

Heat Resistance And Inactivation of *Neosartorya fischeri* and *Talaromyces flavus* ascospores in water, phosphate buffers, fruit juices and fruit juices fortified with sugars and preservatives.

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Abstract: This work was designed to study some heat resistance characteristics of the isolates obtained in relation to fruit processing parameters. The effect of heat (85⁰, 90⁰, 92.5⁰ and 95⁰C) in combination with common fruit products preservatives (sodium benzoate and citric acid) and sugars (fructose and sucrose) on inactivation of ascospores of *N. fischeri* and *T. flavus* was studied in water, phosphate buffers, grapefruit, orange and pineapple juices. The rate of thermal inactivation was highest in medium containing sodium benzoate. The presence of sugars (sucrose and fructose) in juices protected the ascospores against heat inactivation when compared with plain juices. Ascospores of *T. flavus* had D₈₅⁰_C values which ranged from 10.6 to 78.9min; D₉₀⁰_C values of <1 to 13.6min and D_{92.5}⁰_C values of <1 to 8.1min. Ascospores of *N. fischeri* had D₈₅⁰_C values which ranged from 61.9 to 217.5min; D₉₀⁰_C values of 5.0 to 62.6min; D_{92.5}⁰_C values of 1.1 to 19.7min and D₉₅⁰_C values of <1 to 13.6min. These results mark the high heat resistance of ascospores of heat resistant fungi isolated from the tropics. Heat resistant fungi not only cause spoilage of fruit products but also produce highly toxic and sometimes carcinogenic compounds; these findings are of utmost importance to fruit processing industries which are presently increasing in number in Nigeria and other developing countries.

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1. Introduction

Ascospores of heat resistant fungi (HRF) vary in their capacity to withstand thermal treatment. Heat resistance is influenced by many factors that include species, strain, nature of heating medium, the temperature of treatment, age, nature of suspending/storage medium and the presence of anti-mycotic agents among others. Ascospores from different cultures differ in heat resistance even though they represent same specie and are grown under similar conditions (King and Whitehand, 1990).

This work was designed to study some heat resistance characteristics of the HRF isolated from the soils of vegetable and fruit producing zones of South Eastern/Middle belt in Nigeria in relation to fruit processing parameters.

2. Materials and methods

2.1. Isolation and Preparation of ascospores

Heat resistance fungi were isolated from soil samples collected from gardens, orchards and farmlands in South Eastern/ Middle belt zones in Nigeria according to the method of Okagbue(1989). Large amounts of fully developed and mature ascospores were obtained by inoculating selected isolates on PDA slants and incubating for 30 days at room temperature (Splittstoesser *et al.*, 1970; Amaeze *et al.*, 2010). Ascospores in asci or cleistothecia were then dislodged by flooding each slant with 2-3ml of

sterile 0.1M potassium phosphate buffer (PB), pH 6.0, followed by scrapping the culture surface with a sterile wire loop. Heat resistance was confirmed by heating ascospores for 1hour at 70⁰C with the water level in the bath at least 3cm above the level of suspension in the tubes. The heating achieved inactivation of mycelia and conidia and activation of ascospores if any heat resistant ones were produced. Fungal growths, which occurred after the incubation, were considered to be heat resistant fungi. The colonies were further purified on PDA plates and transferred to slide cultures for preliminary identification. For further identification, plates of czapek yeast extract agar (CYA), malt extract agar (MEA) and 25% glycerol nitrate agar (G25N) were used for the identification of *Penicillium* spp and their teleomorphs according to the methods and keys of Pitt (1979). Identification of common *Aspergillus* spp and their teleomorphs was based on growth and cultural characteristics on three media CYA, MEA and czapek yeast extract agar with 20% (CY20S) at two temperatures 37⁰C and 25⁰C as described by Raper and Fennel (1965). Cultures were also put on slants of PDA for preservation and deposited with Prof. J.O. Ugwuanyi of the Department of Microbiology, University of Nigeria, Nsukka.

Pure PDA cultures of representative confirmed heat resistant isolates (3C, E7, 2E and 5A), were inoculated onto slants of PDA in 50ml capacity universal bottles. The cultures were incubated at room

temperature (28°-30°C) for 30 days to ensure ample time for ascospore formation and maturation. To harvest ascospores at the end of the incubation, slants were flooded with sterile deionized water and asci and/or cleistothecia were scrapped off the culture surface with sterile wire loop. The scraping was poured off into another 50ml universal bottle. The procedure was repeated several times to achieve 40ml of asci/cleistothecia suspension and also ensure complete harvest of spores. The cleistothecia / asci suspension prepared were treated to release free ascospores. The suspensions were separately sonicated in a sterile 50ml beaker while being cooled by ice at 0-2°C with a 5 min treatment of 60w to disrupt the asci and cleistothecia and release the free ascospores using a Virsonic model 16-850 cell disrupter (The Virtis Company Gardiner New York). The suspensions were filtered separately through sterile glass wool to remove hyphal fragments. After this, the ascospore suspensions were stored in sterile 0.2cm (diameter) glass beads to minimize clumping in 50mls capacity universal bottles. Ascospore suspensions were stored at 4°C in the refrigerator.

2.2. Enumeration of ascospore

Direct microscopic counts were determined with a haemocytometer. Ascospores were readily recognizable and countable at 40-fold magnification. Direct microscopic counts were taken and total ascospore population/ml of sample was determined as described in the manual of haemocytometer.

2.3. Preparation of fruit juices

Ripe healthy pineapple, grapefruit and orange fruits were purchased from Nsukka main market, Nsukka Local Government Area, Enugu State, Nigeria. Under aseptic conditions in the laboratory their juices were extracted, filtered through sterile filter cloth and the clear juice collected in 250ml screw capped conical flasks and stored at -5°C in a freezer. 10mls of the juice was removed for determination of pH and total soluble carbohydrate before storage. The stored juice was used as such without any further treatment.

2.4. pH of the various fruit juices

The pH values of the three locally prepared fruit juices were determined using a pH meter within the hour of the juices preparation. The pH meter used was model 290mk 2 (PYE UNICAM).

2.5. Total soluble carbohydrate content in prepared fruit juices

Total soluble carbohydrate was determined in the fruit juices using the anthrone reagents method (Deriaz, 1961)

2.6. Preparation of anthrone's of reagent

Anthrone reagent was prepared by stirring in 760ml of sulphuric acid (approx 98% w/w H₂SO₄) into 330ml of water. The mixture was cooled and 1g each of thiourea and anthrone were added and stirred until dissolved. The mixture was stored in a refrigerator until needed. Immediately before use, 0.4g of anhydrous D (+)-glucose was dissolved in tap water and diluted to 500ml. 0, 5, 10, 15, 20 and 25ml of glucose stock solution were measured into 100ml graduated flasks and diluted to 100ml.

To 2ml of appropriately diluted juice and glucose solutions in test tubes, 10ml of anthrone reagent was rapidly added and mixed by inversion. The test tube was loosely covered with a glass bulb stopper and immediately placed in a boiling water bath for 20min. The mixture was cooled and the absorbance measured in a 10mm optical cell at 620nm in a digital photocolormeter (Milton Roy). A standard curve of absorbance (nm) against concentration of glucose (mg/ml) was plotted from which total soluble carbohydrate of the juices were deduced.

2.7. Thermal resistance of ascospores

2.7.1. Thermal resistance/inactivation of ascospores in locally prepared fruit juices water and buffers

Thermal inactivation characteristics (D-values) of each test mold were investigated in fruit juices, water and acetate buffers (AB) prepared to correspond to the pH of the various fruit juices. 0.1ml of the counted ascospores was added to 9.9mls of each juice or buffer in sterile 10mm wide and 20ml capacity screw capped test tube adjusted to the desired temperature (85°, 90°, 92.5° or 95°C) in water bath. A similar test tube with 10ml of distilled water with a thermometer was used as a control to ensure that the desired temperature was obtained before addition of ascospores and maintained during the heating period. For *N. fischeri* (3C and E7) the selected sampling intervals/duration of heating were 20min for 480min at 85°C; 5 or 10min for 140min at 90°C; 3min for 24min at 92.5° and 95°C respectively. For *T. flavus* (2E and 5A) the selected sampling intervals/duration for heating was 10min at 85°C and 3min for 30min at 90° and 92.5°C. At sampling intervals, heating mixtures were agitated with the sterile micropipette inside the water bath and 0.1ml was withdrawn into sterile 4.9ml of water in test tubes maintained at room temperature. 0.1ml of each sample was withdrawn and plated out by pour plate method using single strength PDA. Plates were incubated at room temperature for 3-5 days before mold colonies were counted. The decimal reduction values were calculated using the equation

$$D_{\text{-value}} = \frac{t}{(\log a - \log b)} \quad (\text{Hersom and}$$

Hulland, 1969)

Where a and b are the initial count and the remaining survivors of ascospores/ml and t the heating time in minutes, at a given temperature, T. The highest obtained after heat activation before inactivation started was used as the term a

2.7.2. Effect of fructose, sucrose, citric acid and sodium benzoate on thermal resistance of ascospores in locally prepared juices

A concentration of 0.1% of citric acid and sodium benzoate and two concentrations (10 and 40%) of sucrose and fructose each in orange, pineapple and grapefruit juices were prepared. (All reagents used were obtained from BDH Poole England).

The preservatives (0.1%) and the sugars (10% and 40%) were prepared in sterile 250ml Erlenmeyer flask. 9.9ml of each solution was withdrawn into sterile test tubes and adjusted to the desired temperature (85°, 90°, 92.5° and 95°) in a water bath. 0.1ml of ascospore suspension of known total viable population (of each test isolate) was added. Samples were heated for same periods and withdrawn at intervals as described above and plated out by pour plate method. Incubation was at room temperature for 3-5 days.

3. Results

The isolates obtained were either *Aspergillus* spp (*Neosartorya fischeri* and *Emericella rugulosa*) or *Penicillium* spp (*Paecilomyces variotii*, *Talaromyces flavus*, *Eupenicillium* spp) (Amaeze *et al*, 2010).

3.1. Total count of ascospores (haemocytometer count)

Standardized ascospores population for each test organism is shown on Table 1.

Table 1. Haemocytometer counts of ascospores/ml

Isolate	No of ascospores /ml
<i>N. fischeri</i> (E7)	1.1 x 10 ⁸ /ml
<i>N. fischeri</i> (3C)	8.2 x 10 ⁷ /ml
<i>T. flavus</i> (5A)	5.8 x 10 ⁷ /ml
<i>T. flavus</i> (2E)	1.3 x 10 ⁷ /ml

3.2. The pH and soluble carbohydrate content of locally prepared fruit juices

The pH and soluble carbohydrate content of locally prepared fruit juices are shown in Table 7. The pH ranged from 3.6 to 4.2 and soluble carbohydrate from 22.50mg/ml to 111.25mg/ml.

3.3. Thermal resistance of ascospores of *N. fischeri* and *T. flavus*

The decimal reduction data (D-values) for test isolates are shown in Tables 2, 3, 4 and 5.

Ascospores of *N. fischeri* (3C) were more heat resistant than ascospores of *N. fischeri* (E7) in most of the medium in which the heat treatment was administered at 85° but at higher temperatures used the reverse was observed. The rate of thermal destruction for the *N. fischeri* was very slow at 85°C. D-values for the two isolates of *N. fischeri* (E7 and 3C) at 85°C ranged from 61.9 to 217min. D_{90°C} values of 5.0 to 62.6min. D_{92.5°C} values of 1.1 to 19.7min and D_{95°C} values of <1 to 13.6min depending on the heating medium.

Ascospores of *T. flavus* (2E and 5A) exhibited considerably less heat resistance than those of *N. fischeri*. Complete inactivation was achieved at 95°C in all the media. Ascospores of *T. flavus* had D_{85°C} values which ranged from 10.6 to 78.9min, D_{90°C} values of <1 to 13.6min and D_{92.5°C} values of <1 to 8.1min. Ascospores of *T. flavus* (2E) were considerably more sensitive to heat than those of *T. flavus* (5A).

4.0. Discussion

There was a general low yield of ascospores of *N. fischeri* and *T. flavus* (Table 6) compared to enumeration in the haemocytometer (Table 1). A similar result has also been reported by Splittstoesser *et al.* (1993) and Amaeze and Ugwuanyi (2011). The non-uniform response of ascospores to heat shows heterogeneity in physiology in the ascospores. The low recoveries did not appear to be related to the strain or specie of the isolates. Low recoveries may indicate that the many spores were truly non-viable and thus do not have the potential to produce new vegetative growth.

Ascospores of *T. flavus* were more heat labile. At 95°C, ascospores did not survive 1min heat treatment in the entire heating medium.

The rate of thermal inactivation of *N. fischeri* and *T. flavus* appeared to be slowest in pineapple juice, followed by grape fruit juice. Relating the inactivation rates calculated for the ascospores heated in plain juices with the result of pH and soluble carbohydrate of the juices on Table 7 showed a correlation between D-values and pH and soluble carbohydrate. Pineapple juice which had the highest soluble carbohydrate (111.2mg/ml) and pH 4.2 had the greatest protective effect against heat on the ascospores. Grape and orange juice had not only lower soluble solids but also lower pHs. The contrast between the D-values of ascospores of test isolates in PBs and plain juices goes to point out that the presence of soluble carbohydrate affected the D-values since the pH of the buffers was same as that of the juices. The rate of thermal reduction was greater in

phosphate buffers than in the plain juices. Ascospores of *N. fischeri* and *T. flavus* were most resistant to heat in juices fortified with sugars. This resistance increased with increasing sugar. A close look at the D-values calculated for the ascospores in juices fortified with sugars showed that sucrose conferred more protection on the ascospores than fructose. Rate of inactivation was most rapid in juices fortified with preservatives, sodium benzoate and citric acid.

Comparison of D-values showed that D-value decreased in the order of juices fortified with 40% sugar > juices fortified 10% sugar > plain juices > juices fortified with preservatives or distilled water or phosphate buffers. This indicates that some components in juices e.g. sugars had a protective effect on ascospores during heating and may have influenced rates of ascospore inactivation. Beuchat and Toledo (1977) reported that ascospores of *B. nivea* were protected against inactivation when heated in fruit products supplemented with sucrose. The mechanism was due in part to establishment of an osmotic pressure differential between the heating medium and ascospores, which favoured heat resistance.

Increased resistance of ascospores in sucrose agrees with Corry (1976) report on osmophilic yeasts. Resistance of *Saccharomyces rouxii* and *Schizosaccharomyces pombe* were maximum in solutions of sucrose, less in sorbitol and least in solutions of glucose, fructose and glycerol. This could be due to differences in ability of solutes to penetrate the cell membrane. Corry (1974) also reported sucrose to be the most protective solute for yeasts during heating. This could be responsible for the somewhat increased resistance of ascospores in pineapple juice as it contains in nature 10.5% of sucrose as opposed to 8.1% of sucrose in orange and 7.3% glucose in grape (Holland *et al.*, 1992). Splittstoesser and Churey (1991) have however reported Dvalue in pineapple juice to be same as in grapefruit juice.

Splittstoesser and Churey (1991) reported $D_{85^{\circ}\text{C}}$ of 12, 13 and 12 min in grape fruit, orange and pineapple juices respectively; Tournas and Traxler (1994) reported $D_{85^{\circ}\text{C}}$ of 19.6 to 29.5 min; $D_{90^{\circ}\text{C}}$ of 4.7 to 7.6

min; $D_{95^{\circ}\text{C}}$ of 1.7 to 2.3min in deionized water, pineapple and pineapple juice concentrate. In this study Dvalues as high as 217.5min for *N. fischeri* and 78.9 mins for *T. flavus* at 85°C were obtained. Even though these values are high, there are several other comparable results in literature. Beuchat (1986) reported slightly similar Dvalues especially at 85°C ($D_{85^{\circ}\text{C}}$ for *N. fischeri* ranged from 15.1 to 116min and 20.4 to 68.1mins for *T. flavus* in different fruit juices). Some *Neosartorya* ascospores have extra-ordinary high resistance to heat. Some of them reportedly survived heating at 100°C for 60min or longer when suspended in distilled water (Kavanagh *et al.*, 1963). McEvoy and Stuart (1970) reported a 100% survival after heating in distilled water at 80°C for 20min and 0.002% survival after heating for 15min at 100°C . Ascospores were found to survive for > 300min of heating at 70°C , 75°C and 80°C in various fruit juices (Rajashkhara *et al.*, 2000). This marks the extraordinary heat resistance of the ascospores of the test isolates in this study. There is therefore a possibility the ascospores of HRF isolated in the tropics exhibit unusual resistance to heat and needs to be investigated further.

The poor recovery of ascospores raised a question as to the validity of heat resistance determinations that has been conducted in different media; water, buffers juices and juices fortified with sugars and preservatives. If only a fraction of the dormant spores were being cultured, it was possible that the Dvalues that were obtained do not reflect the resistance of the spore population in general. Splittstoesser *et al.* (1993) have investigated this and reported that although a large proportion of the ascospores population may not be cultured, this does not necessarily introduce a significant error into heat-resistance measurement.

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Table 2: Decimal reduction values (D- values) for *Neosartorya fischeri* (E7) at 85° , 90° , 92.5° and 95°C in different heating medium.

Heating Medium	Dvalues at 85°C (min)	Dvalues at 90°C (min)	Dvalues at 92.5°C (min)	Dvalues at 95° (min)
Distilled H ₂ O	111.4	28.5	19.7	1.1
Acetate buffer (pH 3.6)	79.7	9.9	2.9	<1
Acetate buffer (pH 4.2)	61.9	18.0	5.0	3.9
Orange juice	119.7	34.8	5.3	4.9
Grapefruit juice	144.8	16.7	6.8	6.5
Pineapple juice	217.5	37.5	14.3	5.1
Grapefruit juice + 10% fructose		29.4	12.9	2.9
Grapefruit juice + 40% fructose		40.2	16.3	10.7

Grapefruit juice + 10% sucrose	21.2	16.3	4.8
Grapefruit juice + 40% sucrose	28.5	8.4	10.7
Orange juice + 10% fructose	20.5	10.7	5.3
Orange juice + 40% fructose	45.0	10.1	9.6
Orange juice + 10% sucrose	33.2	5.5	6.2
Orange juice + 40% sucrose	41.6	8.4	10.0
Pineapple juice + 10% fructose	50.8	6.9	12.3
Pineapple juice + 40% fructose	62.6	17.6	13.6
Pineapple juice + 10% sucrose	46.0	13.9	9.4
Pineapple juice + 40% sucrose	46.2	16.3	6.6
Grapefruit juice + 0.1% citric acid		5.1	
Grapefruit juice + 0.1% sodium benzoate		<1	

Table 3: Decimal reduction values for *Neosartorya fischeri* ascospores (3C) at 85°, 90°, 92.5° and 95°C in different heating medium

Heating Medium	Dvalue at 85°C(min)	Dvalues at 90°C(min)	Dvalues at 92.5°C(min)	Dvalues at 95° (min)
Distilled H ₂ O	197.4	28.8	18.4	10.2
Acetate buffer (pH 3.6)	85.9	5.5	3.1	<1
Acetate buffer (pH 4.2)	106.8	5.0	2.2	<1
Orange juice	169.6	18.0	5.4	3.0
Grapefruit juice	205.2	9.9	6.1	2.3
Pineapple juice	216.4	23.4	10.3	1.6
Grapefruit juice + 10% fructose		27.4	19.5	4.2
Grapefruit juice + 40% fructose		45.1	7.7	8.8
Grapefruit juice + 10% sucrose		20.1	6.3	2.6
Grapefruit juice + 40% sucrose		28.8	2.2	7.3
Orange juice + 10% fructose		10.9	2.6	2.3
Orange juice + 40% fructose		40.2	8.9	6.3
Orange juice + 10% sucrose		21.2	1.2	4.2
Orange juice + 40% sucrose		29.4	1.1	11.3
Pineapple juice + 10% fructose		10.0	1.7	2.9
Pineapple juice + 40% fructose		32.5	15.6	5.8
Pineapple juice + 10% sucrose		36.4	5.2	5.1
Pineapple juice + 40% sucrose		51.7	15.5	5.5
Grapefruit juice + 0.1% citric acid			6.0	
Grapefruit juice + 0.1% sodium benzoate			<1	

Table 4: Decimal reduction values for *Talaromyces flavus* ascospores (2E) at 85°, 90° and 92.5° C in different heating medium

Heating Medium	Dvalue at 85°C(min)	Dvalues at 90°C(min)	Dvalues at 92.5°C(min)
Distilled H ₂ O	31.4	5.6	<1
Acetate buffer (pH 3.6)	12.3	2.7	<1
Acetate buffer (pH 4.2)	11.6	4.0	<1
Orange	19.9	3.7	<1
Grapefruit	20.7	5.6	<1
Pineapple	41.6	7.2	<1
Orange juice + 0.1% citric acid	12.0	3.5	<1
Orange juice+0.1% sodium benzoate	11.0	3.3	<1
Grapefruit juice 0.1% citric acid	12.4	5.2	<1
Grapefruit juice+0.1% sodium benzoate	12.1	<1	<1
Pineapple juice +0.1% citric acid	12.1	<1	<1
Pineapple juice+0.1% sodium benzoate	12.1	<1	<1

Orange juice + 10% fructose	26.6	6.6	<1
Orange juice + 40% fructose	31.9	6.7	<1
Orange juice + 10% sucrose	15.3	6.8	<1
Orange juice + 40% sucrose	26.4	8.4	<1
Pineapple juice + 10% fructose	15.0	8.2	<1
Pineapple juice + 40% fructose	23.3	13.6	<1
Pineapple juice + 10% sucrose	15.6	13.0	<1
Pineapple juice + 40% sucrose	26.8	10.3	7.2
Grapefruit juice + 10% fructose	20.8	7.2	<1
Grapefruit juice + 40% fructose	20.8	8.8	3.5
Grape fruit juice+ 10% sucrose	31.3	8.5	<1
Grapefruit juice + 40% sucrose	35.7	13.6	<1

Table 5: Decimal reduction values (D-values) for *Talaromyces flavus* ascospores (5A) at 85°, 90° and 92.5°C in different heating medium

Heating Medium	Dvalues at 85°C(min)	Dvalues at 90°C(min)	Dvalues at 92.5°C(min)
Distilled H ₂ O	36.4	5.6	2.7
Acetate buffer (pH 3.6)	18.0	2.3	<1
Acetate buffer (pH 4.2)	10.6	4.2	<1
Orange juice	34.3	4.6	5.0
Grapefruit juice	24.6	4.5	2.3
Pineapple juice	28.7	12.6	5.3
Orange juice + 0.1%citric acid	12.7	3.3	<1
Orangejuice+0.1%sodium benzoate	12.3	2.6	<1
Grapefruit juice 0.1%citric acid	20.9	4.6	<1
Grapefruit juice+0.1%sodium benzoate	17.6	3.0	<1
Pineapple juice +0.1%citric acid	30.1	1.5	<1
Pineapple juice+0.1%sodium benzoate	20.2	6.3	6.0
Orange juice + 10% fructose	26.6	8.4	7.5
Orange juice + 40% fructose	15.4	10.5	7.1
Orange juice + 10% sucrose	28.7	13.2	7.4
Orange juice + 40% sucrose	64.5	7.9	6.3
Pineapple juice + 10% fructose	82.5	11.4	7.1
Pineapple juice + 40% fructose	42.3	7.4	7.1
Pineapple juice + 10% sucrose	78.9	13.2	8.1
Pineapple juice + 40% sucrose	41.0	10.0	3.6
Grapefruit juice + 10% fructose	52.7	12.3	7.1
Grape fruit juice+ 40% fructose	50.9	10.5	5.9
Grapefruit juice + 10% sucrose	65.5	10.2	6.2
Grape fruit juice+ 40% sucrose			

Table 6: Maximum population counts (x 10⁵cfu ^{-ml}) of ascospores obtained at 85⁰ , 90⁰ , 92.5⁰ and 95⁰C.

Isolate	85 ⁰	90 ⁰	92.5 ⁰	95 ⁰ C
5A	105(orange juice) ²⁰	70(orangejuice+0.1%NaB) ³	87(grapejuice) ³	
2E	81(grapefriut) ²⁰	65(orangejuice+0.1%NaB) ³	55(pineapplejuice+10%fructose)	
3C	110 AB (pH4.2) ⁴⁰	126(Distilled H ₂ O) ¹⁰	98 AB (pH 4.2) ³	92(grapejuice) ²
E7	85 AB (pH 4.2) ⁴⁰	127(orangejuice+40% sucrose) ¹⁰	72 (orangejuice) ³	76 AB(pH4.2) ²

(A)^B : A= heating medium

B= duration of heating (min)

Table 7: pH and soluble carbohydrate content of locally prepared fruit juices

Fruit juice	pH	soluble carbohydrate (mg/ml at 28°C)
Grape	3.6	22.50
Orange	3.6	68.75
Pineapple	4.2	111.25

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