Microbiological (Bacteria) Evaluation of Street Vended Ready to Eat Mixed Fruit Salads in Calabar South Local Government Area of Cross River State

Ikor, Peter Ulimujuoushang.¹, Dr. Lebari Barine Gboeloh.², Chijioke, Ikechukwu.³, Jumbo, Elizabeth I.⁴, Chijioke, Nkeiruka Adaeze.⁵, Monye, Vivian E.⁶. Department of Science Laboratory Technology (Applied Biology Unit) Federal Polytechnic of Oil and Gas Bonny Island, Rivers State, Nigeria

Abstract:- The ascent in incidence of meals borne sickness to avenue distributed natural product servings of mixed fruits in unindustrialized international locations is of great well-known wellness difficulty. This examine changed into for this reason assessed on street dispensed organized toeat organic product servings of blended fruits in Calabar south, to decide their bacteriological well worth. Twenty (20) examples of pre-bundled organic product servings of mixed fruits produced the usage of various blends of new natural products were amassed (purchased) and assessed for bacterial impurities making use of pour plate approach. The unique microscopic organisms genera were counted on becoming media and recognized using preferred techniques. The maximum extended heterotrophic bacterial be counted (8.5 x 106) became gotten from test 16 (sixteen) and the least (1.6 x one hundred and five) from take a look at eight (eight). The confine with most extended pervasiveness changed into Staphylococcus Sp thirteen (65%) with Salmonella Sp and Enterobacter Sp being the most un-3(15%), one by one. The presence of microscopic organisms pollutants in numbers surpassing the cautioned microbiological principles is an influence of unwholesome item, eventually the requirement for valid bacteriological security examination of herbal product servings of blended greens prepared for human utilization. Merchants and consumers are exhortation to scrub their new natural merchandise as it should be prior to stripping, cutting, or reducing. Natural merchandise need to be placed away in refrigerators if any postpone previous usage. Great man or woman cleanliness and effective danger research and basic manipulate factor (HACCP) software should be ahead to decrease the possibility of tainting of organized to-devour natural product servings of combined veggies and Government administrative bodies have to make comes to a decision that will tie dealers of organic merchandise plates of blended fruits.

Keyword: Microscopic Organisms, Calabar South, Merchants, Mixed Fruit, and Evaluation.

I. INTRODUCTION

Universally, Organic product are awesome dietary wellspring of supplements, micronutrient, vitamins and fiber for human, for this reason they're extremely fundamental for the general prosperity of man. The usage of privately prearranged blended natural merchandise, prominently referred to as organic product servings of mixed veggies, has expanded in the course of the long term in many regions of the planet (Mahale *et al.*, 2008).

In Nigeria, natural product salads is known as organized to-consume street meals when you consider that they're often bought straightforwardly from street dealers or providers or at avenue facet cubicles and eaten fast in the shape they're purchased minus any additional coping with, washing, stripping or slicing (Adams et al., 2000).

Numerous consumers belittle the road disbursed herbal product plates of mixed fruits because of the way that they are less expensive than complete natural products, helpful to eat incompletely and in light of the reality that they are correctly accessible. Natural product salads is typically a mixture of different new natural products like apples, watermelon, Pineapples, pawpaw, cucumber, and orange. They are generally cut into little pieces and bundled in little trustworthy plastic dishes. The plate of blended greens may be eaten utilising fork or enamel pick out, no matter milk introduced to it (Bean et al., 2001). Since natural product servings of combined veggies comprises of diverse natural products, the commonly speaks nutritional fame of the serving of combined greens mirrors the wholesome nature of the singular natural merchandise applied in its readiness (beuchat, 1998).

As in line with Edward et al. (2012), natural product plates of mixed fruits are low in cholesterol, sodium and soaked fat but excessive in nutrients A, C. Additionally, D. Manganese, copper and dietary fiber. In Calabar south, natural product servings of combined greens is ready and bought in road side stands, carrying centers and occupied roads in Calabar South. The various natural merchandise utilized in putting in the serving of mixed veggies are normally kept on the ground near the reducing tables with practically no type of guarantee. Thus, the microbiological (bacteriological) nature of the pre-organized organic product plates of blended veggies remains a ways fetched (Beuchat and Scouten., 2002).

Natural produces are willing to bacterial defilement considering that they're continually in contact with soil, residue and water, and by way of taking care of at accumulate or in the course of put up-acquire coping with. Pathogenic microorganisms (microscopic organisms) can also likewise enter the natural merchandise thru harmed surfaces, like cuts, wounds, cuts and parts. Such microorganisms may emerge as internationalized, make due and increase interior the goods of the soil grow to be well-being hazard to purchasers (FDA, 1999). Salmonella spp. Has been accounted for to get by means of and broaden quickly on watermelon held at room temperature and the degree of tainting didn't exchange while the melon was shop at refrigeration temperature (FDA, 1999) Episodes of listeriosis and salmonellosis have moreover been associated with the usage of prepared to-feline natural product plates of mixed greens (Jones, 1990).

In Nigeria wherein road meals dispensed is particularly everyday, there is shortage of information on frequency of meals borne sicknesses related with the street disbursed meals sorts. Nonetheless, bacterial examinations on such food resources in American, Asian, European and a few African nations have uncovered accelerated bacterial microbe in organic product salad (Mahale et al., 2008)

Considering the well-being hazard offered by means of the bacterial microbes in natural product salad and the growing hobby for such avenue allotted natural product salad, the present day overview was embraced to survey the bacteriological exceptional or well worth of newly preorganized prepared to-devour herbal product servings of mixed fruits bought in Calabar South local government Area.

A. Proclamation of the problem

In Nigeria, It has been laid out that the problem of herbal product distributing has made positions and contributes altogether to the casual region of the economies and also settle a hard problems defying serious issues due to the location's jobs of giving reasonable dinners to consumers. There are no reachable statistics on the assessment of the progress of these mediations as some distance as retaining merchants from organized to-devour organic product servings of combined greens in Nigeria. There is a pivotal want to take a look at these occasions specially in high gamble place of the us of a. Information comprised of the assessment will move quite some distance in supporting to control microscopic organisms on street allotted herbal product plates of combined veggies. In this manner, the need to decide microbial bacterial appraisal of street disbursed organized to-eat natural product servings of blended greens peddled in Calabar South Local Government Area of Cross River State.

B. Scope of Study

The evaluate become confined to the bacteriological appraisal of avenue distributed read to devour herbal merchandise plates of combined fruits in Calabar South Local Government Area.

C. Justification

Organic product servings of mixed greens are highquality nutritional wellspring of supplements, micronutrients, vitamins, and fiber for human beings and are therefore crucial for health and properly creatures, and are accounted for to be debased typically with pathogenic microorganisms (microbes) (Mahale *et al.*, 2008). It is thinking about this that this take a look at turned into led to assess the bacteriological fine or gadgets in herbal product plates of blended greens sold in Calabar South with the end aim of giving extra facts at the frequency of such enteropathogenic microorganisms for fundamental pastime with recognize to food traders, clients and specialists.

D. Aim and Objectives

The factor of the overview become to survey the microbes found in the street dispensed prepared to-consume natural merchandise servings of combined greens in Calabar South, Cross River State with the accompanying desires as;

- To isolate microorganisms (bacteria) from organized todevour herbal product salad showcased by sellers in Calabar South local Government Area.
- To characterize and identify the isolates using culture media, microscopy and biochemical analysis.
- To mention observable data and suggestions to Calabar South Government administrative bodies to give directing requirements to fruit merchants.

II. METHODOLOGY

A. Sampling and Data Collection

A sum of 20 samples of prepared - to-consume natural product servings of blended fruits were bought from avenue merchants in Calabar South Local Government Area of Cross River State.

The samples had been collected in events for the duration of which twenty samples each have been gotten from traders. All samples have been accrued in sterile polythene sacks and moved in ice-pressed cooler to the research facility, microbiology laboratory, Cross River University of Technology, Calabar for examination within 1hour after assortment.

B. Data Analysis.

Both the possible and corroborative assessments became accomplished to decide the presence of microbial (microscopic organisms) pollutants. The bacteriological investigation was carried out by the methods of multipletube technique. Nutrient Agar, MacConkey agar, and Methylene Blue Agar was used for determining the total viable count and for detection of coliforms respectively, as other selective media was also used for selective plating. After inoculation, all plates was incubated at 35°C for 24 hours. Presumptive colonies was confirm by gram staining and biochemical test reactions

C. Biochemical Characterizations

The accompanying biochemical assessments became utilized to describe the disconnects up to their species degree by way of contrasting their responses and that of the noted taxa as archived in Bergey's guide of determinative key in bacteriology. The exams include; Gram Staining, Catalase take a look at Indole check, Methyl purple take a look at, Voges Proskauer check, Citrate test, Coagulase check and Sugar maturation check (Triple sugar Iron Agar check) exams as portrayed with the aid of Cheesbrough (2004)

> Determination of Total Heterotrophic Bacterial Count

This was carried out on nutrient agar using pour plate method. A ten fold serial dilution was carried out for each of the sample. This was done by measuring 90ml of distilled water into a conical flask and it was labeled accordingly. Ten gram (10g) of sample was weighed and transferred into the flask containing 90ml of distilled water to make one part of sample and 9 part of distilled water.

From the 3^{rd} (10⁻³) and 5^{th} (10⁻⁵) test tables of each samples, one ml was removed and poured into separate sterile Petri dishes and labeled properly, then 20ml of nutrient agar was poured into each plates and were incubated at 37^{0} C for 18-24 hours. Pink colonies were counted and the mean of the each sample was recorded as the total heterotrophic bacterial count.

> Determination of Total Coliform Count

This was done using MacConkey Agar by pour plate Method. 1ml of each sample was transferred into a sterile Petri dish from the 3^{rd} (10^{-3}) and 5^{th} (10^{-5}) dilution and MacConkey Agar was poured into each plates and incubated at 37^{0} C for 24-48 hours.

Colonies were counted and the mean of each of the sample was calculated and recorded as colony forming unit (CFU).

D. Characterization and Identification of Isolate

Chosen colonies of all morphological kinds changed into aseptically picked from nutrient agar and MacConkey agar and again and again subcultured on media and incubated at 37°C for twenty-four hours. The pure cultures obtained from

the nutrient agar and MacConkey agar were culture on slants in maccarteny bottle and preserved in the refrigerator at 4^{0} C for further use.

Bacterial isolate were characterized and identify following the method recommended by Chessbrough, (2004).

In this approach, bacterial segregates have been defined and identified via Gram stain and some biochemical tests, for instance, catalase take a look at, indole test, methyl pink test, voges proskauer, citrate check, coagulate test, and triple sugar iron agar take a look at.

Gram Staining Methods

This was used in the preliminary identification of bacterial isolate. The principle is based on the ability of the bacteria to retain the basic dye which is crystal violet after decolourization with alcohol. The Gram stain divides the bacteria into Gram positive and Gram negative.

A thin film of each isolate was smeared on degrease slide. Each slide was heat fixed by passing the slide over the blue flame of a Bunsen burner. Then each slide was flooded with crystal violet stain and allow for 1 minute and rinsed with clean water. It was then flooded with grams iodine solution and was allowed for 1 minute to react and was rinsed with clean water. It was decolorize rapidly with 95% of ethyl alcohol until no crystal violet color was drain out and was rinsed immediately under gentle running tap water. It was then be counter stain with safranin for 1 minute and then it was rinsed with clean water and was both with blotting paper. A drop of immersion oil was added on each slide and examined using objective lens of x100 (oil immersion) of a light microscope. Bacteria that retained the crystal violet dye appeared purple or deep blue and were considered as gram positive bacteria while those that were decolorized and picked the pink safranin appeared red or pink and were considered as gram negative bacteria.

➤ Catalase Test

This test is used to differentiate those bacteria that produced the enzymes catalase from non-catalase producing bacteria. This enzyme breaks down hydrogen peroxide and oxygen (O_2) and water (H_2O) (Cheesbrough; 2004).

97ml of water changed into expected proper right into a recepticle and 3ml of hydrogen peroxide modified into delivered in unique to form the affiliation. Then, at that factor, 4ml of the association became anticipated right into a sterile check tubes and with the manual of a sterile twine circle, microscopic organisms confines were picked and delivered to the association. The notion of air pockets demonstrated the advent of fuel and the air pocket turn out to be taken into consideration as positive microorganisms while no air pocket changed into considered as regrettable microscopic organisms.

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➤ Indole Test

Indole test is used to detect the production of indole from the amino acid tryptophan (Prescotts *et al*,. 2011)

A colony of bacteria was inoculated on peptone water and incubated at 37^oC for 18-24 hours. O.5ml (10 drops) of Kovac's indole reagent was added and shake gently. Then a deep red pale yellow colorations indicate positive result while red coloration shows negative result.

> Methyl Red

Methyl red is a pH indicator which showed whether the bacterium carries out mixed acid fermentation (Prescotts *et al*; 2011). 4ml of the culture in peptone water was measured into a test tube. 0.2ml (5 drops) of methyl red indicator was added to the test tube containing the culture. A red ring at the top shows a positive result while pale yellow or no color change show negative result.

> Voges Proskauer

Voges Proskauer detects the production of acetoin from glucose fermentation (Prescott *et al*; 2011). 4ml of the culture in peptone water was measured into a test tube for each sample. 0.6ml (6 drops) of VP (A) (Barritt's solution was dropped into the test tube and 0.2ml) (4 drops) of Barritt's solution B (VPB) was dropped into the test tube and shake gently. Then a red coloration within 15-20 minutes indicates a positive result while no change in colour shows negative result.

Citrate Test Citrate Test

Citrate Test Citrate Test is used determine whether or not the bacterium can used sodium citrate as a sole source of carbon. Each Simmons citrate agar plate was labeled with the name of the isolate. The culture was inoculated for 24 to 48 hours at 35° C. The development of a deep blue colour shows positive while green shows negative result.

Coagulase Test

This test is used to differentiate *Staphylococcus aureus* (positive) from coagulase staphylococci (negative) coagulase that causes blood plasma to clot by converting fibrinogen to fibrin. The isolate saline and plasma was added, positive result indicated by the clumping of the bacteria cell showing agglutination whereas negative result shows no clumping.

Sugar Fermentation Test (Triple Sugar Iron Agar Test)

Triple sugar iron agar is used to determine whether a gram negative rod utilizes glucose and lactose or sucrose fermentatively and forms hydrogen sulphide.

With the aid of a straight inoculating wire, a well isolated colony was inoculated firstly by stabbing through the center of the medium to bottom of the tube and the streaking the surface of the agar slant of each prepared TSI agar prepared for each isolate. No change in butt and slant, negative for glucose, lactose, H_2S and sucrose. Red slant and yellow butt, only glucose positive. Red slant and yellow butt with gas production, glucose, sucrose, and lactose positive, H_2S negative.

III. RESULTS

A. The Mean of Heterotrophic Bacterial Count

The mean of heterotrophic count number (THBC) fromto-eat natural product servings of combined fruits samples as displayed in table1 uncovers that sample sixteen (16) had the most expanded commonness of foreign substances (8.5 x 10^6) even as take a look at 8 (eight) had the least pollutants (1.6 x 10)

B. The Mean of Total Coliform Count

The suggest of total coliform count (TCC) from read-toconsume organic product plates of mixed fruit demonstrated in table 2 confirmed that sample three (three) had the most noteworthy overseas contaminants (11.7 x 10^6) at the same time as test one (1) and test 4 (four) had the least pollutants with (1.5 x 10^5) one at a time.

C. Biochemical Reactions of Bacteria Isolates

The biochemical take a look at in table 3 (three) uncovers the accompanying existence shape, ultimately *Escherichia coli*, *Staphylococcus Sp*, *Bacillus Sp*, *Streptococcus Sp*, *Enterobacter Sp*, and *Salmonella Sp* are available

D. Bacterial Species Isolated

Microorganisms that were secluded from assessments include *Bacillus Sp, Escherichia coli, Staphylococcus Sp, Enterobacter Sp, Streptococcus Sp,* and *Salmonella Sp.*

Staphylococcus Sp had the maximum expanded occasion of 13(32%) even as Salmonella Sp and Enterobacter Sp had minimal event of three (7.15\%) one at a time.

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SAMPLE CODE	THBC CFU/G	
1	3.5 x 10 ⁶	
2	$4.4 \ge 10^6$	
3	3.3×10^6	
4	3.6 x 10 ⁵	
5	6.3 x 10 ⁵	
6	$1.7 \ge 10^5$	
7	2.7×10^5	
8	1.6 x 10 ⁵	
9	3.1 x 10 ⁶	
10	8.6 x 10 ⁶	
11	3.1 x 10 ⁶	
12	5.2 x 10 ⁶	
13	3.3 x 10 ⁶	
14	$5.2 \text{ x} 10^6$	
15	$3.0 \ge 10^6$	
16	8.5 x 10 ⁶	
17	$3.1 \ge 10^6$	
18	$2.2 \ge 10^{6}$	
19	$3.6 \ge 10^6$	
20	$5.6 \ge 10^6$	

Table 1: Total Mean of Heterotrophic Bacterial Counts from Ready-to-eat Salads

Table 2: Total Coliform Counts from Ready-to-eat Fruit Salads

SAMPLE CODE	Total Coliform Count	
1	1.5 x 10 ⁵	
2	2.4×10^5	
3	$11.7 \ge 10^{6}$	
4	$1.5 \ge 10^5$	
5	9.5 x 10 ⁶	
6	$11.1 \ge 10^{6}$	
7	$5.2 \ge 10^6$	
8	6.1 x 10 ⁶	
9	$6.5 \ge 10^6$	
10	$2.2 \ge 10^{6}$	
11	$3.8 \ge 10^6$	
12	$3.4 \ge 10^6$	
13	$3.8 \ge 10^6$	
14	$4.1 \text{ x} 10^6$	
15	$4.7 \ge 10^6$	
16	$3.0 \ge 10^6$	
17	$4.2 \ge 10^6$	
18	$3.4 \ge 10^6$	
19	3.7×10^{6}	
20	$4.1 \ge 10^6$	

Table 5: Result of microscopic and Biochemical Analysis of Isolates											
Gram	CAT	IND	CIT	MR	VP	H ₂ S	CO	Fermentation	SLANT	BUTT	Micro
stain											Organism
								L G S GAS CR			
-Ve	+	+	+	+	-	-	-	+ + + + +	R	Y	Escherichia
Short rod											coli
+Ve cocci	+	+	+	+	-	-	-	+ + + + +	R	Y	Staphylococ
cluster											cus Spp.
+Ve short	+	-	+	+	+	-	-	- + - + -	R	Y	Bacillus spp.
rod											
+Ve cocci	-	+	+	-	+	-	-	+ + + + -	R	Y	Streptococc
in chin											us spp.
+Ve short	-	-	-	-	+	-	-	- + +	Y	Y	Enterobacte
rod											r Spp
+Ve Long	+	-	+	+	-	-	-	- + +	R	Y	Salmonella
rod											Spp

 Table 3: Result of microscopic and Biochemical Analysis of Isolates

 $MR = Methyl red, VP = Voges Proskauer, H_2S = Hydrogen sulphide, L = Lactose, G = Glucose, S = Sucrose, R = Red, Y = Yellow, CAT = Catalase, IND = Indole, CIT = Citrate, CO = Coagulase.$

Table 4: Prevalence of bacterial species isolated from ready-to-eat fruit salads						
Total no of samples studies	20					
Total no of samples contaminated	20					
Percentage of the samples contaminated	100					
Number and percentage of samples contaminated with:						
Bacillus Sp	7 (17.5%)					
Staphylococcus Sp	13 (32.5%)					
Escherichia coli	8 (20%)					
Salmonella Sp	3 (7.5%)					
Enterobacter Sp	3 (7.5%)					

Table 4: Prevalence of bacterial species isolated from ready-to-eat fruit salads

IV. DISCUSSION

Streptococcus Sp

The usage of organized to-devour herbal product servings of mixed fruits straight forwardly from road merchants or peddlers probably expands the gamble of meals borne sickness brought about through a wide collection of microorganisms since it's miles challenging to bear witness to the cleanliness of those dealers or to the sterile instances at the motive behind dealing with in addition to the bundling materials. The large contrasts saw in tainting of gadgets from diverse roads might be an impact of the diploma of openness and dealing with strategies inside the dispensing locales, the items are opened as often because the client's request, open to expose of item to draw inside the customers energizes irregular visit through flies.

The presence examination uncovers high microbial (bacterial) load in the organic product plates of mixed fruits pondered. The bacterial impurities incorporate of numerous microbes, as an example, *Escherichia coli, Staphylococcus Species, Streptococcus Species, Bacillus Species, Salmonella Species, and Enterobacter Species.* The mean bacterial counts

of the organic product servings of blended fruits assessments is introduced in table1.

6 (15%)

The outcome suggests that one hundred percent of the samples had high all out viable counts going from 1.6×10^5 to 11.7 x 10⁶ settlement forming units for each gram of the servings of combined fruit homogenate. The presence of the existence paperwork in huge numbers inside the organic product plates of mixed fruits is of significant safety worry about the utilization of roads distributed food assets. A part of the residing beings experienced in this observe are like the ones revealed in comparative examinations from numerous nations (Mahale, 2008., Edward et al., 2012). This verify that organic product servings of combined veggies are surprisingly defenseless to microbial (bacterial) tainting. Microorganisms can sully herbal product servings of mixed fruits thru distinctive assets, as an example, unsanitary situations, unhygienic managing and coping with, usage of low pleasant of water to wash the natural products can purpose go pollution from different leafy ingredients or utilization of dirty managing utensils like blades, cutting sticks, slicing tables and plate. In this evaluation, Staphylococcus Species, Bacillus Sp

Salmonella Sp, and Escherichia coli were secluded from the natural product servings of combined veggies, those existence bureaucracy are acknowledged motives for food borne ailments and might have been added into the meals by way of unhygienic dealing with to the gamble of food borne disorder. The presence of waste coliform E.Coli in the plates of mixed veggies tests manner that waste tainting regularly related with faecally polluted water, squander water, or sewage muck. It is conceivable that traders didn't wash herbal merchandise appropriately or they could have applied faecally tainted water or waste water to clean organic products utilized in setting up the plates of mixed fruits. recommended washing of the component herbal merchandise provides those microbes into the plates of blended fruits bringing approximately tainting. The presence of coliform in natural product servings of combined fruits isn't authorized by way of secure food utilization wellknown (Andres et al., 2004).

Table four indicates the diploma of bacterial defilement of natural product plates of blended vegetables tested. Out of the 20 samples broke down, each one of the samples were tainted with one-of-a-kind forms of microorganisms, from the defiled samples, *Bacillus Sp* blanketed 17.5% while 32.5% and 20% of the sample yielded *Staphylococcus Sp* and *Escherichia coli* one after the other. Tainting with *Staphylococcus Sp* can be thru overseers while *Bacillus Sp* may want to have interacted with the herbal product through soil. The well worth of microorganisms pollution were given in this take a look at aren't exactly the ones announced by Edward *et al.*, (2012) in comparable evaluate port-Harcourt, Nigeria. The differences may be because of dissimilarity in the dealing with strategies and sterilization of the creation place and man or woman cleanliness of the street merchants.

The end result of this observe suggests that road dispensed organic product servings of mixed vegetables gift serious well-being risk to customers as they include multiplied diploma of unfavorable microorganism (microbes) which may purpose difficult disease. Since herbal product servings of blended veggies is normally eaten minus any additional handling, valid dealing with and reception of extreme aseptic strategies and tremendous man or woman cleanliness have to be stuck with the aid of avenue traders on the association degree to lower microbes load and do away with microscopic organisms defilement of the eventual final results.

V. CONCLUSION

The excessive microorganisms burden and provides of those natural entities, for sample, *staphylococcus Sp* in the organized to-eat herbal product plates of mixed vegetables exams should act as a marker for the want to boost mindfulness about the manageable threat that would be motive due to unlucky treatment of those natural product servings of combined greens. There is finally, the requirement for administrative our bodies to guarantee that microbiological preferred are laid out and rehearsed by way of ranchers and advertisers (merchants/carriers) for the taking care of and dissemination of those organic product plates of blended greens.

RECOMMENDATION

The sellers, water and poor washing of arms and utensils appear, by way of all accounts, to be a sizeable hazard related with those natural product plates of combined greens and ought to be deal with appropriately. In this manner Sellers and customers are exhortation to wash their new natural products as it should be previous to stripping, cutting, or reducing. Organic products must be given with wiped clean palms, utensils, and moreover put away in refrigerators if any put off previous usage. Great character cleanliness and viable hazard exam and fundamental control factor (HACCP) utility have to be ahead to lower the opportunity of tainting of prepared toconsume natural product plates of mixed veggies and Government administrative our bodies should make comes to a decision in an effort to tie traders of natural products plates of combined fruits.

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