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# Assessment of Nutritional Quality and Physico-Chemical Properties of Semi-Commercially Produced Mixed Fruit Juice From *Citrullus lanatus*, *Citrus sinensis* and *Citrus paradisi*

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## Abstract

Nutritional quality, a value added measure has recently been emphasized as an important consideration in the production of commercially available fruit juices in order to promote health and wellness of the consumers. Therefore, this study was designed to investigate the nutritional value of a laboratory produced mixed fruit juice using fresh samples of water melon, *Citrullus lanatus*; grape, *Citrus paradisi* and sweet orange, *Citrus sinensis*, under strict cleanliness. The microbiological assessment of fruit juices was carried out and raw untreated fruit juices were all coliform free. The mixed fruit juice had a pH value of 4.31, acidity; 5.30 g/ kg tartaric acid; specific gravity of 1.06 and organoleptic values (low), respectively. Pasteurization for 30 min at 75 $^0$C with added 40 ppm of sodium metabisulphite apparently optimized the product, being highly microbiologically stable. On the basis of combined favourable organoleptic, physicochemical and microbiological properties, this product could be suggested suitable for human consumption and therefore, recommended for commercial purposes.

**Keywords:** Watermelon; Grape fruit; Mixed fruit juice; Organoleptic values; Physico-chemical analysis.

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## 1. Introduction

Tropical regions of the world, including Nigeria, are very rich in fruit-bearing trees such as achee apple, bread fruit, avocado, grape fruit, pawpaw, walnut, coconut, *citrus* (orange, lemon), cashews, mangoes, pearl, etc [1]. Equally important also are vines, which are abundantly planted and consumed especially in Nigeria, the most prominent being the watermelon fruit (*Citrullus lanatus*). For a long time, water melon was commonly propagated in northern Nigeria from where it was transported and supplied to the southern part. Currently, it's largely planted in the southern Nigeria. Watermelon contains a significant amount of an amino acid *citrulline* [2]. It is also known to be mildly diuretic and contains large amount of carotene [3]. Water melon is characterized by thick rind (epicarp) and large, soft mesocarp and endocarp in which many seeds are embedded [4]. The rind is usually light green, white or speckled with the reddish mesocarp / endocarp as a remarkable source of lycopene [5].

Grape fruit (*Citrus paradisi*) is a subtropical citrus known for its tartness. It's typically in season from October-June, and exists in varieties which are determinable by color caused by pigmentation due to its state of ripeness [6]. The most popular varieties cultivated today are red, pink and white hues commonly exhibited by their fruit's pulp. The pink and red hues of the mesocarp are embedded some seeds and beneficial antioxidant lycopene, just as found in watermelon [5]. Their flavor ranges from highly acidic and somewhat bitter to sweet Marcaptan [7]. Grape's Marcaptan is Sulphur-containing terpene that imparts a strong influence on the taste and color of grape fruit compared with other citrus fruits [8].

The sweet orange (*Citrus sinensis*) is the most economically important fruit crop, as well as the most popular widespread fruit in the world [9]. Just like the grape fruit, citrus fruits also exist in varieties, the two most important being the loose-skinned and tight-skinned types [10]. The orange fruit is believed to be native to southern China from where it was probably carried into USA, Africa, Israel, Japan, Palestine and Brazil. The fruit juices are known to be rich in vitamin C, calcium, potassium, magnesium, zinc and iron [9]. Sodium is completely absent in fruit species [11].

However, fruit juices are suitable substrates for heterogenic microorganisms, particularly bacteria and yeast which are capable of surviving high temperature treatments and able to grow at moderately low pH [12]. Yeasts are part of the natural microbiota of grapes [13]. Among the most commonly encountered spoilage bacteria in fruit juices and beverages are the species of *Acetobacter*, *Alicylobacillus*, *Lactobacillus*, *Leuconostoc*, *Bacillus* and *Clostridium* [14]. Non-spore forming vegetative form of anaerobic microorganisms (*Propionibacterium cyclohexanicum)* have been reported to be resistant to high pasteurization in orange juice [15]. Deteriogenic bacteria species frequently associated with processed fruit juices have been found to include microorganisms from the genera *Lactobaccillus* and *Leuconostic*, which constitute the most important lactic acid bacteria (LAB) which has been reported to be responsible for producing off-flavor, off-odor and off-color [16]. It has been established that the diacetyl produced by LAB is partially responsible for that undesirable flavor and odor in orange juice and it's also the main indicator of deterioration in orange juice [17].

Microorganisms invade plant tissues during various stages of fruit development. Contamination of fruit surfaces and juices by microorganisms has been shown to occur via soil during harvest, postharvest processing, including transport, storage and processing [18, 19]. A second factor contributing to microbial contamination of fruits has been connected to their post-harvest handling inadvertently from production personnel or food processing materials and fermentation of products [20]. Yeasts present in fruit juice have been implicated in the fermentation process [21].

To limit fruit damages and losses, small cottage industries maybe necessary to change the fruit juice to a more enduring, commercial product to boost our economy as well as our nutritional status. Therefore, the main objective of this work was to fabricate a mixed fruit juice from sweet orange, melon and grape fruits based on some physico-chemical, organoleptic and microbiological properties.

## 2. Materials and Methods

### 2.1. Sample Collection and Preparation

#### 2.1.1. Sample Collection

Fresh raw water melon (*Citrullus lanatus*), orange (*Citrus sinensis*) and grape (*Citrus paradisi*) fruits were bought from Ago Aduloju market in Ado-Ekiti, Ekiti State, Nigeria. They were washed with chlorinated water (about 10 ppm) and towel-dried.

### 2.2. Preparation of Juice Samples

With the aid of a sterilized knife, sweet orange and grapefruit were peeled, cut into halves and their juices removed separately with a juice extractor (Fruit juicer - model no - JE-380). The watermelon was sliced into pieces, the outer hard epicarp removed while the softer mesocarp and endocarp were fed into juice extractor to remove its juice. The sweet juices were filtered to separate pulp from juice. They were kept in refrigerator while some preliminary unit operations were on-going.

### 2.3. Production of Mixed (combined) Fruit Juice (MOG)

The raw fruit juices of watermelon juice, orange juice and grape juice were combined in ratio 2: 2: 1, respectively. Mixed fruit juice (MOG) was made from ratio 1:1 of the mixed juices to previously boiled, cooled water sweetened with 0.4 g/100 ml, n-low sweetener.

### 2.4. Physico-Chemical Analyses of MOG

Evaluation of pH, specific gravity and crude nitrogen were carried out according to the methods of Kumar et al. (2018). Acidity was estimated by methods of Ighodaro, *et al.* [22]. Estimation of yeast assimilable nitrogen (YAN) was made by formol titration technique.

### 2.5. Microbial Analysis of MOG

Raw fruit juices prior to their processing into desired end product (semi-commercial fruit juice product) were assayed for bacteria and yeasts using standard methods for the examination of water and wastewater, according to American Public Health Association (APHA) described by Lopez [23], and coliforms were determined by the method of Wohlsen, *et al.* [24].

### 2.6. Treatment Designed and Protocols

#### 2.6.1. Treatment, Storage and Microbial Assay of MOG

The under-listed treatment protocols were critically examined, stored under laboratory conditions and assayed for bacteria, yeasts and coliforms as in microbial assay stated above:

## 2.7. Treatment Steps

i. Fresh MOG without heat or chemical treatment.
ii. Fresh MOG bottled, corked and kept on laboratory bench for five (5) days.
iii. Fresh MOG treated with Sodium Metabisulphite at 3 mg/100 ml and 4 mg/100 ml bottled, corked and studied for 2-4 weeks (15-30 days).
iv. Fresh MOG pasteurized in thermostatically controlled water bath at 75% for 30 min. Cooled under running water and observed for 0-30 days.
v. Fresh MOG subjected to combined sterilization and chemical preservative ($SO_2$) treated and studied as in (iv).
vi. Fresh MOG bottled and autoclaved at 121°C for 15 min cooled and studied for 0-30 days.

## 3. Data Analyses

Data were analyzed using SPSS 18 software (SPSS Institute Inc., Cary, NC, USA), using one-way analysis of variance (One-way ANOVA) followed by the Duncan multiple range test. Values were considered statistically significant at $p < 0.05$ with results expressed as mean ± standard deviation (SD).

# 4. Results

## 4.1. Physico-Chemical Properties of Raw Fruits and MOG

Physico-chemical properties of individual fruit juices and mixed fruit juice are presented in Table 1. In the results, it was shown that all the raw fruit juices and their mixed product are moderately acidic compared with citric and tartaric acids as standards (references). Similarly, the specific gravity of the juices is close to 1.0. The formol value as well as their crude protein are very low.

## 4.2. Microbial Analyses on Raw Fruit Juices and MOG

Microbial analyses on raw fruit juices and MOG in culture medium are represented in Table 2. It was revealed that within the holding period, these microorganisms have increased from 16-25 to 33 (CFU/ml) and yeast cells from between 3-6 to 8 (SFU/ml) in separate fruit juices.

## 4.3. Microbiological Stability and Organoleptic Properties of MOG Only

Microbiological stability and organoleptic properties of mixed fruit Juice (MOG) are presented in Table 3. The results show the microbiological and organoleptic properties of MOG through the assessment of various yield counts and parameters, i.e, nutrient agar TBC (coliform count, CFU/ml), malt agar yeast count (spore forming units, SFU/ml), MacConkey agar (CFU/ml) and organoleptic signs.

## 4.4. Comparative Organoleptic of MOG with Commercially Available Juice

Evaluation of organoleptic of MOG with commercially available juice is presented in Table 4. As indicated in the results, organoleptic properties of the new product (MOG) revealed a significant ($p < 0.05$) difference compared to a well-known commercially available juice (Fumman juice), in parameters such as color/appearance, taste, mouth feel and odour.

# 5. Discussion

A few number of reports have shown that fruit juices have nutritional advantages and promote good health [25, 26]. However, the prevalence of commercially produced fruit juices in the present days has drastically discouraged the nutritional importance of the naturally available fruit juices [27].

In our study, it is clearly revealed that all the ripened, raw fruits involved in the work were not completely free from bacterial and yeast cells but all were coliform – free (Table 1). The invasion of plant tissues during fruit developmental stages and or invasion of fruit surface during harvest and storage may be a reason for the initial microbial load reported in this study in agreement with previous reports [17]. Another factor that may contribute to microbial contamination of fruits, apart from yeasts being the innate microflora of fruits, may pertain to the physico-chemical composition of fruit juices and mixed fruit juices, however, considerably satisfied chemical properties were revealed in MOG in this study (Table 2) [28]. Yeasts present in fruit juice have been implicated in the fermentation process by attacking low molecular sugars found in fruit juice to cause production of alcohol and carbon dioxide [29]. Due to their generally faster growth rate than moulds, yeasts often precede the latter organisms in the spoilage process of fruit in certain circumstances [18]. The destruction of the higher molecular weight fruit constituents is only brought about more by moulds than yeasts. Inevitably the natural microbial components of fruits, coupled with the holding period needed to process the juice may be the cause of their prolific growth rate in raw or inadequately processed juice and hence microbial spoilage (off-flavour, off- colour) as found under five-day storage of raw fruit juice [30]. In this study however, sterilization of new product at the chosen temperature destroyed all colonial microorganisms and the product remained sterile during storage periods, between 0-30 days displaying no off colour or off taste. The resulting colour fade may be a big snag suggesting undesirable chemical reaction. This may also warrant application of colorant not ideally desired to change the natural hue of the product.

Similarly, the product to which lower concentration (30 ppm) of $SO_2$ was applied (Table 3), manifested substantial bacterial damage without any noticeable effect on yeast cells. At 40 ppm of $SO_2$ treatment, no bacterial cells were found and yeast cells considerably reduced. This work shows that $SO_2$, the chemical preservative applied in this work is more potent on bacteria than yeast cells [24]. With the level of residual yeast cells, there was no alteration in the organoleptic properties of the new product. The tart or rather pungent juice taste, believed to be due to the presence of grape's marcaptan was not, however, suppressed in the finished product. This taste may be taken as the product's peculiar property for its identification. Although pasteurization had a pronounced effect on bacterial growth, TBC of 0-91 CFU/ml and TYC of 46- 96 SFU/ml signaled resistance of organisms to the conditions obtainable at chosen temperature and time. This level of treatments may cause product damage during storage, even at a short expiry date [31].

Application of combined chemical treatment and pasteurization produced the most acceptable result post process at room storage period, especially when $SO_2$ was applied at 40 ppm followed by pasteurization at $75^0$C for 30 min. Extended shelf stability effected by these two synergistic applications is believed to represent the common practice in industries producing similar fruit juices preparatory to their wide distribution in order to forestall deterioration before their expiry dates [32, 33]. The organoleptic properties of the new product (Table 4), except for its taste, were, however, considerably lower than the factory produced Funman juice. Similarly the sensory scores exhibited by the raw product (MOG) and other parameters are considerably adjudged to be acceptable being above recomended minimum acceptable sensory score.

# 6. Conclusion

The new product revealed acceptable parameters based on its shelf stability, physico-chemical and sensory properties. Non-appearance of coliforms in the finished products simply indicates the meticulous hygienic practice on the part of the processing personnel. Therefore, it could be recommended for public consumption, value-added and profit-making venture.

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## Conflicts of Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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**Table-1**. Physico-chemical composition of fruit juices and mixed fruit juices

| Parameter | Water melon Juice | Orange Juice | Grape Juice | Mixed MOG Juice |
|---|---|---|---|---|
| pH | 5.17 | 4.03 | 3.81 | 4.31 |
| Acidity (g/kg) | 1.05 ± 0.05 as Citric acid | 7.30 ± 0.03 as Citric acid | 9.50 ± 0.10 as Tartaric acid | 5.30 ± 0.10 as Tartaric acid |
| Specific gravity | 1.05 | 1.10 | 1.04 | 1.06 |
| Formol value (amino acid value) | 1.55 | 2.30 | 2.60 | 2.80 |
| Crude protein | 0.05% | 0.08% | 0.09% | 0.07% |
| Colour produced | Amaranth | Yellow | Cream | Pink orange |

**Table-2**. Analysis of microbial assay in culture medium

| Juice Source | Total bacterial count in nutrient agar (CFU/ml) | Yeast/mold in malt agar (SFU/ml) | Coliforms in MacConkay agar (CFU/ml) |
|---|---|---|---|
| Grape | 16 | 3 | Nil |
| Orange | 21 | 4 | Nil |
| Watermelon | 25 | 6 | Nil |
| Combined MOG | 33 | 8 | Nil |

**Table-3.** Microbiological stability and organoleptic properties of mixed fruit Juice (MOG)

| Treatments | Storage Period ( Days) | Nutrient Agar TBC (CFU/ml) | Malt Agar Yeast count (SFU/ml) | MacConkey agar coliform count (CFU/ml) | Organoleptic signs/colour | Odour | Taste |
|---|---|---|---|---|---|---|---|
| Raw untreated | Zero | 33 | 8 | Nil | OK | OK | OK |
| Raw bottled and corked | 5 | TNTC | TNTC | Nil | CL | Off odour | Off Taste |
| **Chemical treatment** | | | | | | OK | OK |
| 30 ppm of $SO_2$ | 15 | 04 | TNTC | Nil | OK | OK | ST |
| 40 ppm of $SO_2$ | 30 | Nil | 296 | Nil | OK | | |
| **Pasteurization** | | | | | | | |
| Pasteurized at 75°C in water bath (30 min) | Zero | Nil | 17 | Nil | OK | OK | OK |
| | | | | | | | |
| Pasteurized at 75°C in water bath (30 min) | 15 | 01 | 46 | NIL | OK | OK | OK |
| | | | | | | | |
| Pasteurized at 75°C in water bath (30 min) | 30 | 91 | 96 | Nil | OK | OK | OK |
| **Combined pasteurization and chemical treatment** | 15 | Nil | 02 | Nil | OK | OK | OK |
| 30 ppm of $SO_2$ + | 30 | Nil | Nil | Nil | OK | OK | OK |
| 40 ppm of SO2 + | | | | | | | |
| **Raw autoclaved** | | | | | | | |
| at 121 °C for 15 min | Zero | Nil | Nil | Nil | OK | OK | OK |
| at 121 °C for 15 min | 15 | Nil | Nil | Nil | FD | OK | OK |
| at 121 °C for 15 min | 30 | Nil | Nil | Nil | FD | OK | OK |

**Keynotes:** TNTC, too numerous to count; CL, cloudy/slurry deposit; SL, slimy; FD, faded; OK; no off odour, no off colour and no off taste.

**Table-4.** Comparison of organoleptic properties of the new product with a known commercially available juice

| Parameter | Fumman Juice | MOG |
|---|---|---|
| Color/appearance | $5.00 \pm 0.00$ [a] | $3.6 \pm 0.42$ [b] |
| Taste | $5.00 \pm 0.00$ [a] | $4.4 \pm 0.17$ [a] |
| Mouth feel | $5.00 \pm 0.00$ [a] | $4.0 \pm 0.41$ [b] |
| Odour | $5.00 \pm 0.00$ [a] | $3.7 \pm 0.85$ [c] |

**Note:** Sensory score of panel members for the new product was noted to be 3.6 - 4.4 which is significantly ($p < 0.05$) higher than 2.5, the minimum acceptable sensory score.

The values were expressed as mean ± SE. Values not commonly superscripted along horizontal axis are significantly ($p < 0.05$) different.