extraction

Oil was extracted according to the method described by Nielson (2002). Petroleum ether was used to extract the oil, using Sohxlet assembly connected to a reflux condenser in which petroleum ether dissolved the fat in the sample and washed it down into the flask and the ether can easily be evaporated and recollected.

GC-MS Analysis

Derivatization and GC-MS analysis (using GC-MS-QP2010 SHIMADZU, JAPAN) followed alkaline hydrolysis of 0.2 g of the extracted oil as described by Rexanka *et al.*, [13], hence, 0.2 g of the oil sample was used for methylation of fatty acids. Methyl esters of the corresponding fatty acids were prepared according to Kitson *et al.*, [14], by using 0.3 mg of sodium sulphate, 2 cm^3 of n-hexane/dimethyl carbonate mixture (1:1), and 1 cm^3 of sodium methylate and shaken for one minute. To the whole preparation 3 cm^3 of water was added with shaking and finally centrifuged at 2500 rpm for 3 minutes, and 1 µL of the filtrate was used for GC-MS analysis by aspirating to the HP 5 capillary column (25 mm × 0.32 mm, film thickness 0.53 µm) via an inlet where the heat chamber acted to volatilize the sample. Hydrogen gas (1 mL/min) transported the sample into the HP 5 capillary column and the molecules in the analytes were separated as they moved along the length of the column due to different chemical properties. The molecules eluted separately from the gas chromatograph owing to the differences in their retention time. The eluted molecules were captured, ionized, accelerated, deflected, and detected by the mass spectrometer, by breaking each molecule into ionized fragments and the fragments were detected using their mass to charge ratio by the detector.

RESULTS AND DISCUSSION

Results

The amino acid concentration, amino acid score, and other parameters in respect of amino acid contents of Bambaranut are presented in Tables 1 through 3.

Table 1. Amino acid concentrations of Bambaranut (*Vigna subterranea* (L) Verdc.) grown in Madobi Local Government, Kano State, Nigeria

Amino acid	g/100 g Protein
Lysine (Lys)*	6.42±0.25
Histidine (His)*	3.00±0.06
Arginine (Arg)*	6.45±0.18
Aspartic acid (Asp)	10.70±0.27
Threonine (Thr)*	3.46±0.14
Serine (Ser)	4.72±0.20
Glutamic acid (Glu)	15.50±0.61
Proline (Pro)	3.63±0.29
Glycine (Gly)	3.73±0.19
Alanine (Ala)	4.24±0.11
Cystine (Cys)	1.37±0.08
Valine (Val)*	4.47±0.20
Methionine (Met)*	1.31±0.07
Isoleucine (Ile)*	3.91±0.12
Leucine (Leu)*	7.47±0.20
Tyrosine (Tyr)	3.28±0.18
Phenylalanine (Phe)*	4.71±0.25

*essential amino acids

Results are mean ± standard deviation, n=3

Table 2. Amino acid scores for Bambaranut (*Vigna subterranea* (L) Verdc.) grown in Madobi Local Government, Kano State, Nigeria

Amino acid	Amino acid (g/100 g protein)	WHO Ideal Protein (g/100 g protein)		[(%Amino acid/ideal) × 100]	
		Children	Adult	Children	Adult
Isoleucine	3.91	2.8	1.3	139.64	300.76
Leucine	7.47	8.3	6.6	90.00	113.18
Lysine	6.42	4.2	5.8	110.68	110.69
Histidine	3.00	1.9	1.6	157.89	187.50
Valine	4.47	4.4	3.5	101.59	127.71
Threonine	3.46	3.0	3.4	115.33	101.76
Total Sulphur	2.68	1.6	2.5	167.50	107.20
Total Aromatic	7.99	7.4	6.3	107.97	126.83

Discussion

Amino Acid Profile

The concentrations of amino acids in the studied Bambaranut are presented in Table 1. Only seventeen amino acids were detected in the current study and this might be due to a complete destruction of tryptophan during acid hydrolysis and the conversion of amide glutamine and asparagine to their corresponding amino acids [15]. The percentages of the essential amino acids and the total sulphur and aromatic amino acids were compared with the reference standard values established by [16] for both adult and school children (Table 2). The values obtained in the current study for the essential amino acids and the sulphur and aromatic amino acids were higher than the reference values for both adults and children except that of leucine in children (Table 2). The total non-essential amino acids (47.17 g/100 g protein) was higher than the essential amino acids (41.20 g/ g/100 g protein) (Table 3). The findings of the present study agree with Alhassan *et al.*, [6] that Bambaranut could be an excellent source of protein.

Table 3. Protein parameters of Bambaranut (*Vigna Subterranea* (L) Verdc.) grown in Madobi Local Government, Kano State-Nigeria

Amino acid	g/100 g protein
Total amino acid (TAA)	88.37
Total non-essential amino acid (TNEAA)	47.17
Total essential amino acid (TEAA)	41.20
With His	41.20
No His	37.77
% TNEAA	53.37
% TEAA	46.62
With His	46.62
No His	42.74
Total neutral amino acids (TNAA)	9.55
%TNAA	10.81
Total acidic amino acid (TAAA)	26.20
% TAAA	29.64
Total basic amino acid (TBAA)	15.87
% TBAA	17.95
Total sulphur amino acid (TSAA)	2.68
% TSAA	3.03
% Cys in TSAA	48.92
Total aromatic amino acid (TArAA)	7.99

% TArAA	9.04
Predicted protein efficiency ratio (P-PER)	2.71
Leu/Ile ratio	1.91
Leu-Ile (difference)	3.56
% Leu-Ile	4.02

The total amino acid TAA (88.37 g/100 g protein) found in this study was higher than that of degutted white grubs (82.89g/100g protein) [17], cowpea (64.0 g/100 g protein) [18], *A. occidentale* and *C. acumina* (65.90 g/100 g protein and 35.60 g/100 g protein, respectively) [19], and that of *Monodoramyristica* (65.60 g/100 g protein) reported by Ekeanyanwu [2]. The total amino acid reported in the current study was lower than that of African yam bean (91.70 g/100 g protein) [18]. The results of the current study indicated that Bambaranut has an excellent essential amino acid composition. Dietary essential amino acids deficiency decreased food intake remarkably [20, 21] and depletion of a single essential amino acid led to quick deacylation of its (essential amino acid) tRNA and the levels of charged tRNA fell, leading to disruption of global protein synthesis [22].

The total essential amino acid (TEAA) 41.20 g/100 g protein found in this study was comparable to that of soya bean (44.40 g/100 g protein [23], pigeon pea (45.20 g/100 g protein) [24], and cowpea (42.90 g/100 g protein) [25]. The TEAA of 41.20 g/100 g protein was higher than that of *Monodoramyristica* (31.25 g/100 g protein) [2], Kersting's groundnut (32.70 g/100 g protein) [16] and degutted white grubs (31.48 g/100 g protein) [14]. These findings indicated that Bambaranut can be used in children with malnutrition especially in kwashiorkor where there are decreases in essential amino acids, usually the branched chain amino acids leucine, isoleucine, and valine. Looking at the amino acid profiles (Table 2), Bambaranut can be used to replenish these amino acids. The results also indicated that Bambaranut used in the study was rich in lysine (Table 2), which is an amino acid that enables the synthesis of carnitine, converts fatty acids into energy, and also plays an important role in the production of hormones, antibodies, and enzymes [17]. Deficiency in lysine can lead to niacin deficiency and causes pellagra, which is characterized by dermatitis, diarrhea, and dementia. Valine is necessary for muscle metabolism and the repair of tissues and can be useful in the treatment of liver and gallbladder disorders. Methionine aids in the production of sulphur, which is necessary for normal metabolism and it is also essential for the synthesis of hemoglobin and glutathione that fights against free radicals, while isoleucine is important in the regulation of blood sugar. Leucine increases muscle mass, helps muscle recover after exercise and regulates blood sugar. Clinically leucine helps the body heals, affects brain function, and can be used in place of glucose in fasting states. Phenylalanine is a precursor to catecholamines that regulate the central and peripheral nervous systems, while threonine is important for antibody production [26]. Significant decrease in leptin and insulin is related with the consumption of essential amino acid deficient diet [21]. The hepatic triglyceride levels tend to be elevated upon consumption of diet deficient in lysine whereas consumption of diet deficient in methionine, tryptophan, valine, and threonine tend to decrease the hepatic triglycerides. Feeding of essential amino acid-deficient diet was also reported to disturb estrous cycles in female rats [20].

The total non-essential amino acids of 47.17 g/100 g protein found in the current study was lower than that of degutted white grubs (51.41 g/100 g protein) [17] and higher than that of Kersting's groundnut and cowpea (41.40 g/100 g protein and 36.10 g/100 g protein, respectively) [16], *A. occidentale* (32.30 g/100 g protein), African yam bean (45.4 g/100 g protein) [17] and *Monodoramyristica* (34.35 g/100 g protein) [2]. In kwashiorkor, the non-essential amino acids tyrosine and arginine are below the normal value, therefore, Bambaranut could be used to correct the deficiency. The total sulphur containing amino acids (TSAA) of 2.68 g/100 g protein was lower than that of degutted white grubs (3.35 g/100 g protein) [14] and higher than that of *Monodoramyristica* (1.19 g/100 g protein) reported by [2], and cowpea and Kersting's groundnut (2.10 g/100 g protein and 2.60 g/100 g protein, respectively) [18]. In terms of total aromatic amino acids (TArAA), the value of 7.99 g/100 g protein found in this study was lower than that of degutted white grubs (8.02 g/100 g protein) [14], and higher than that of cowpea and Kersting's groundnut (3.90 g/100 g protein and 4.40 g/100 g protein, respectively) [16] and *Monodoramyristica* (3.98 g/100 g protein) [2].

Fatty Acid Profile

The GC-MS analysis of Bambaranut oil revealed the presence of saturated and unsaturated fatty acids that varied in chain length numbers, position of double bonds and configurations (Table 4). The presence of odd number carbon fatty acids disagreed with the impression that fatty acids of plant and animal origin contain even number of carbon atoms (16-22), with zero to six double bonds of *cis* configuration; and methylene-interrupted double bond systems predominate [17]. These, however,

agreed with those reported earlier [27,28]; of which many exception including odd and even numbered fatty acids exist in nature, with up to almost 100 carbon atoms and double bonds which can assume the *trans* configuration, with acetylenic and allenic bonds, and other structural features including branch point, rings, oxygenated functions and so forth. The fatty acid methyl tetradecanoic acid detected in this study is similar to myristic acid, an ubiquitous component of lipids in many living organisms, more abundant in cow's milk fat, fish oils, and seed oils enriched in medium-chain fatty acids like coconut and palm kernel oils. Myristic acid is specifically found in certain proteolipids and is essential to the function of the protein components [29]. It is also a very important stabilizer for many different body proteins; including immunoglobulins and fight tumors through myristoylation [30]. 1-(+)-Ascorbic acid-2,6-dihexadecanoate (vitamin C fatty acid conjugate) detected in the studied Bambaranut indicated the presence of vitamin C. The fatty acid hexadecanoic acid methyl ester detected in this study is similar to palmitic acid and is considered the most abundant saturated fatty acid in nature, found in animals, plants, and lower organisms. It functions as specific proteolipids in molecular level [30]. Octadecanoic acid methyl ester detected in the current study is similar with stearic acid found in the lipids of most organisms. Stearic acid occurs in lipids of some commercial importance, found in the highest concentrations in ruminant fats (milk fat and tallow) or in vegetable oils such as cocoa butter, and in hydrogenated fats and comprises 80% of the total fatty acids in gangliosides [30]. The fatty acid eicosanoic acid (arachidic acid) detected in the current study, exists at low levels in most animal lipids, and more common to plants and microorganisms. Eicosanoic acid is sourced from the essential fatty acids, linolenic acid and linoleic acid; this indicated that there might be a presence of essential fatty acids in Bambaranut oil. This could have been detected if derivatizing agents other than methyl were used. The unsaturated fatty acids detected in Bambaranut oil: *cis*-9- hexadecenal is found in almost all fats; *cis*-9-octadecenol is the most common fatty acid in natural fat; and *trans*-9-octadecenoate is mainly found in hydrogenated and ruminant fats. Other unsaturated fatty acids detected but are not commonly found in natural fats (Table 4) may play physiological roles in living organisms. Bambaranut oil could play an important clinical role, since monounsaturated fats help to decrease the blood LDL cholesterol level and increase the level of HDL cholesterol. This can lower the risk of developing heart disease and stroke, and provide nutrients, such as vitamin E. Monounsaturated fats can also decrease the risk of breast cancer, help in losing weight, and relieve the pain of rheumatoid arthritis. Monounsaturated fats help the body in absorbing nutrients, especially the fat-soluble vitamins (A, D, E, and K). Fats are needed for normal growth and development in children and keep the brain and central nervous system healthy. They also produce hormone-like substances that regulate blood pressure, blood clotting, and the immune system [31]. A study on women suffering from breast cancer indicated that consumption of high amounts of long chain polyunsaturated fats from food produced a 25% reduced risk. These women were also shown to have reduced risk of all-cause mortality [32]. Bambaranut oil could therefore play an important role in cancer management.

Bambara nut may have many health benefits including lowering high blood cholesterol, lowering high blood pressure, and relieving digestive difficulties, attention disorders, some cancers, asthma, arthritis and depressive disorders [33, 34, 35, 36]. Incidence of insulin resistance is lowered with diets high in monounsaturated fats (especially oleic acid). Hence, Bambaranut may show the same effects. Eicosanoids are biologically active and oxygenated metabolites of arachidonic acid, eicosapentanoic acid (EPA), or dihomo-γ-linolenic acid. They act as modulators of numerous physiological processes including reproduction, blood pressure, homeostasis, and inflammation [37].

Table 4. Fatty acid composition of Bambaranut (*Vigna subterranea* (L) Verdc.) grown in Madobi Local Government, Kano State, Nigeria

Rt. (min)	Compound Identified	Corresponding acid
13.75	Methyl tetradecanoate (Myristic acid) $C_{15}H_{30}O$	Tetradecanoate C14:0
16.33	1-(+)-Ascorbic acid 2,6-dihexadecanoate $C_{38}H_{68}O_8$	Dihexadecanoate C38:0
17.600	Methyl 16-methyl heptadecanoate $C_{19}H_{38}O_2$	Heptadecanoate C17:0
17.600	Cis-9-hexadecenal $C_{16}H_{30}O$	Hexadecenal C16:1
18.000	Cis-9-Octadecen-1-ol (Oleyl alcohol) $C_{18}H_{36}O$	Cis-9-Octadecenol C18:1

18.000	9-Tetradecenel (Z) $C_{14}H_{26}O$	9-Tetradecenel C14:1
18.000	Oxacycloheptadec-8-en-2-one $C_{16}H_{28}O_2$	Oxacycloheptadecenone C16:1
19.133	Methyl (11E)-11- icosenoate $C_{21}H_{40}O_2$	11- icosenoate C20:1
19.133	Methyl 8- (2-hexylcyclopropyl) octanoate $C_{18}H_{34}O_2$	Cyclopropyloctanoate C17:0
19.133	Methyl cis-9- Octadecenoate (oleic acid) $C_{19}H_{36}O_2$	cis-9- Octadecenoate C18:1
19.133	9- Hexadecenoic acid methyl ester (Z) (palmitoleate) $C_{17}H_{32}O_2$	Hexadecenoate C16:1
19.317	n-heptadecanoic acid methyl ester (Margaric acid) $C_{18}H_{36}O_2$	n-heptadecanoate C17:0
19.317	Methyl 15-methylhexadecanoic acid $C_{18}H_{36}O_2$	Hexadecanoate C16:0
20.917	Nonadecanoic acid methyl ester $C_{20}H_{40}O_2$	Nonadecanoate C19:0
20.917	Docosanoic acid methyl ester (Behenic acid) $C_{23}H_{46}O_2$	Docosanoate C22:0
22.583	Heneicosanoic acid methyl ester $C_{22}H_{44}O_2$	Heneicosanoate C21:0
22.583	Tetracosanoic acid methyl ester (Lignoceric acid) $C_{25}H_{50}O_2$	Tetracosanoate C24:0

CONCLUSION

Based on the findings of this study, it could be deduced that Bambaranut has the amino acid and fatty acid profiles that could be used to support the nutritional needs of the society and can also be used as a raw material in baby foods and in pharmaceutical industries.

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