Journal of Experimental Sciences Vol. 1, Issue 7, Pages 26-32 [2010]
www.jexpsciences.com

Regular Article

Development of Tropical Fruit Bars and Assessment of its Shelf Life

C. Ramalingam*, Prenitha Mercy I., Sweta Rai and Sonica Gupta

School of Biosciences and Technology, VIT University, Vellore, T.N., India

ABSTRACT: Five tropical fruits are chosen to make fruit bars on three important aspects namely high nutritive value, easy availability and cost effective. These different fruit bars prepared from a new process with better textural and sensory properties are assessed for storage stability with three different packaging materials. The bars are subjected to accelerated shelf life study by varying temperature and relative humidity. Changes in the soluble solid content, pH, titrable acidity and microbiological attributes are monitored. Minimally processed fruit bars are microbial sensitive entities. The data given provide an useful tool for describing microbial spoilage and seems expandable to specific microorganisms. Microbial growth is affected by pH, storage temperature or relative humidity. The sugar content did not change during the first weeks of storage but significant small declines are observed after prolonged storage. The proliferation of mesophilic bacteria, which are dominant in the product will explain these slight depletions.

Key words: Fruit bars; Shelf life study; Packaging material; Microbial spoilage

Abbreviations: AAS , Alomic Absorption Spectroscopy; TS ,Total Solids; TSS,Total Soluble Sugars; RS, Reducing Sugar; EMB, Eosin-methylene blue agar; LB, Luria-bertani broth; IMViC, Indole-methyl red-voges proskauer-citrate test; MRVP, Methyl Red Voges Proskauer broth; HCl, Hydrogen chloride; NaOH, Sodium hydroxide; KOH, Pottassium hydroxide; H2SO4, Sulphuric acid; GM, Growth media; SG, Specific Gravity; AAR, Accelerated aging rate; AATD, Accelerated aging time duration; DRTA, Derived real time aging; AAT, Accelerated aging temperature; AT, Ambient temperature

Introduction

Tropical fruits are rich source of vitamins and minerals. The tropical fruits chosen for the bar preparation are apple, grapes, orange, papaya and sapodilla. They are the rich source of essential nutrients. These fruits are chosen based on three important aspects: high nutritive value, easy availability and cost effective. These fruits were peeled off and made into pulp form. The pulp was then subjected to different tests like physical, chemical, microbiological tests to find out the nutritive quality of fruits. The pulp was then mixed with the required substances like pectin, sugars and respective flavours. The pulp was allowed to set for 24 hours at 40 degree Celsius. Three set of trials were done for the bar formation. The bars formed finally were wrapped in three different kinds of wrapping material- silver foil, polythene bag, and industrial packaging material. Accelerated shelf life study is done on the bars for a period of one month by varying the temperature and relative humidity which gave the results for four months and the results were analysed to find the best packaging material. The fruit bars formed were again subjected to repeated set of physical, chemical, microbiological tests. The physical tests include the measurement of parameters like specific gravity, density, kinematic viscosity and dynamic viscosity. The chemical tests include the measurement of reducing sugars, total sugars, sucrose, mineral content, pH, titratable acidity, crude fibre and dietary fibre. Microbiological tests include the screening of the microflora naturally occurring in the fruits. Oraganoleptic tests include the grading of the bars based on texture, taste, appearance, odour and colour. Accelerated shelf life study is done with different packaging material at different temperature and relative humidity in accelerated chamber. It was found that there is a decrease in the composition of sugar and increase in the composition of titratable acids at the time of decay. The organism present was found to be *E.coli* and it was responsible for the decay of the bar.

Materials and methods

Physical parameters

Specific gravity

Fruit pulp is taken and kept in a specific gravity bottle closed with a lid and which has a fixed volume. The fixed volume of the sample is weighed.

SG = density of the liquid/density of water
Density=mass/volume (kg/meter^3)

Viscosity:

The pulp is passed through the viscometer and the time taken in sec for the flow of 50 ml of sample is noted.

Kinematic viscosity=At-B/t*10^-6(m^2/sec)
Dynamic viscosity = kinematic viscosity ×density

Where A= 0.25, B= 172, t= time in sec

Chemical parameters

Titratable acidity

Dissolve known weight of the sample in water. Heat on steam bath to dissolve, if necessary. Cool and make up to a known volume. Take aliquots for determination. If insoluble material is present, filter before taking aliquots. Dilute the sample with distilled water and titrate just below end point with 0.1N NaOH, using phenolphthalein indicator. Transfer a measured quantity of this solution into approximately 20ml of neutral water in a small beaker. (In this extra solution, colour of fruit juice becomes so pale that the phenolphthalein colour is easily seen). If the test shows that the end point has not been reached, pour extra diluted portions back into the original solution, add more alkali and continue the titration to the end point. By comparing dilutions in a small beaker, differences produced by a few drops of 0.1N alkali can be easily observed.

$$\% \text{ Total acid} = \frac{\text{Titre} \times \text{Normality of alkali} \times \text{Vol made up} \times \text{Equiv wt. Of acid} \times 100}{\text{Vol of sample taken for estimation} \times \text{Wt. or Vol of sample taken} \times 1000}$$

Reducing sugars

Place 50g of sample in a 500 ml beaker and add 400 ml of water. Neutralise the solution with 1N NaOH using phenolphthalein indicator. Boil gently for 1 hour with occasional stirring. Add boiling water to maintain the original level. Cool and transfer to a 500 ml volumetric flask. Make upto the volume and filter through No.4 Whattman paper. Pipette 100 ml aliquot into 500 ml volumetric flask. Add 2 ml of neutral lead acetate solution and add about 200 ml of water. Let it stand for 10 mins, then precipitate the excess of lead with potassium oxalate solution. Make upto mark and filter. Pipette 10 ml of mixed Fehling's solution into each of two 250-ml conical flasks. Fill the 50-ml burette with the solution to be titrated. Run into the flask almost the whole volume of sugar solution required to reduce the Fehling's solution, so that 0.5ml to 1.0 ml is

* Corresponding Author, Email: cramalingam@vit.ac.in

ISSN: 2218-1768

required later to complete the titration. Mix the contents of the flask, heat to boiling and boil moderately for 2min. Then add 3 drops of methylene blue solution, taking care not to allow it to touch the side of the flask. Complete the titration within 1min by adding 2 to 3 drops of sugar solution at 5 to 10 sec intervals, until the indicator is completely decolourized. At the end point, the boiling liquid assumes the brick red colour of precipitated cuprous oxide, which it had before the indicator was added. Note the volume of the solution required.

Total sugars

Pipette 50 ml of the clarified solution into a 250-ml conical flask. Add 5g of citric acid and 50 ml of water. Boil gently for 10 min to complete the inversion of sucrose, then cool. Transfer to a 250 ml volumetric flask and neutralize with 1N NaOH using phenolphthalein as indicator. Make upto volume. For inversion at room temperature, transfer 50 ml aliquot of clarified and deleaded solution to a 250-ml flask. Add 10 ml of HCl (1+1) and allow to stand at room temperature (20 C or above) for 24 hours. Neutralize wuth concentrated NaOH solution and make upto volume.Take an aliquot and determine the total sugars as invert sugars.

- % Reducing sugars = $\frac{\text{mg of Invert sugar} \times \text{dilution} \times 100}{\text{Titre} \times \text{wt or volume of the sample} \times 100}$
- % Total sugars as = calculate as in (a) making use of the titre value obtained invert sugar in the determination of total sugars after inversion.
- % sucrose = (% Total invert sugars - % reducing sugars originally present)× 0.95

% Total sugars = (% Reducing sugars + % sucrose)

pH

Dip the electrode into the different fruit pulp sample whose pH is to be find out. Release the check switch to read the pH value of the unknown sample.

Moisture content

This method consists in measuring the weight loss by foods due to thye evaporation of water. Drying methods are generally used as they give accurate results. The proportion of free water lost increases as the temperature of drying increases. So it is important to compare the results obtained using the same conditions of drying. It is advisable to use lower drying temperatures 60-70. C . Use a oven whose temperature can be controlled accurately. Since the temperature tends to be differrent on different shelves, a drying oven fitted with internal fan for circulation of air should be preferred.

Microbiological parameters

The fruit was peeled, cut into small pieces and Grinded in the mixer. The pulp taken was passed through the sieve. The pulp is then subjected to serial dilution followed by pour plate technique and then subculturing. The following tests were performed to assess the presence of *E.coli* in the sample: Gram staining, spore staining, methyl red test, indole test, voges-proskaeur test, citrate utilization test.

All these parameters are analysed with both pulp and fruit bars.

Formation of dehydrated tropical fruit bar

Tray drying method

Take the pulp of the fruit which has to be used in which TSS should be 15%. Add the sugars (=15%) and pectin(0.5%) which is premixed. Preservative like citric acid , colours and flavours are added into the pulp. Take a tray with 0.2-0.3 feet and lubricate with liquid paraffin and spread the pulp on the tray. Keep it in the drier at the temperature of 50-55'c for 25-28 hrs.

Retexturization method

Take a fruit pulp and concentrate it in non stick pan so that TSS ranges from 15% to 30%. Dissolve the maltodextrin in water at 60 - 70 degree celsius (4-5%). Mix the sugar (15%) and pectin(0.8%)separately. Add the mixture of sugar and pectin in the fruit pulp which already contains the maltodextrin. Preservative like citric acid , colours and flavours are added into the pulp. Now heat the mixture so that the solid content is 85%. Take a tray with 0.2-0.3 feet and lubricate with liquid paraffin and spread the pulp on the tray. Keep it in the drier at the temperature of 50-55'c for 25-28 hrs.

Shelf life study

This can be done by varying the temperature and relative humidity.

AAR=Q10((AAT-AT)/10)

AATD=DRTA/AAR

where,

AAR= Accelerated aging rate, AATD= Accelerated aging time duration, DRTA= Derived real time aging, AAT= Accelerated aging temperature, AT= Ambient temperature, Q10= Accelerated aging factor, (Q10= 2= Industrial standard, Q10= 1.8= More conservative option)

Results and Discussions

Pulp characterisation

Table 1.1 Physical parameters

Fruits	Density	Specific Gravity	Kinematic viscosity*10^-6 m^2/sec	Dynamic viscosity*10^-3 m^2/sec
Apple	1080	1.08	1.8502	1.998
Grapes	1240	0.98	1.687	1.653
Orange	980	1.24	0.725	0.899
Papaya	1010	1.01	4.495	4.539
Sapodilla	1310	1.31	2.482	3.251

In table 1.1, it is observed that the sapodilla has the highest density and specific gravity. Papaya has the highest kinematic viscosity and dynamic viscosity. Orange has the lowest density, specific gravity and viscosity.

Table 1.2 Chemical parameters

Fruits	% Reducing Sugar	% Sucrose	% Total sugar	pH	%Titratable acidity
Apple	9.9	3.3	13.2	4.2	2.0
Grapes	14.2	3.4	17.6	3.8	1.2
Orange	4.7	4.2	8.9	3.7	1.0
Papaya	4.5	1.8	6.3	4.8	0.8
Sapodilla	4.7	2.1	6.8	5.4	0.2

Grapes shows the maximum reducing sugar content which is followed by apple. Sucrose content is maximum in orange. Grapes has the highest total sugar content and Papaya has the least sugar content. Sapodilla has the highest pH and hence has the lowest percentage for titratable acidity.

Three trials were done to study the shelf life with different packaging material at varying temperature and relative humidity. In the first trial, the temperature was maintained at 50◦c and relative humidity at 80%. In the second trial, the temperature was maintained at 55◦c and relative humidity at 60%. In the third trial, the temperature was maintained at 60◦c and relative humidity at 60%.

Table 2.1 Sugar content on zeroth day in all three trials

Fruits	% Reducing Sugar			% Sucrose			% Total Sugar		
	Poly-thene bag	Alumi-nium foil	Indus. material	Poly-thene bag	Alumi-nium foil	Indus. material	Poly-thene bag	Alumi-nium foil	Indus. Material
Apple	11.07	11.07	11.07	5.68	5.68	5.68	16.75	16.75	16.75
Grapes	12.30	12.30	12.30	5.80	5.80	5.80	18.10	18.10	18.10
Orange/ Papaya	6.51	6.51	6.51	5.14	5.14	5.14	11.65	11.65	11.65
Sapodilla	6.70	6.70	6.70	5.20	5.20	5.20	11.90	11.90	11.90

Fig 1. Chemical parameters observed in the bars kept in polythene bag on zeroeth day

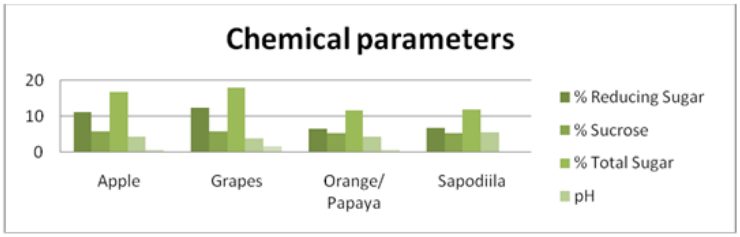


Table 2.2 pH and % titratable acidity on zeroth day in all three trials

Fruits	pH			% Titratable Acidity		
	Polythene bag	Aluminium foil	Indus. material	Polythene bag	Aluminium foil	Indus. Material
Apple	4.19	4.20	4.20	0.81	0.80	0.80
Grapes	3.79	3.80	3.80	2.02	1.99	2.00
Orange/ Papaya	4.17	4.23	4.23	0.68	0.65	0.65
Sapodilla	5.38	5.40	5.40	0.24	0.20	0.20

The concentration of sugars remained constant for two weeks and from the third week there was a decrease in the sugar concentration which subsequently decreased the pH and increased the percentage of titratable acidity in polythene bag. In case of aluminium foil, the concentration of sugars remained constant for three weeks and from the fourth week it started to decline which in turn decreased the pH and increased the percentage of titratable acidity. In case of industrial packaging material, all the parameters remained constant.

Table 3.1 Sugar content on twenty first day in first trial maintained at 50◦c and relative humidity at 80%

Fruits	% Reducing Sugar			% Sucrose			% Total Sugar		
	Poly-thene bag	Alum-inium foil	Indus. material	Poly-thene bag	Alum-inium foil	Indus. material	Poly-thene bag	Alum-inium foil	Indus. Material
Apple	9.87	10.07	11.06	4.68	4.86	5.67	14.55	14.93	16.75
Grapes	9.30	10.30	12.30	3.80	4.91	5.80	13.10	15.21	18.10
Orange/ Papaya	4.51	5.23	6.45	4.59	4.59	5.15	9.10	9.80	11.60
Sapodilla	5.70	5.80	6.80	3.20	4.60	5.15	8.90	10.40	11.95

Fig 2. Chemical parameters observed in the bars kept in polythene bag on twenty first day

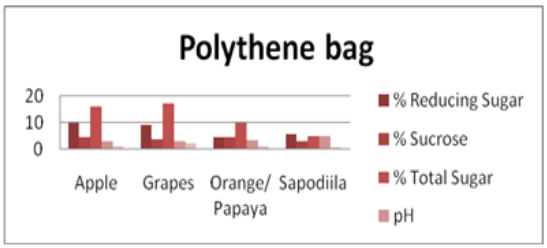
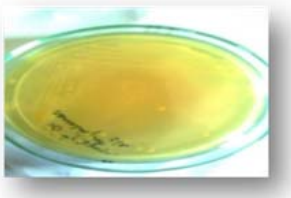
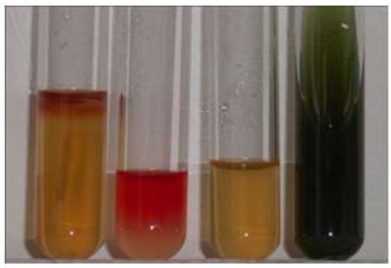


Table 3.2 pH and % titratable acidity on twenty first day in first trial maintained at 50◦c and relative humidity at 80%

Fruits	pH			% Titratable Acidity		
	Polythene bag	Aluminium foil	Indus. material	Polythene bag	Aluminium foil	Indus. Material
Apple	3.20	3.80	4.20	1.3	1.10	0.80
Grapes	3.12	3.02	3.80	2.52	2.10	2.00
Orange/ Papaya	3.55	3.75	4.20	1.0	0.99	0.65
Sapodilla	5.01	5.11	5.40	0.5	0.38	0.20

In first trial where the temperature is relatively low and relative humidity is high, there is a drastic decrease in the sugar of the bars in polythene bag on twenty first day with respect to the zeroth day. There is a significant change in sugar content of the bars in polythene bag and aluminium foil. The percentage of titratable acidity is observed to be maximum in grapes. It is noted that on the twenty first day, there is a reduction in the value of pH and increase in the value of titratable acids in the fruit bars packed in polythene bag and aluminium foil. And the sugar content in industrial packaging material remains the same with respect to the zeroth day. It is found to be due to the activity of *E.coli*. It degrades the reducing sugar and the sucrose which in turn reduces the total sugar content. And this has been confirmed by the microbiological tests.





It is observed that *E. coli* shows positive result to indole test and methyl red test. It shows negative result to Voges-Proskauer test and citrate utilisation test.

Conclusion

Three different conditions were set for the study of shelf life of the fruit bars with three different packaging materials.
First trial was carried out at a constant temperature of 50◦C and 80% relative humidity. In case of polythene bag, the concentration

of sugars remained constant for two weeks and from the third week there was a decrease in the sugar concentration which subsequently decreased the pH and increased the percentage of titratable acidity. In case of aluminium foil, the concentration of sugars remained constant for three weeks and from the fourth week it started to decline which in turn decreased the pH and increased the percentage of titratable acidity. In case of industrial packaging material, all the parameters remained constant.

Second trial was carried out at a constant temperature of 55◦C and 60% relative humidity. In case of polythene bags, the concentration of sugars remained constant for three weeks and showed a slight decrease in the concentration of sugar and pH and slight increase in titratable acidity from the third week. In case of aluminium foil and industrial packaging material, all the parameters remained almost constant.

Third trial was carried out at a constant temperature of 60◦C and 60% relative humidity. Since there is an increase in temperature, charring of fruit bar occurred. And there were no significant differences in the parameters in all the three packaging material.

The bars were then subjected to microbiological tests. *E.coli* colonies were observed in nutrient agar and EMB agar medium during first trial. The presence of *E.coli* was confirmed through the Gram staining by the appearance of pink colonies.

It was noted that the decrease of concentration of sugars and pH and increase in the titratable acidity is due to the activity of *E.coli.*

Monosaccharides can be oxidized by relatively mild oxidizing agents such as ferric or cupric ion The carbonyl carbon is oxidized to a carboxyl group. Glucose and other sugars capable of reducing ferric or cupric ion are called reducing sugars**.**

Disaccharides (such as maltose, lactose, and sucrose) consist of two monosaccharides joined covalently by an *O*-glycosidic bond**,** which is formed when a hydroxyl group of one sugar reacts with the anomeric carbon of the other. Glycosidic bonds are readily hydrolyzed by acid but resist cleavage by base. Thus disaccharides can be hydrolyzed to yield their free monosaccharide components by dilute acid. *E.coli* has the capacity to degrade both glucose and sucrose. It degrades the glucose and converts into the intermediates of citric acid cycle such as acetate, formate and pyruvate.

E. coli has the full complement of enzymes for the glyoxylate and citric acid cycles in the cytosol and can therefore grow on acetate as their sole source of carbon and energy. The phosphoprotein phosphatase that activates isocitrate dehydrogenase is stimulated by intermediates of the citric acid cycle and glycolysis and by indicators of reduced cellular energy supply. The same metabolites *inhibit* the protein kinase activity of the bifunctional polypeptide. Thus, the accumulation of intermediates ofthe central energy-yielding pathways, indicating energy depletion, results in the activation of isocitrate dehydrogenase.

Hence, when the concentration of the intermediates of citric acid cycle increases, the pH decreases. The greater the acidity of a solution, the lower its pH. Weak acids partially ionize to release a hydrogen ion, thus lowering the pH of the aqueous solution.

So, it is concluded that the industrial packaging material gives more shelf life without any depletion of nutrients.

References

P. Vijayananda, A. R. Yadavb, N. Balasubramanyamb and P. Narasimhama,Storage Stability of Guava Fruit Bar Prepared Using a New Process, *Lebensmittel-Wissenschaft und-Technologie*, *Volume 33, Issue 2*, *March 2000*, *Pages 132-137*

M. I. Gil, , a, M. A. Conesaa and F. Artésb,Quality changes in fresh cut tomato as affected by modified atmosphere packaging, *Postharvest Biology and Technology*, *Volume 25, Issue 2*, *June 2002*, *Pages 199-207*

Laura K. Stran, Michelle D. Danyluk, Fate of Escherichia coli O157:H7 and Salmonella spp. on fresh and frozen cut mangoes and papayas, *International Journal of Food Microbiology*, *Volume 138, Issues 1-2*, *31 March 2010*, *Pages 78-84*

P. García-García, a, A. López-Lópeza and A. Garrido-Fernández,Study of the shelf life of ripe olives using an accelerated test approach, Journal of Food Engineering Volume 84, Issue 4, February 2008, Pages 569-575

María Ros-Chumillasa, Yulissa Belissarioa, Asunción Iguaza and Antonio López,Quality and shelf life of orange juice aseptically packaged in PET bottles, Journal of Food Engineering Volume 79, Issue 1, March 2007, Pages 234-242

Teferaa, T. Seyoum , a, and K. Woldetsadika, Effect of Disinfection, Packaging, and Storage Environment on the Shelf Life of Mango , *Biosystems Engineering*, *Volume 96, Issue 2*, *February 2007*, *Pages 201-212*

Patnaik R, Roof WD, Young RF, Liao JC,Stimulation of glucose catabolism in Escherichia coli by a potential futile cycle.

Robert C. Soliva-Fortuny, Pedro Elez-Martínez and Olga Martín-Belloso,Microbiological and biochemical stability of fresh-cut apples preserved by modified atmosphere packaging , *Innovative Food Science & Emerging Technologies*, *Volume 5, Issue 2*, *June 2004*, *Pages 215-224*

C. Van der Steen, L. Jacxsens, F. Devlieghere, and J. Debevere,Combining high oxygen atmospheres with low oxygen modified atmosphere packaging to improve the keeping quality of strawberries and raspberries, *Postharvest Biology and Technology*, *Volume 26, Issue 1*, *August 2002*, *Pages 49-58*

Véronique COMA, a,aUS2B, UMR 5103 Bioactive packaging technologies for extended shelf life of meat-based products, *Meat Science*, *Volume 78, Issues 1-2*, *January-February 2008*, *Pages 90-103*

F. Devlieghere, , a, A. H. Geeraerdb, K. J. Versyckb, H. Bernaerta, J. F. Van Impeb and J. Debeverea, Shelf life of modified atmosphere packed cooked meat products: addition of Na-lactate as a fourth shelf life determinative factor in a model and product validation, *International Journal of Food Microbiology*, *Volume 58, Issues 1-2*, *30 June 2000*, *Pages 93-106*

James P. Smitha, Hosahalli S. Ramaswamya and Benjamin K. Simpsona, Developments in food packaging technology. Part I: Processing/cooking considerations, *Trends in Food Science & Technology*, *Volume 1*, *July 1990*, *Pages 107-110*

Zeki Berk, Food packaging,Food Process Engineering and Technology, 2009, Pages 545-559

M. Simona and A.P. Hansen, a, Effect of Various Dairy Packaging Materials on the Shelf Life and Flavor of Pasteurized Milk, *Journal of Dairy Science*, *Volume 84, Issue 4*, *April 2001*, *Pages 767-773*

D. Sun-Waterhouse, A. Teoh, C. Massarotto, R. Wibisono, S. Wadhwa, Comparative analysis of fruit-based functional snack bars, Food Chemistry, Volume 119, Issue 4, 15 April 2010, Pages 1369-1379

Robert C. Soliva-Fortuny, Pedro Elez-Martínez and Olga Martín-Belloso , Microbiological and biochemical stability of fresh-cut apples preserved by modified atmosphere packaging, *Innovative Food Science & Emerging Technologies*, *Volume 5, Issue 2*, *June 2004*, *Pages 215-224*

M.T. Treeby, R.E. Henriod, K.B. Bevington, D.J. Milne, R. Storey, Irrigation management and rootstock effects on navel orange [Citrus sinensis (L.) Osbeck] fruit quality,Agricultural Water Management, Volume 91, Issues 1-3, 16 July 2007, Pages 24-32

Bin Fu and Theodore P. Labuz,sShelf-life prediction: theory and application,Food ControlVolume 4, Issue 3, 1993, Pages 125-133

Bin Fu, Theodore P. Labuza, Shelf-life prediction: theory and application,Food Control, Volume 4, Issue 3, 1993, Pages 125-133

Pexaraa,I. Ambrosiadisa, S. Georgakisa, C. Genigeorgisa and Ch. Batziosb, Basic parameters of a new production technology for "gyros". A shelf life study of the product at 4 °C , *Journal of Food Engineering*, *Volume 79, Issue 2*, *March 2007*, *Pages 681-688*

Elisa I. Benítez, Diego B. Genovese, Jorge E. Lozano,Effect of typical sugars on the viscosity and colloidal stability of apple juice,*Food Hydrocolloids*, *Volume 23, Issue 2*, *March 2009*, *Pages 519-525*
Elisa I. Benítez, Diego B. Genovese, Jorge E. Lozano

Feng-Jui Kuo, Chung-Teh Sheng, Ching-Hua Ting, Evaluation of ultrasonic propagation to measure sugar content

and viscosity of reconstituted orange juice,*Journal of Food Engineering*, *Volume 86, Issue 1*, *May 2008*, *Pages 84-90*

D.K. Tandon, B.P. Singh, S.K. Kalra,Storage behaviour of specific-gravity-graded guava fruits,*Scientia Horticulturae*, *Volume 41, Issues 1-2*, *December 1989*, *Pages 35-41*
D.K. Tandon, B.P. Singh, S.K. Kalra

Yeasts and lactic acid bacteria microbiota from masau (*Ziziphus mauritiana*) fruits and their fermented fruit pulp in Zimbabwe,*International Journal of Food Microbiology*, *Volume 120, Issues 1-2*, *30 November 2007*, *Pages 159-166*

Loveness K. Nyanga, Martinus J.R. Nout, Tendekayi H. Gadaga, Bart Theelen, Teun Boekhout, Marcel H. Zwietering,Purification and characterization of polygalacturonase-inhibitory proteins from Psidium guajava Linn. (guava) fruit,*Plant Science*, *Volume 164, Issue 2*, *February 2003*, *Pages 147-156*

Jeffrey M. Becker, Guy A. Caldwell, Eve Ann Zachgo,Aseptic Technique and Establishing Pure Cultures: The Streak Plate and Culture Transfer,*Biotechnology (Second Edition)*, *1996*, *Pages 9-16*

Otto Lüderitz, Marina A. Freudenberg, Chris Galanos, Volker Lehmann, Ernst Th. Rietschel, Derek H. Shaw,Lipopolysaccharides of Gram-Negative Bacteria,*Current Topics in Membranes and Transport*, *Volume 17*, *1982*, *Pages 79-151*

Herbert C. Friedmann ,Escherich and EscherichiaEscherich and Escherichia, *Advances in Applied Microbiology*, *Volume 60*, *2006*, *Pages 133-196*

Adam Driks,The *Bacillus anthracis* spore,*Molecular Aspects of Medicine*, *Volume 30, Issue 6*, *December 2009*, *Pages 368-373*

M. L. Sanz, M. Villamiel, I. Martínez-Castro, Inositols and carbohydrates in different fresh fruit juices *Food Chemistry*, *Volume 87, Issue 3*, *September 2004*, *Pages 325-328*

Inés Mato, Silvia Suárez-Luque, José F. Huidobro,A review of the analytical methods to determine organic acids in grape juices and wines *Food Research International*, *Volume 38, Issue 10*, *December 2005*, *Pages 1175-1188*

Elisa I. Benítez, Diego B. Genovese, Jorge E. Lozano,Effect of typical sugars on the viscosity and colloidal stability of apple juice *Food Hydrocolloids*, *Volume 23, Issue2*, *March 2009*, *Page519-525*

Lehninger Principles of Biochemistry, fourth edition, David L. Nelson (University of Wisconsin–Madison)Michael M. Cox (University of Wisconsin–Madison)

Handbook of analysis and quality control for fruit and vegetable products.S.Ranganna.