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Examples:

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998;

1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)
References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

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Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant Staphylococcus aureus in community-acquired skin infections. Emerg. Infect. Dis. 11: 928-930.

Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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# African Journal of Food Science

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## ARTICLES

*Full Length Research Paper*

# Quality of pears with permeability of Bio-Fresh[TM] edible coatings

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**Bio-Fresh[tm] edible coatings in four concentrations (0.5, 0.8, 1.0 and 1.2%) were investigated on quality characteristics of conference pears (*Pyrus communis* L. cv. Conference) with regards to its permeability. The Bio-Fresh[TM] was applied on pears by dipping. It was found that the effect of Bio-Fresh[TM] on pears was significantly effective in maintaining the skin color of green and only coating 1.2% Bio-Fresh[TM] delayed the changes of firmness, soluble solid content and retardation of shriveling and weight loss. Coating of 1.2% Bio-Fresh[TM] showed low permeability to respiratory gases ($O_2$ and $CO_2$) and created modified atmosphere, reduced decay and improved the quality of pears. Increasing concentration of coating on the surface skin of pears blocked the pores and lenticels, providing a drop-down in $O_2$ partial pressure. As a consequence, coating of 1.2% showed low permeability with good quality of pears. The results of this study suggest that coating of 1.2% Bio-Fresh[TM] increased shelf life of pears after 30 days without significant losses in quality.**

**Key words:** Edible coating, dipping, respiration, permeability, quality, gas exchange.

## INTRODUCTION

Pears (*Pyrus communis* L. cv. Conference) are known as pome fruits and are very perishable and susceptible to deterioration accompanied by shriveling, softening and decay. Rapid postharvest physiological changes in conference pears are responsible for short ripening period, rapid senescence that results to short shelf life commodity and pose a challenge to their marketing (Lin et al., 2003), and also a serious constraint to efficient handling and transportation (Hassan and Nurhan, 2004). Controlled atmospheres (CA) and modified atmosphere packaging (MAP) like several storage techniques have

been developed over the years to extend the storage life of fruits. However, these techniques are not free from drawback. For instance, $O_2$ and $CO_2$ injury, increase ethanol production, flavour problem due to anaerobic respiration have been reported (Bender et al., 1994). Therefore, alternative practices are required for prolonging the shelf life of fresh pears.

The potential alternative storage methods for fresh agricultural produces could be edible coatings which could increase attention because of environmental consideration and the trends towards the use of

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convenience foods (Ozden and Bayindirli, 2002). Semi-permeable coating can create a modified atmosphere similar to CA (Nisperos-Carriedo and Shaw, 1990). The atmosphere created by coating can change in response to environmental conditions due to combined effect on fruits respiration and coating permeability. Coatings are also used to extend the shelf life of fruits and improve appearance (Baldwin et al., 1999). Surface coatings can also improve the postharvest quality of horticultural com-modities by reducing water loss (Hagenmaier and Baker, 1993), improving the finish of the skin (Hagenmaier and Baker, 1995; Amarante, 1998) and reducing skin susceptibilities (Amarante et al., 2001).

The effects of coatings on shelf life extension of fruits have been studied by several researchers such as with apples (Rojas-Grau et. al., 2008), mango (Srinivasa et. al., 2002; Dang et. al., 2008), kiwi (Xu et. al., 2001). Coatings have been known to prevent fruits and vege-tables from deterioration by inhibiting respiration, redu-cing dehydration, maintaining textural quality, retaining volatile flavor and decreasing microbial growth (Han et al., 2004).

However, in some cases, edible coatings were not successful and have degraded fruits quality (Hagenmaier, 2005). The occurrence of physiological disorders such as core flush, flesh breakdown was induced by improper coatings (Park, 1999). Modification of internal atmos-phere by the use of edible coatings can increase dis-orders associated with high carbon dioxide or low oxygen concentration (Ben-Yehoshua, 1969).

The effect of edible coating on internal gas composition and their interactions on quality parameters must be determined for coated pears for effective application of Bio-Fresh[TM] edible coatings on pears to prolong the shelf life and avoid postharvest losses. The main objective of this study was to evaluate the quality of coated pears with the permeability of coatings.

## MATERIALS AND METHODS

Pears (*P. communis* L. cv. Conference) were harvested and stored at ultra low oxygen (ULO) condition at temperature of -1°C and 90% relative humidity in the cold storage. Bio-Fresh[TM] is an edible coating solution which is composed of sucrose fatty acid ester and carboxymethyl cellulose (CMC) in a concentrated liquid form (distributed by De Leye, Agrotrade, Netherlands).

### Preparation of Bio-Fresh[TM] and coating of fruits for experiment

Bio-Fresh[TM] was diluted in water with a temperature of 37 - 38°C to obtain the desired concentration: 0.5, 0.8, 1.0 and 1.2% (0.83, 1.328 1.66 and 1.992 L Bio-fresh[TM] per 100 L water). The diluted solutions were mixed for a few minutes and then the pears were dipped in the dilution for a few seconds so that the pears can be thoroughly wetted on all sides. After that, the pears were dried by air blowing. The coated pears are used for studying ripening and gas exchange characteristics. Each batch contained four groups of treatments with 30 single fruit replicates for each treatment. The pears were evaluated for quality parameters color, firmness, soluble solid contents, weight loss and incidence of disorders and for coating

permeability. The experiments were conducted immediately after coating called Shelf-0 and after 30 days of storage of coated pears called shelf-1.

### Firmness measurement

The firmness of pears was measured by using a universal texture analyzer ((LRX, Lloyd Instruments, Hampshire, UK) by measuring the maximum penetration force required for a 11 mm diameter self cutting plunger to penetrate 1 cm into the pear at a rate of 8 mm/s. The values were taken at two points on the equator of each pear.

### Soluble solid content measurement

Soluble solid content was measured from the pressed juice of the pear by means of a refractometer (HANNA, UK) and the results were expressed as °Brix.

### Color measurement

The surface color of pears was directly measured with a spectrophotometer (CM-2500d, Minolta, Japan). The equipment was set up for illuminant D65 and 10° observer angle and calibrated using a standard black reflector plate for zero and white reflector plate for one. The color changes were quantified in the L*, a*, b color space (defined by CIE in 1976). The hue value was also calculated in order to compare color change among different treatments. On each pear, five readings in five different areas were taken. The numerical values of a* parameter was employed to calculate hue angle.

### Incidence of disorders

Pears were cut longitudinally for measuring the internal browning and internal cavities using 30 pears. The flexibility of neck was measured by observing the shrinkage in neck by pressing. The visual evaluation was done for external flexible necks, and internal browning and cavities for pears by hedonic scale. The samples were evaluated using the following hedonic scale: 0 = excellent, 1 = very good, 2 = good and 3 = fair good 4 = bad for flexible necks. A value of 2 is considered as the commercial acceptability threshold.

### Weight loss measurement

The samples were weighted using 30 pears individually with a laboratory balance. The results were expressed as the percentage loss of the initial weight. Weight loss was calculated from the initial weight using the formula:

$$Weight\ loss\ (\%) = \frac{(W_i - W_s)}{W_i} \times 100$$

Where, $W_i$ is the initial weight and $W_s$ is the weight in the sampling period.

### Gas exchange measurement

Pears were weighed by a laboratory balance and placed in the jar. Each jar of 1.7 ml contains pear fruits resulting in approximately 250 g pear $L^{-1}$ jar. The jar were stored in a temperature controlled room and connected to a flow through system. Two conditional airs were applied at 20 kPa $O_2$, 0 kPa $CO_2$ and 0 kPa $O_2$, 0 kPa $CO_2$ by

gas mixtures. The gas mixtures were made from pure gases using an in house built mixing panel equipped with mass flow controllers (Brooks Instrument, The Netherlands). The compositions of the mixtures were measured by using gas analyser (Checkmate II, PBI Dansensor, Denmark). The gas analyser has an accuracy of ±0.1% absolute of $O_2$ reading and ±0.5% absolute of $CO_2$ reading, respectively and calibrated against calibrated mixtures (Air products N.V., USA). For each condition, five jars were connected in series and flushed with conditional air with a flow rate of 10 L per hour for at least one day. The air stream through the jar was stopped after equilibrium of gas mixtures and the jars were closed. The partial pressure of $O_2$ and $CO_2$ changes in the jars with time were measured twice in the day with checkmate II and the exact time of measurement was recorded.

After weighing, intact coated fruits were put into the five jars, sealed and placed at two different gas conditions: 20 kPa $O_2$, 0 kPa $CO_2$ for oxidative respiration and 0 kPa $O_2$, 0 kPa $CO_2$ for fermentation at temperature of 10°C. The $O_2$ and $CO_2$ gas concentration profiles in the sealed jar due to respiration and fermentation of the fruits were measured as a function of time using gas analyzer checkmate II. The gas percentages were converted to partial pressure by multiplying with the measured total pressure. The gas permeability of coating was estimated from a difference in the gas profiles between the measured gas concentration profiles of coated and uncoated fruits.

The modified Michaelis Menten kinetics model has been applied to describe the respiration characteristics of intact pears (Peppelenbos et al., 1996; Peppelenbos and van't Leuven, 1996). A non-competitive inhibition model was used to describe the respiration of the pears as follows:

$$R_{O_2} = -\frac{V_{m,O_2}.P_{O_2}}{(K_{m,O_2}+P_{O_2})\left(1+\frac{P_{CO_2}}{K_{mn,CO_2}}\right)} \tag{1}$$

$$R_{CO_2} = -r_{q,ox}.R_{O_2} + \frac{V_{mf,CO_2}}{\left(1+\frac{P_{O_2}}{K_{mf,O_2}}\right)} \tag{2}$$

Where, $V_{m,O_2}$ (mol/m$^3$s), the maximum $O_2$ consumption rate; $V_{mf,CO_2}$ (mol/m$^3$s), the maximum $CO_2$ production; $K_{m,O_2}$ (kPa) and $K_{mf,O_2}$ (kPa), the Michaelis-Menten constant for $O_2$ consumption and $CO_2$ production, respectively; $P_{O_2}$ (kPa), the $O_2$ partial pressure; $P_{CO_2}$ (kPa), the $CO_2$ partial pressure; $K_{mn,CO_2}$ (kPa), the Michaelis-Menten constant for non-competitive $CO_2$ inhibition, $r_{q,ox}$ the respiration quotient at high $O_2$ partial pressure, and $R_{O_2}$ (mol/m$^3$s) and $R_{CO_2}$ (mol/m$^3$s), the $O_2$ consumption rate and $CO_2$ production rate of the sample, respectively.

Changing of the gas concentrations inside the jar due to respiration of fruit was described as follows:

$$(V_{jar} - V_{pear})\frac{\partial C_{O_2}}{\partial t} = V_{pear}.R_{O_2} \tag{3}$$

$$(V_{jar} - V_{pear})\frac{\partial C_{CO_2}}{\partial t} = V_{pear}.R_{CO_2} \tag{4}$$

Where, $C_{O2}$ (mol/m$^3$s) and $C_{CO2}$ (mol/m$^3$s) are the $O_2$ and $CO_2$ concentration, $t$ (s) is the time; $V_{jar}$ (m$^3$) and $V_{pear}$ (m$^3$) are the volume of jar and pears.

Difference in gas concentration between the coated membranes can be expressed base on the Fick's first law of diffusion as follows:

$$V_{pear}R_{O_2} = S_{pear}h_{O_2}\left(C_{O_2,o} - C_{O_2,i}\right) \tag{5}$$

$$V_{pear}R_{CO_2} = -S_{pear}h_{CO_2}\left(C_{CO_2,o} - C_{CO_2,i}\right) \tag{6}$$

Where, $h_{O2}$ (m/s) and $hc_{O2}$ (m/s) are the permeability for $O_2$ and

$CO_2$, $S_{pear}$ is the surface area of the pear (m$^2$), $C$ is the mean $O_2$ and $CO_2$ concentration (mol/m$^3$). Subscript $i$ and $o$ indicate inside and outside the coated membrane.

Changing of the gas concentrations inside the jar as function of time was described as follows:

$$(V_{jar} - V_{pear})\frac{\partial C_{O_{2,o}}}{\partial t} = -S_{pear}h_{O_2}\left(C_{O_2,o} - C_{O_2,i}\right) \tag{7}$$

$$(V_{jar} - V_{pear})\frac{\partial C_{CO_{2,o}}}{\partial t} = -S_{pear}h_{CO_2}\left(C_{CO_2,o} - C_{CO_2,i}\right) \tag{8}$$

The Michaelis-Menten kinetics was used to describe the respiration characteristics of pears. The Michaelis-Menten constant $K_m$ values for $O_2$ and $CO_2$ was assumed to be constant in each batch of experiment. The maximum $O_2$ consumption rate $V_{m,O_2}$ and maximum $CO_2$ fermentative production rate $V_{mf,CO_2}$ vary from batch to batch depending on the maturity of pears. So, the respiration was carried out to determine the $V_{m,O_2}$ and $V_{mf,CO_2}$ in a close jar.

**Statistical analysis and model parameter estimation**

Analysis of variance (ANOVA) was applied to the data obtained from each treatment to detect significance of differences at 5% level of significance ($P<0.05$) to analyze color, firmness and soluble solid contents and also Turkey mean comparison ($P<0.05$) was used to show the comparison of each treatment mean. Statistical software, the S-PLUS 8.0 version was used.

An iterative least square estimation procedure written in MATLAB (The Mathworks, Inc., Natick, USA) was used to determine respiration parameters and gas permeability of coating by fitting the model solutions to measure $O_2$ and $CO_2$ concentration change profiles.

# RESULTS AND DISCUSSION

## Firmness

The effect of Bio-Fresh[TM] coating on the firmness of pears was measured. The statistical analysis showed that all coating concentrations were effective for maintaining firmness. The mean comparison test confirmed that only coating of 1.2% Bio-Fresh[TM] had significant ($p<0.05$) firmness values than control sample during shelf life study (Figure 1).

## Color

The color changes have been quantified in the L*, a*, b color space. The a*-values were correlated best with visual observance of green color: more negative a*-values indicated more green color. Therefore, color data were expressed as a*-values. The statistical analysis revealed that Bio-Fresh[TM] was significantly ($p<0.05$) effective for retaining the green color of pears (Figure 2). All coating concentrations had significantly more negative a*-values than control sample among different shelf life conditions, and coating of 1.2% Bio-Fresh[TM] had higher negative values than others concentration.

## Firmness of pears for shelf-0



## Firmness for shelf-1
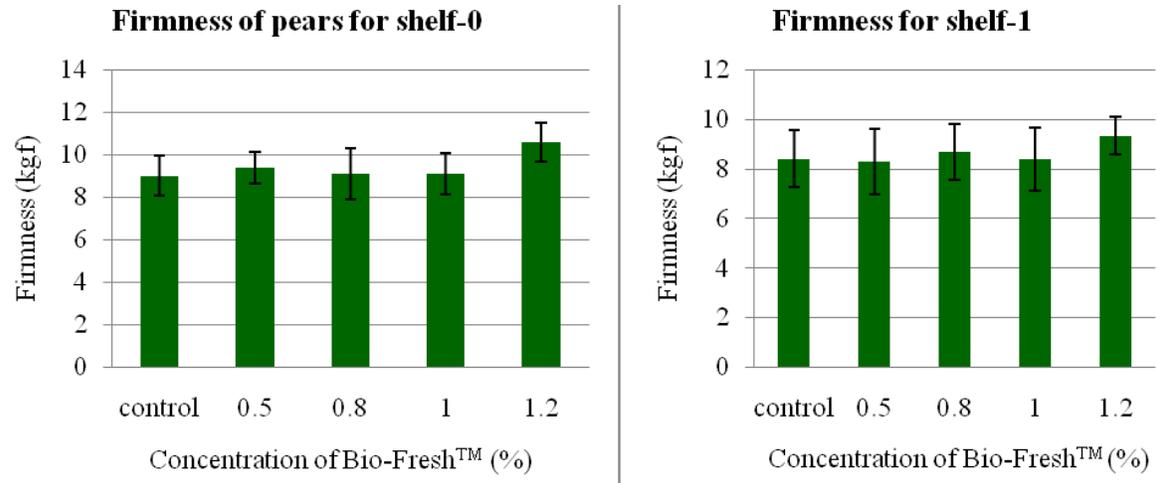


**Figure 1.** Changes of firmness among different concentration of Bio-Fresh[TM] during shelf life study.

## Color change for shelf-0



## Color change for shelf-1
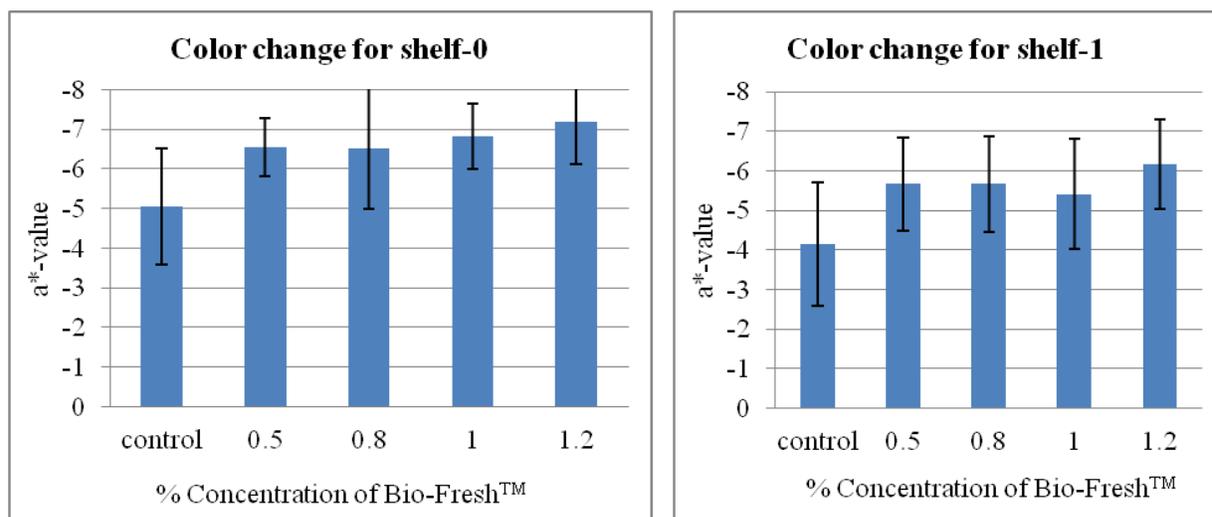


**Figure 2.** Changes of color among different concentration of Bio-Fresh[TM] during shelf life study.

## Soluble solids content (SSC)

The soluble solids content is a common physical quality (maturity) indicator for fruits and fruit juices. Statistical analysis of the data revealed that all coating concentrations were not significantly different for retention of the soluble solid content during different shelf conditions but showed similar level of soluble solid content (Figure 3).

## Weight loss

The weight loss has a strong impact on the pears appearance due to shrinkage or shriveling. Coating of pears with Bio-Fresh[TM] showed the variation of weight loss with storage time for coated and uncoated pears.

The results (Figure 4) showed that coating of 1.2% Bio-fresh[TM] significantly reduced the weight loss among all coating concentration.

## Incidence of disorders

Edible coating can increase disorder of pears associated with high $CO_2$ or low $O_2$ by modifying the internal atmospheres of pears. To check the effects of Bio-Fresh[TM] edible coating, the following disorders were observed

### Cavities and internal browning

The analysis of variance revealed that there were no

## Soluble solid content for shelf-0



## Soluble solid content for shelf-1
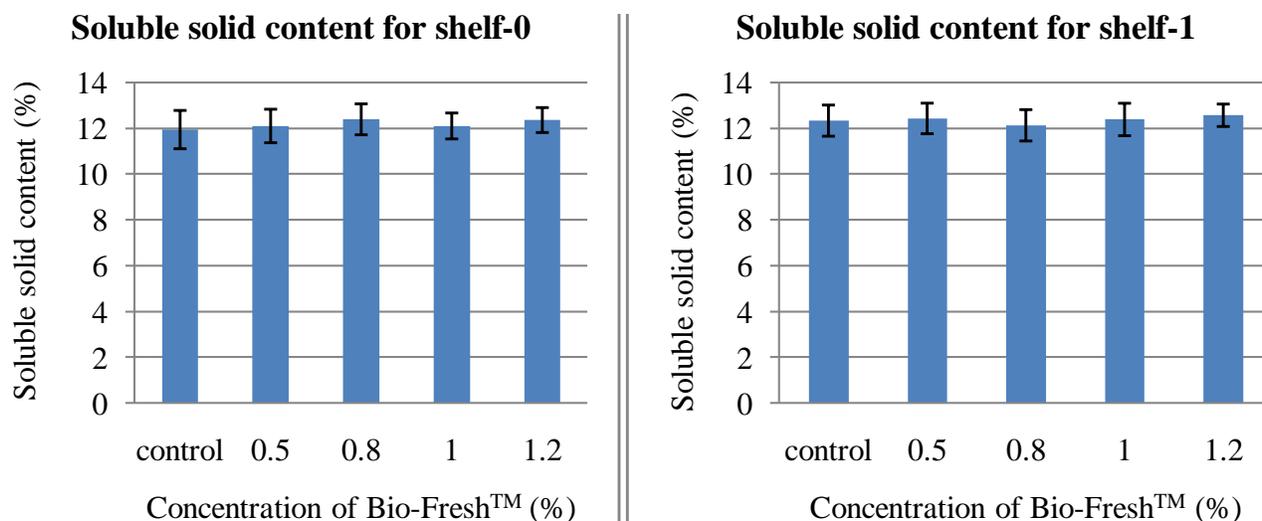


**Figure 3.** Changes of soluble solid content among different concentration of Bio-Fresh[TM] during shelf life study.
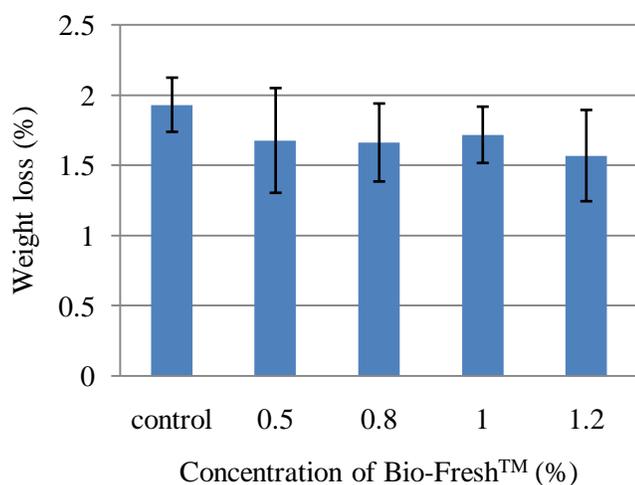


**Figure 4.** Weight loss among different concentration of Bio-Fresh[TM].

significant observations of cavities and internal browning in pears during different shelf conditions as compared to control sample in pears for different concentration of Bio-Fresh[TM] (Figure 5).

### *Shriveling*

Fresh produce is susceptible to shriveling due to water loss. The effects of Bio-Fresh[TM] coating on pears to reduce shriveling which allows the retardation of water loss were evaluated. The statistical analysis showed that high standard error among all coatings concentration during different shelf conditions that could be due to few amounts of shriveling observation (Figure 6).

### Gas exchange

The results of gas exchange measurement have been discussed with respiration and the permeability of Bio-Fresh[TM] coating of pears described by means of Michaelis-Menten kinetics.

### *Permeability of coatings*

Pears were coated by dipping in coating solution after storage and the permeability of Bio-Fresh[TM] was measured. The effects of Bio-Fresh[TM] coating on pears showed the permeability to gases were low at concentrations of 0.8, 1.0 and 1.2% Bio-Fresh[TM] as compared to 0.5% coating (Table 1).

Oxygen permeability was higher than carbon dioxide for these coating concentrations. Coating of 1.2% Bio-Fresh showed the low permeability as compared to other treatments. Coating of 0.5% Bio-Fresh[TM] was not successful due to high standard error. The concentration profile of $O_2$ (Figure 8) showed the more $O_2$ gradient which had substantial effect to modify the internal atmosphere of pears. The respiratory gas concentration profile with time for coating of 1.2% Bio-Fresh[TM] is shown in Figure 7.

### DISCUSSION

### Quality evaluation

Previous research indicated that the inhibition activities of pectin degrading enzymes was closely related to fruit softening and contributed to firmness maintenance by reducing the rate of metabolic process during ripening (Zhou et al., 2008). The results indicate that 1.2% coating

**Cavities of pears for shelf-0**



**Browning of pears for shelf-1**
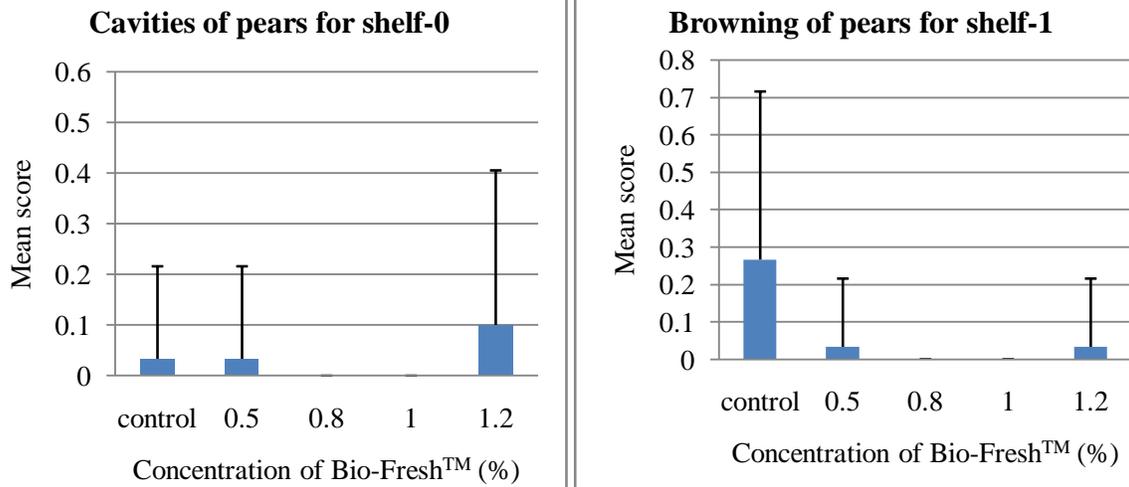


**Figure 5.** Incidence of cavities and browning on pears among different concentration of Bio-Fresh™ during shelf life study.

**Shriveling of pears for shelf-0**



**Shriveling of pears for shelf-1**
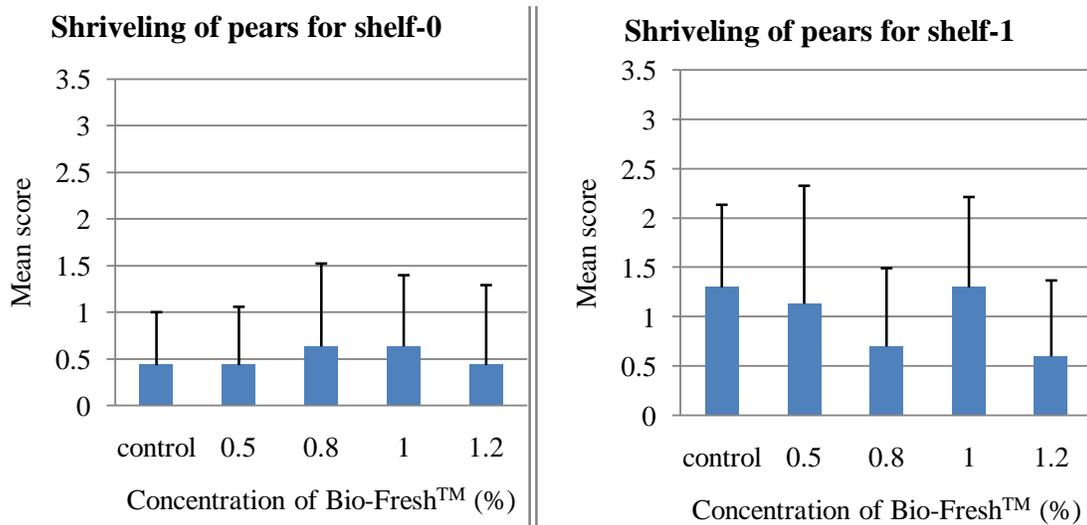


**Figure 6.** Incidence of shriveling on pears among different concentration of Bio-Fresh™ during shelf life study.

**Table 1.** Estimation of permeability parameters of coated pears after 6 months of storage and the values represent ± 95% confidence interval.

| Treatment | Permeability parameter | | | |
|---|---|---|---|---|
| Optimally picked | $h_{O2}$ (m/s) | $h_{CO2}$ (m/s) | stdev $h_{O2}$ (m/s) | stdev $h_{CO2}$ (m/s) |
| 0.5% Bio-Fresh™ | 3.66E-05 | 0.000751 | 0.0034311 | 2.2604 |
| 0.8% Bio-Fresh™ | 1.25E-07 | 5.00E-08 | 2.48E-08 | 9.24E-09 |
| 1.0% Bio-Fresh™ | 1.21E-07 | 2.76E-08 | 2.35E-08 | 3.53E-09 |
| 1.2 % Bio-Fresh™ | 1.25E-07 | 1.37E-08 | 1.39E-08 | 6.47E-10 |

concentration of Bio-Fresh™ may maintain firmness by inhibiting the activities of pectin degrading enzymes and inhibiting water loss (Figure 1) on pears coated by dipping. Coating of 1.2% Bio-Fresh™ may also make
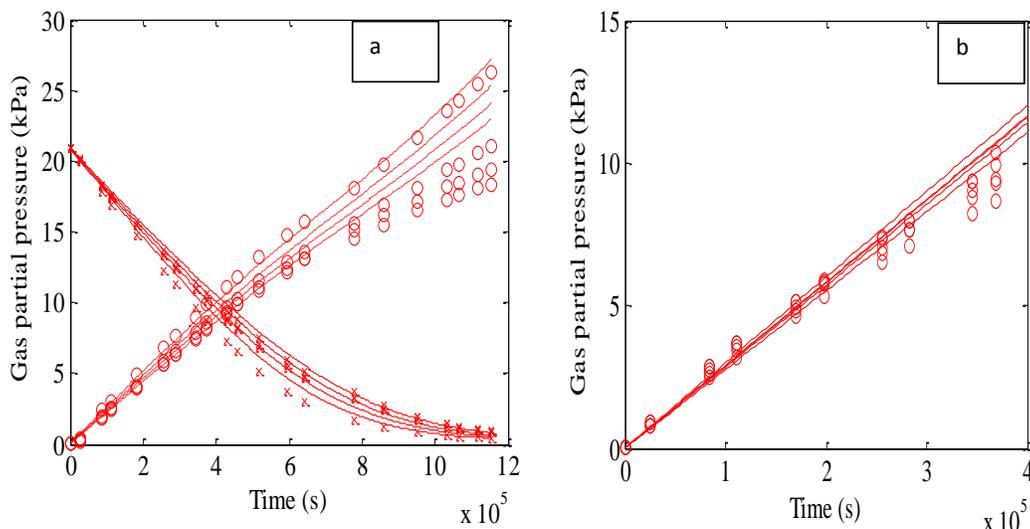
**Figure 7.** Gas concentrations profile as a function of time in jars contained coated pears with 1.2% Bio-Fresh[TM] at storage temperature of 10°C. Solid (—) and dashed (- -) lines indicate modeled $O_2$ and $CO_2$ partial pressure while symbols (x) and (o) indicate $O_2$ and $CO_2$ measurement. The initial conditions were (a) 20 kPa $O_2$, 0 kPa $CO_2$ and (b) 0 kPa $O_2$, 0 kPa $CO_2$.
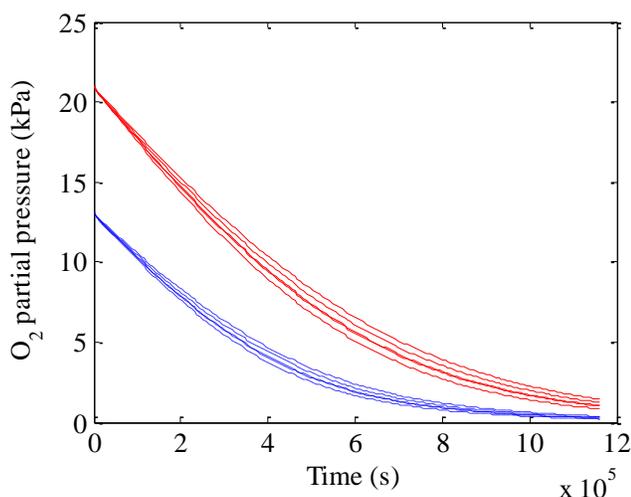


**Figure 8.** Mean concentration profile of $O_2$ with time in the outer (solid line: —) and inner (dashed line: - -) side of coatings on pears of 1.2% Bio-Fresh[TM].

internal atmosphere modification (low oxygen and high carbon dioxide concentrations) on pears. Hence, results of the research nicely reflect the findings of Yaman and Bayindirli (2002) for cherries, Sumnu and Bayindirli (1995) for Amasya apples coated with Semperfresh[TM], and Amarante et al. (2001) for pears coated with carnauba bases wax.

Coatings of Bio-Fresh[TM] were more pronounced for the substantial effect on changes in skin color. All coating concentrations were significantly good for maintaining the green color of pears during different shelf life. Coating of

1.2% Bio-Fresh[TM] was more effective for retention of green color than control sample and other treatments (Figure 2). The beneficial effect of Bio-Fresh[TM] coatings on skin color can be explained by proper blockage of pores (lenticels and stomata) as well as cracks of the skin (Amarante, 1998). Similar results were found on banana coated with sucrose fatty acid esters (Momen et al., 1997), cherries coated with Semperfresh[TM] (Yaman and Bayindirli, 2002), and on pears (Amarante et al., 2001).

Soluble solids and organic acids of fruits are substrates that are consumed by respiration during storage (Ozden and Bayindirli, 2002; Yaman and Bayindirli, 2002). In this study, only 1.2% coating were effective for the retention of soluble solid content as compared to the control (Figure 3) and other treatment because lower respiration rates retarded the overall metabolic activities of pears during storage. Similar results were found by Zhou et al. (2008), Hasan and Arslan (2004) and Ju et al. (2000).

The main mechanism contributing to the weight loss is the evaporation activated by a gradient in water vapor at different location in fruit (Yaman and Bayindirli, 2002). In this study, pear coated by dipping method showed coating of 1.2% Bio-Fresh[TM] was effective for inhibition of weight loss during storage (Figure 4). The reason for the reduction in weight loss may be the blockage of lenticels and stomata (Amarante, 1998; Amarante et al., 2001) as evidenced by the reduction in respiration and gas exchange (Hagenmaier and Baker, 1993).

Cavities arise from brown tissue because of time course of internal browning (Lammertyn et al., 2000). Browning disorder caused by imbalance oxidative and reductive processes due to metabolic gas gradients inside the fruit, lead to an accumulation of reactive

**Table 2.** Comparison of estimated permeability parameters of coated pears after 6 months of storage between two methods and the values represent ± 95% confidence interval.

| Treatment | Permeability parameter | | | |
|---|---|---|---|---|
| | *Method 1 | | *Method 2 | |
| Optimally picked | $h_{O2}$ (m/s) | stdev $h_{O2}$ (m/s) | $h_{O2}$ (m/s) | stdev $h_{O2}$ (m/s) |
| 0.5% Biofresh | 3.66E-05 | 0.0034311 | 7.80E-05 | 0.0004099 |
| 0.8% Biofresh | 1.25E-07 | 2.48E-08 | 8.75E-08 | 3.73E-09 |
| 1.0% Biofresh | 1.21E-07 | 2.35E-08 | 1.08E-07 | 7.20E-09 |
| 1.2 % Biofresh | 1.25E-07 | 1.39E-08 | 5.41E-08 | 2.80E-09 |

*Method 1: Estimation $O_2$ and $CO_2$ permeabilty simultaneously and *Method 2: Estimation with assuming $O_2$ and $CO_2$ permeability following Graham's law ($h_{CO2}=0.8528h_{O2}$).

oxygen species which may induce loss of membrane integrity through the enzymatic oxidation of phenolic compounds to brown color polymer compound (Franck et al., 2007). Shriveling is due to water loss by respiration and transpiration (Woods, 1990). The Bio-Fresh[TM] edible coating was not statistically significant but showed good for retardation of shriveling, cavities and internal browning during shelf life study of pears (Figures 5 and 6).

**Permeability of coatings**

The respiration is a good index for the quality of fruits during storage. Edible coating of Bio-Fresh[TM] with a concentration of 1.2% coated by dipping reduced the respiration rate which contributes to longer shelf life with good quality. Similar results were found on green pepper coated with Semperfresh[TM] (Ozden and Bayindirli, 2002). The suppression of respiration was likely due to the modification of the internal atmosphere of pears caused by the semi-permeable characteristics of the Bio-Fresh[TM] coating to the respiratory gases (Banks, 1984). The concentration profile of $O_2$ (Figure 8) confirmed that coatings slightly modified the internal atmosphere of pears.

Effect of coating on $O_2$ consumption can be found by decreasing the slope of $O_2$ concentration profiles (Figure 8) but difficult to evaluate for $CO_2$ due to fermentation at low $O_2$ concentration. Estimated $h_{CO2}$ is much lower than those values of $O_2$ (Table 1).

Assuming $O_2$ and $CO_2$ per-meability follows Graham's law, estimated $h_{O2}$ was reported is Table 2. Rather similar $h_{O2}$ values between two methods (Table 2) indicated that $O_2$ permeation (Equations 5 and 7) was less affected by $CO_2$ concen-tration. Note that at 0.5% Bio-Fresh[TM], coating was not successful due to high permeability with high variation.

Oxygen is the key factor for oxidation which is responsible for changes in color and firmness. Therefore, coatings that provide proper oxygen barrier can help in improving food quality and extending shelf life. The oxygen permeability is too low, anaerobic respiration will commence, resulting to production of ethanol and off flavor as well as product deterioration, if coating showed too high permeability, the internal atmosphere will not be modified to have beneficial results to extend the shelf life (Baldwin et al., 1999).

On the other hand, carbon dioxide is very important for respiration and higher permeability value can delay fruits softening (Kader, 1986). The permeability (Table 1) of 1.2% Bio-Fresh[TM] coating for $O_2$ and $CO_2$ was good as compared to other coating concentrations for maintaining the quality of pears.

**Conclusion**

The benefits of different concentrations of Bio-Fresh[TM] applied by dipping for the extension of shelf life with good quality were significantly effective for maintaining the color of pears. Coating of 1.2% Bio-Fresh[TM] modest delayed the changes of firmness, soluble solid contents and inhibited cavities, internal browning, shriveling and weight loss of pears during storage than other concentrations.

Although, all coating concentrations of Bio-Fresh[TM] exhibited better reduction of respiration rate and permeation of coating except 0.5% coating and 1.2% Bio-Fresh[TM] showed low permeability of coating with good quality of pears. With the view of the above findings, 1.2% Bio-Fresh[TM] can be used for extending the shelf life of pears without significant loss of quality.

**Conflict of Interests**

The authors did not declare any conflict of interests.

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*Full Length Research Paper*

# Effect of extruded soy-cocoa and corn starch based complementary food on some haematological, biochemical and histopathological parameters of rats

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Extruded soy-cocoa and corn-starch based complementary diet with a protein-energy ratio of 21% holds great promise in alleviating malnutrition so prevalent among Nigerian children under five years. There is however, paucity of scientific information on its safety- a necessary prelude to trials on human subjects. This study reports a controlled feeding trial involving 29 adult *wistar* rats housed in standard cages and acclimatization for 10 days under tropical room temperature conditions. Following a subsequent daily intubate feeding, 50 - 5000 mg/kg body weight for 21 days, sub-acute toxicity studies (anthropometric, biochemical, haematological and histopathological) were done. Results show that the diet had a statistically significant salutary effect on growth (weight gain 56.9%) of test rats when compared with control (37.85) at an optimum daily intake of 100 mg/kg body weight. Haematological characteristics such as mean corpuscular hemoglobin concentration (MCHC) range from 276-294 g/l for treatment groups as against 282 g/l for control with no significant difference ($P \leq 0.05$). The values (0.56 - 7.74 µ/kat/l) obtained for alkaline phosphate (ALP)- a key biochemical marker in liver function tests were within permissible limits. Moreover, rat biopsy (histopathology) revealed no necrosis. Evidently extruded soy-cocoa corn starch-based complementary food has no established deleterious effect and may therefore be safe for humans.

**Key words:** Complementary food, extruded soy-cocoa, sub-acute toxicity, food safety, rat biopsy.

## INTRODUCTION

The end of the 20th century was marked by drastic increase in the incidence of chemical hazards as well as other safety issues (Motarjemi and Lelieveld, 2013). In a bid to establish the safety or otherwise of raw or processed foods for human consumption, it is customary to test same on animal models which could provide relevant indirect information. New complementary foods meant to provide nutritional support for improving growth and for continued reduction in child morality/morbidity are by no means an exception (SACN/COT, 2012). Against backdrop of research findings such as enlargement of pancreas and inhibited growth of rats fed sub-optimally

**Table 1.** The complementary food stored under similar condition as the basal diet had the following composition.

| Parameter | Composition (%) |
|---|---|
| Moisture | 0.1±0.09 |
| Protein | 21.7±0.4 |
| Fat | 3.7±0.2 |
| Ash | 3.0±0.1 |
| Crude fibre | 0.5±0.1 |
| Carbohydrate** | 71.6±0.8 |
| Potassium | 1.4±0.2 |
| Phosphorus | 0.39±0.1 |
| Calcium | 0.21±0.1 |
| Magnesium | 0.15±0.1 |
| Sodium (mg/10 g) | 8.3±0.4 |
| Manganese | 2.5±0.2 |
| Iron (mg/100 g) | 13±1.0 |
| Copper (mg/100 g) | 3±0.1 |
| Zinc (mg/100 g) | 2.1±0.1 |
| Polyphenol (mg/100 g) | 50.41±0.1 |
| Energy Kcal/100 g | 406.5±5.6 |

**Carbohydrate was by difference. Source: Arueya (2013).

processed soybean foods (Lusas, 2004), theobromine (as in cocoa) induced fetal malformation (Eteng et al., 1997), preclinical experimentation using animals are therefore in order.

Ogazi et al. (1990) in reporting the effects of 'soya musa' (an extruded soybean-plantain baby food) on sixteen *wistar* rats observed that they had normal growth, packed cell volume and white blood cell counts. No immunological reaction occurred following haemagluti-nation tests. In evaluating the biological effects of a cassava-soy weaning food on rat organs (small intestines, pancreas, liver and heart), Babajide et al. (2001) saw no significant difference in the organ weight of the rats fed the test diet as compared to those fed cerelac - a commercial weaning food. Rats fed on extruded weaning foods based on peanut, maize and soybean were observed by Plahar et al. (2003) to have between 60-100 fold increases in mean weight gain over the control. It was reported that haematological data of test animals showed normal values for white blood cells (WBC) count, red blood cell (RBC) count, haemoglobin (Hb) levels and packed cell volume (PCV) for all the weaning foods studied except the control. In an experiment involving the nutrient status of the protein of corn-soy based extruded products evaluated by rats bioassay, Baskaran and Battacharaya (2004) reported higher body weight gain by rats fed the test diet as compared to the control (fed skimmed milk powder).

Information on the possible safety or otherwise of a complementary food based on extruded soy-cocoa is virtually non-existent. There is need to ascertain this in view of the likely potential benefit of such a product harnessing properties of these two agricultural produce (Dillinger et al., 2000; Lusas, 2004).

## MATERIALS AND METHODS

### Experimental animals

The animals used in this study were adult male and female albino *wistar* rats (100-200g) obtained from the animal house, department of Clinical Pharmacy, University of Ibadan. The animals were used after an acclimatization period (10 days) to well ventilated room with temperature 30 ± 4°C and relative humidity of 60%. They were housed in standard cages.

### Basal diet

The animals were maintained on standard animal pellets (obtained from Ladokun Feeds Ltd., Ibadan, Nigeria) and potable water *ad libitum*. The proximate composition of the pellet as stored under room temperature (28±2°C): protein 21%, fat 3.8%, fibre 6.0%, calcium 0.8%, phosphorus 0.8%.

### Complementary food

The complementary food stored under similar condition as the basal diet had the following composition (Table 1).

### Acute toxicity studies

Following clearance by the UI/UCH ethical Review Committee, the fixed dose procedure of Boyd (1976) and organization for European cooperation and development (O.E.C.D) as described by (Botham, 2004) was followed. Gruels (20% w/v) were prepared from the complementary food by making a smooth cold paste and gradually pouring same into boiling water. This was stirred vigorously until the entire mass became viscous. The resulting mass was administered (intubate feeding - syringe feeding through the esophagus into the stomach) to the rats (five per group) in five groups with single dose ranging from 50 - 5000 mg/kg body weight of the rats. The sixth group of five served as control. After 24 h, the animals were inspected for appearance of signs of toxicity and possible deaths.

### Sub-acute toxicity

The intubate feeding reported continued daily for twenty-one (21) days immediately after which the blood samples were taken through induced bleeding (from the orbital sinus) into heparinised and ethylene-diaminetetraacetic (EDTA) coated bottles.

### Biochemical analysis

Some biochemical parameters (total protein, albumin, aspartate (AST), alanine amino transferase (ALT) alkaline phosphatase (ALP) urea and creatinine) were determined using Roche diagnostic test kits. (Indiana, USA).

### Haematological analysis

Haematological (mean corpuscular volume (MCV), mean corpuscular

**Table 2.** Feed treatment effects on mean weights.

| Mean weight (g) | Soyco meal (mg/kg body wt) | | | | | |
|---|---|---|---|---|---|---|
| | Control | 50 | 100 | 300 | 2,000 | 5,000 |
| Initial | 164±2.6[a] | 138±1.9[a] | 116±1.7[a] | 172±2.1[a] | 173±1.4[a] | 124±1.6[a] |
| Final | 226±2.3[b] | 167±2.6[b] | 182±1.8[b] | 232.5±2.3[b] | 196±1.2[b] | 144±1.7[a] |
| Weight gain (%) | 37.8 | 21.0 | 56.9 | 34.8 | 13.3 | 16.1 |

Means not having the same superscript (a, b or c) within a column are significantly different at $P \leq 0.05$.

hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), neutrophils, lymphocytes, packed cell volume (PCV), hemoglobin (Hb), (RBC), (WBC), platelets) were evaluated by Sysmex automated hematology analyzer KX - 21 (Sysmex corporation, kobe, Japan).

### Histopathological analysis

After the induced bleeding, rats from each group (1 - 6) were sacrificed through vascular dislocation. Three organs namely liver, kidney and spleen were removed from the animals. They were weighed and subjected to histopathological analysis (toxicity signs) after fixation in slides using the methods of Adesiji (1999). The prepared slides were viewed under the light microscope (model:420- 420AHF-10) and examined by a pathologist.

### Stool consistency

Samples of stool from the various groups were also compared for consistency.

### Mortality rate

This was done by visual observation and counting of any death among the rats.

### Statistical analysis

All the data obtained were statistically analyzed using the analysis of variance (ANOVA) SPSS software package (No. 13). Means were separated using Duncan's multiple range test (P<0.05).

## RESULTS AND DISCUSSION

### Acute toxicity studies

There were no visible signs of toxicity such as gasping or writhing within the first 24 h of diet administration. There was however decreased motor activity (reduced movement) evident through direct observation within the first 2 min of administration of the diet at 5000 and 2000 mg/kg levels. These animals became more active, ostensibly having overcome the initial shock of sudden expansion of the gastric (stomach) chamber of the rat. There was no death of animal recorded.

### Sub acute toxicity

### Feed treatment effects on mean weights of rats

Changes ranging from 144 - 232.5 g were observed in weights of rats fed the test diet and the control (Table 2). While there was significant gain across all the test groups and control, rats fed 100 mg/kg body weight were exceptionally high, attaining a 56.9% weight gain. This is the only grouping where the figure obtained was greater than the control. The progressive increase in percentage weight gain from 50 mg/kg body weight diet fed rats to 100 mg/kg body weight and thereafter a decrease as compared to the control may indicate that the diet had an optimum salutary effect on rat growth at the 100 mg/kg body weight feeding level. Increase in weight gain is an indication that the diet supported growth and this is in agreement with the findings of Plahar et al. (2003). In the work, they observed between 60-100 fold increases in mean weight gain over control when rats were fed extruded weaning foods based on peanut, maize and soybean. Apparently beyond this level (100 mg/100 g body weight), rats can no longer optimize the benefits of the diet. This may be due to increasing density of antinutritional factors such as polyphenols per unit weight of diet intake. A similar conclusion has been reached in some studies where anti-nutritional factors affected the activity of digestive enzymes *in vivo* (Anantharaman and Finot, 1993). It has also been noted that above serum levels of 25 µg/ml, cocoa polyphenols becomes inhibitory (Mao et al., 2003). The study showed that this level is a critical threshold for some stimulatory/inhibitory effect on growth related secretions from peripheral blood mononuclear cells.

### Organ weights relative to body weights of rats after feeding duration

There was no significant difference ($P \leq 0.05$) in the weights of liver and spleen across the test groups and control after a feeding duration of 21 days (Table 3). This also holds true for kidney weights of the animals fed at 50, 2,000 and 5,000 mg/kg body weight. The implication is that the test diet intake may not have deleterious

**Table 3.** Selected organs relative to body weight of rats after feeding duration (21 days).

| Mean relative weight (%) organs | Control | 50 | 100 | 300 | 2,000 | 5,000 |
|---|---|---|---|---|---|---|
| Kidney | 0.567±0.1[c] | 0.521±0.2[a] | 0.615±0.1[b,c] | 0.525±0.1[b,c] | 0.523±0.1[a, b] | 0.607±0.2[a] |
| Liver | 2.84±0.3[a] | 2.81±0.5[a] | 2.97±0.5[a] | 2.63±0.6[a] | 2.8±0.3[a] | 2.85±0.3[a] |
| Spleen | 0.27[a] | 0.21[a] | 0.40[a] | 0.28[a] | 0.49[a] | 0.41[a] |

Means not followed by the same superscript (a, b or c) across the rows are significantly different at p < 0.05.

**Table 4.** Feed treatment effects on mean values of some haematological parameters between groups of rats.

| Haematological parameters | Soyco-meal (mg/kg body wt) | | | | | |
|---|---|---|---|---|---|---|
| | Control | 50 | 100 | 300 | 2,000 | 5,000 |
| Mean corpuscular volume (MCV) (fL) | 61.4±1.2[a,b] | 59.2±1.9[a] | 65.0±1.6[b] | 63.3±1.5[b] | 65.0±2.9[b] | 69.4±1.6[c] |
| Mean corpuscular hemoglobin (MCH) (pg) | 17.4±1.9[a] | 17.4±2.1[a] | 18.4±1.7[abc] | 17.8±1.6[ab] | 18.8±1.7[bc] | 19.2±1.7[c] |
| Mean corpuscular haemoglobin concentration (MCHC) (g/l) | 282±2.6[a] | 294±1.8[a] | 280±1.3[a] | 280±1.7[a] | 276±1.5[a] | 276±1.5[a] |
| Neutrophils (%) | 18.0±2.1[a] | 21.8±1.8[a] | 13.2±2.1[b] | 21.3±1.6[a] | 26.6±2.3[a] | 20.6±0.9[a] |
| Lymphocytes (%) | 80.8±34[a] | 77.4±3.1[a] | 86.0±2.8[a] | 77.5±3.0[a] | 71.8±3.2[a] | 79.0±3.0[a] |
| Packed cell volume (PCV) (%) | 54.4±2.2[ab] | 52.2±2.1[a] | 56.6±2.8[b] | 61.0±2.0[c] | 57.6±1.8[bc] | 50.4±2.1[a] |
| Haemoglobin (g/l) | 154.8±2.8[b] | 152.6±2.7[b] | 157.8±3.0[b] | 170.5±1.5[c] | 158.8±2.5[b] | 140.6±1.8[a] |
| Red blood cells (x10$^3$/µl) | 8.86±0.1[b] | 8.70±0.1[b] | 8.70±0.2[b] | 9.60±0.1[c] | 8.48±0.2[b] | 7.34±0.1[a] |
| White blood cells (x10$^3$/µl) | 12.26±2.0[a] | 10.3±1.6[a] | 11.44±1.5[a] | 11.48±1.8[a] | 13.32±2.1[a] | 15.3±1.7[a] |
| Platelets (x10$^3$/µl) | 771.6±28.0[a,b] | 916.8±26.1[c] | 673±23.0[a] | 741±30.1[a,b] | 659.4±27.3[a] | 861.6±28.4[bc] |

Mean values not accompanied by the same superscript (a, b or c) across rows are significantly different at ($P \le 0.05$).

effects (such as a tendency toward enlarging those organs). The statistical disparity in the relative organ weight for the kidneys of the treatment groups and the control may be due to responses to other dietary factors which do not in themselves lead to damage. This fact is underscored by the histopathological analysis which revealed no evident signs of necrosis of the cells. It is noteworthy also that the creatinine and urea levels (-indicator of kidney function) compare favourably with control and hence supporting this assertion. It must be stated that exclusive use of relative organ weight for rats and other species of animals in toxicological experiments may lead to faulty conclusion unless corroborated by other facts (Bailey et al., 2004).

**Effect of varying levels of feed intake on some haematological parameters in rats**

The mean values obtained for 50, 100, 300, 2,000 mg/kg body weight treatment groups were not significantly different (p≤ 0.05) from the control (Table 4). The parameters reported for the group fed with 5,000 mg/kg body weight of the rat appears unique. However, the mean corpuscular haemoglobin (MCH) for this treatment group compared favourably with those of 100 and 2,000 mg/kg body weight group but were significantly different

(P ≤ 0.05) from those of the control group. The 294 g/L mean corpuscular hemoglobin concentration (MCHC) obtained for 50 mg/kg body weight treatment group was the highest. This is also true for the neutrophils excluding the 100 mg/kg body weight group where this is lower than others. The reason for this is not obvious especially with the significant weight gained but may be traceable to non-dietary factors (Michael et al., 2008). The highest value of 61.0% obtained as packed cell volume (PCV) for the 300 mg/kg body weight test group compared favourably with the treatment group of 2,000 mg/kg body weight. The haemoglobin concentrations followed the same pattern with the PCV. The red blood cell levels for 50, 100 and 2,000 mg/kg body weight treatment groups of rats were not significantly different from the control. This was also true for white blood cells and platelets.

The MCV, MCH, MCHC values including RBC, PCV and Hb- major indicators of assessment of tendency towards anaemia shows that the diet was neither toxic toward red blood cells nor an impediment towards erythopoesis (Bain et al., 2011). The difference in WBC values of test groups and the control may be attributable to a suppression of leucocytosis in the bone marrow (Antia et al., 2006). The lower platelets levels as compared to control may be due in part to bioavailability of cocoa polyphenols. This conclusion is borne out of a study reported by Rein et al. (2000), where after a cocoa

**Table 5.** Feed treatment outcomes on some mean values of some biochemical parameters in rats.

| Biochemical parameter | Soyco-meal (mg/kg body wt) | | | | | |
|---|---|---|---|---|---|---|
| | Control | 50 | 100 | 300 | 2,000 | 5,000 |
| Alkaline phosphatase (ALP) (μkat/L) | 6.67±0.5[c] | 3.38±0.4[b] | 0.56±0.1[a] | 7.74±0.4[c] | 6.67±0.3[c] | 5.74±0.5[c] |
| Alanine amino transferase (ALT) (μkat/L) | 1.83±0.1[a] | 1.79±0.2[a] | 1.56±0.1[a] | 1.68±0.2[a] | 1.58±0.2[a] | 1.40±0.1[a] |
| Aspartate amino transferase (AST) (μkat/L) | 6.16±1.1[a] | 5.33±0.9[a] | 4.44±0.8[a] | 6.31±1.2[a] | 4.89±1.0[a] | 4.98±1.2[a] |
| Total Protein (T.P) (g/L) | 92±9.2[ab] | 110.8±8.2[c] | 84.4±8.6[a] | 102±10.1[bc] | 105±8.3[b,c] | 93.8±10.4[ab] |
| Albumin (g/L) | 45±3.6[b] | 51.4±3.1[c] | 46±3.5[b,c] | 49±2.9[bc] | 45.8±3.0[b] | 40.2±2.7[a] |
| UREA (mmol/L) | 21.9±1.5[ab] | 21.4±1.8[ab] | 19.0±1.2[a] | 25.0±1.0[.b] | 23..8±1.6[b] | 22.6±1.2[ab] |
| Creatinine (μmol/L) | 100.8±1.5[b] | 99±1.3[b] | 95.5±1.7[a,b] | 103.4±1.1[b] | 93.7±1.8[a,b] | 84.9±1.6[a] |

Mean values not accompanied by the same superscript (a, b or c) across rows are significantly different at P < 0.05.

drink high in polyphenols was taken, a reduction in platelet surface marker (PAC-1) was found in human subjects, an indication of decreased platelet activation. Platelets carry blood-clotting factors and are important for wound healing.

## Effect of feed treatment on some mean values of biochemical parameters in rats

The alkaline phosphatase (ALP) values for treatment groups (0.56- 7.74 μkat/l) were generally not significantly different from the control (6.67 μkat/l) (Table 5). The group of 50 and 100 mg/kg body weight feed treatment were though lower in real values. It is however instructive that the highest weights gained among the rat groupings are associated with this low values which may just be the optimum for fastest growth rate. Experimental results established for alkaline aminotransferase (ALT) across all the groupings were not significantly different at (P ≤ 0.05). A similar pattern was evident for alanine amino transferase. The diet appears to be having a slight depressing activity on these enzymes linked principally with liver function, in view of their comparatively reduced values (Antia et al., 2006). Increased values are associated with higher activity and may likely indicate damage or hyperplasia of liver cells (Benjamin, 1978).

The total protein for groups on 100 and 5,000 mg/kg body weight feed intake compared favourably with that of the control, but differ significantly from the 50, 300 and 2000 mg/kg body weight groups. The albumin levels of 100, 300 and 2,000 mg treatment groups (45.8-49 g/l) were essentially similar to control (45 g/l). Fluctuations in the levels of total protein and albumin in the rats' blood serum are reflections of likely deviation from the normal liver function (Ahmed et al., 1992).

The urea profile for treatment groups 300, 2000 and 5,000vmg/kg body weight were slightly higher but significantly different from that of the control group. The 50 and 100 mg/kg test groups were similar in this respect (21.4 and 19mmol/l respectively). With the exception of the 5,000 mg/kg body weight treatment groups (84.9 μmol/l) all others had creatinine level which were not significantly different (P ≤ 0.05)

## Histopathological findings

The absence of necrosis in the tissues examined as shown in photomicrographs (Figures 1 to 3) clearly established the safety of the diet at the levels administered following feeding duration.

## Mortality rates

There was no recorded death among the rats. This reinforces to some extent the safety of the diet.

## Stool consistency

The treatment groups had better stool consistency (Figure 4). There were no sign of diarrhoea or watery stool. This is significant as a number of food induced diarrhea have been reported (Motarjemi and Lelieveld, 2011). Additionally, excessive gas production by hetero-fermentative degradation of carbohydrate are known to cause loose stool and speed up the intestinal passage. In both cases, the absorption of nutrient become less efficient.

Absence of this occurrence in this study might be linked to the polyphenols/tannins content of the test diet. These have a tendency to interfere with microbial activities by immobilizing their extracellular enzymes and proteins on cell membranes (Gupta and Haslam, 1993; Shahidi and Naczk, 2002). Cocoa procyanidins (condensed tannins) are high in molecular weight and form strong complex with proteins. They are resistant to digestive enzmes and get transported down the intestinal colon (Jimenez-Ramayse et al., 1994). The foregoing may explain the better stool consistency from the test group as compared to the control as the administered diet is the only identified variable factor.

**(a) Control**     **(b) Treatment group (rep.) – 5,000 mg/kg body wt**
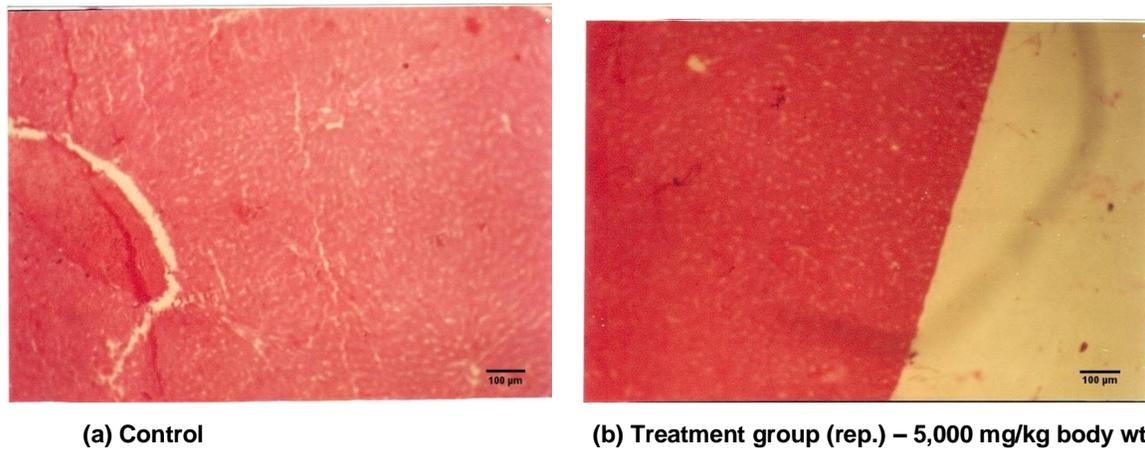
**Figure 1.** Photomicrographs indicating the null effect of the diet on the histopathology of the liver cells (liver architecture intact).



**(a) Control**     **(b) Treatment group (rep.) – 5,000mg/kg body wt**
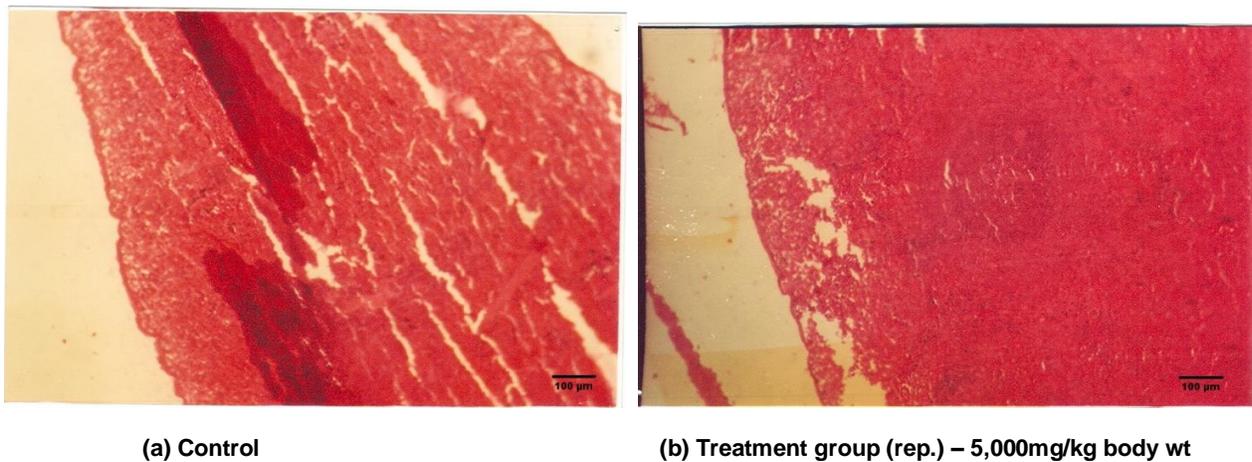
**Figure 2.** Photomicrographs indicating the null effect of the diet on the histopathology of the spleen cells (spleen architecture intact).



**(a) Control**     **(b) Treatment group (rep.) – 5,000mg/kg body wt**
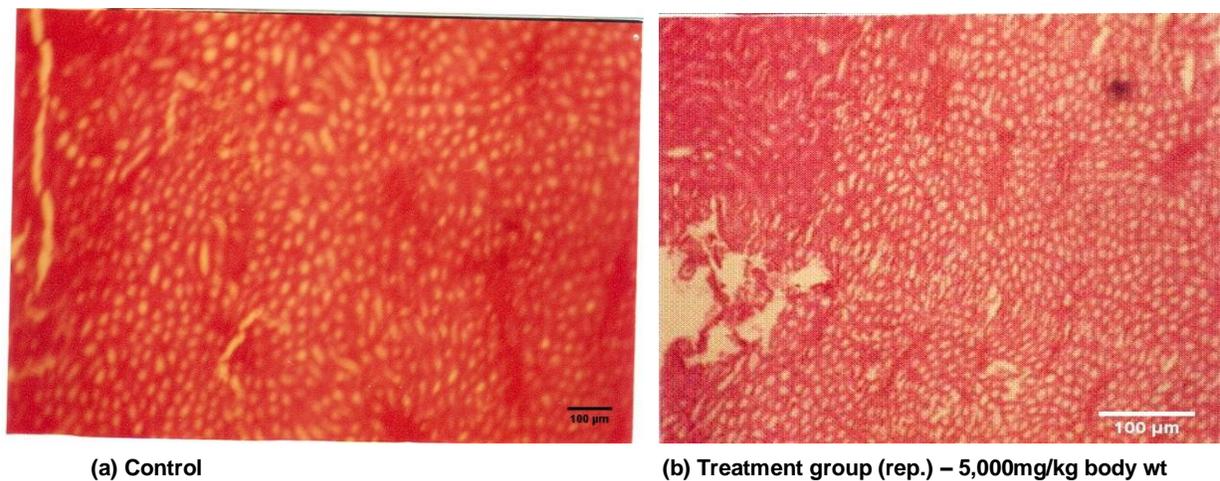
**Figure 3.** Photomicrographs indicating the null effect of the diet on the histopathology of the kidney cells (kidney architecture intact).

**Figure 4.** A pictorial illustration showing consistency of the rat stool (A = treatment group (5, 000 mg/kg body weight); (D = treatment group (100 mg/kg body weight).

## Conclusion

Evidently, the cocoa based complementary diet is safe and beneficial to growth especially at lower feeding levels of administration.

## Conflict of Interests

The authors did not declare any conflict of interests.

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*Full Length Research Paper*

# Phenolic content and antioxidant activity of selected Ugandan traditional medicinal foods

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Twenty one (21) traditional food plants recognized as medicinal by communities in Kamuli and Gulu districts in Uganda were identified and analyzed for their phytochemical content and antioxidant activity. The total phenolic content (2.6 ± 0.1 to 184.2 ± 6.4 mg GAE gDW$^{-1}$) and flavonoid content (0.3 ± 0.1 to 162.2 ± 3.5 mg CE gDW$^{-1}$) as well as antioxidant activity (0.1 ± 0.1 to 57.8 ± 0.5 mg VCE gDW$^{-1}$) varied widely among the food plants. The seeds of *Tamarindus indica* L. and leaves of *Ipomea eriocarpa* R.Br., *Corchorus trilocularis* L., *Ocimum suave* L., *Corchorus olitorius* L., *Acalypha bipartita* Müll.Arg., and *Hibiscus acetosella* Welw. ex Hiern showed the highest phenolic contents and antioxidant activities. Juice prepared from tamarind pulp alone or mixed with tamarind seed powder (0.5, 1 and 3%) and analyzed for sensory acceptability, phenolic content and anti-oxidant activity were found to be highly acceptable, recording an overall hedonic acceptability scores of 7.40 ± 0.4, 6.65 ± 0.3, 7.01 ± 0.1 and 5.34 ± 0.2, respectively on a nine-point scale for juice containing 0, 0.5, 1 and 3% seed powder. Incorporation of tamarind seed powder into tamarind pulp juice resulted in a dose dependent increase in antioxidant activity from 8.5 ± 0.2 mg VCE 100 ml$^{-1}$ for the control to 12.05 ± 0.3, 15.33 ± 0.7 and 17.22 ± 0.7 mg VCE 100 ml$^{-1}$ for tamarind juice containing 0.5, 1 and 3% seed powder, respectively. A similar trend was recorded for the total phenolic and flavonoids.

**Key words:** Medicinal foods, nutraceuticals, antioxidant activity, phenolics, flavonoids.

## INTRODUCTION

The consumption of significant amounts of certain particular foods, especially those of plant origin, including fruits, vegetables and whole grain cereals is associated with reduced risk of developing chronic degenerative diseases, such as cardiovascular diseases, cancers and diabetes mellitus (Espín et al., 2007). The health benefits of consuming such foods are ascribed partly to bioactive compounds found in the foods. For example, lycopene in tomatoes may protect against prostate cancers (Kucuk et al., 2002) and carotenoids in the various fruits and vegetables may also reduce the risk of prostate cancer (Jian et al., 2005) while flavonoids in tea act against cardiovascular diseases (Kris-Etherton and Keen, 2002). The phenolic and polyphenolic compounds comprise of important group of health promoting bioactive compounds, their protective effects are mainly attributed to

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**Figure 1.** Map of Uganda showing the geographic location of Gulu and Kamuli districts, where a study was conducted to identify foods traditionally considered having medicinal properties. Source: http://en.wikipedia.org/wiki/Gulu_District;http://en.wikipedia.org/wiki/Kamuli _District.

their antioxidant activity (Shahidi, 1997).

Several traditional foods from different communities have been analyzed for presence of relevant bioactive compounds and biological activities (Buřičová and Réblová, 2008; D'Antuono et al., 2010; Heinrich et al., 2005, 2006) and such investigations have provided scientific evidence and validation for health claims of the different traditions. In Uganda, many foods are traditionally believed to provide medicinal benefits (Rubaihayo, 2002; Tabuti and Van Damme, 2012). However, there is inadequate scientific evidence for the ascribed medicinal properties of these traditional foods. Thus, there is need to validate the nutraceutical proper-ties of traditional foods, given the rapidly increasing burden of chronic degenerative diseases in the country. It is anticipated that non-contagious chronic diseases will overtake infectious, maternal and nutritional diseases as the most common cause of death in Uganda by the year 2030 (WHO, 2011a, b).

In view of the above, this study was undertaken to analyze the phenolic content and antioxidant activity of selected traditional foods considered by local commu-nities in Uganda to have health benefits. In addition, the potential of using certain traditional food additives in enhancing the natural antioxidant activity of foods was evaluated.

## MATERIALS AND METHODS

### Study area

A study to identify foods traditionally considered to have medicinal properties was conducted among the rural communities in Gulu and Kamuli districts, Uganda. Geographically, Gulu district is situated in the northern part of Uganda (02° 45'N, 32° 00'E) (Figure 1), and is home to the ethnic Acholi tribe who are estimated to be appro-ximately 1.1 million people, representing 4.8% of the population of Uganda (UBS, 2002). Kamuli district on the other hand, is located in the eastern part of Uganda (00° 55'N, 33° 06'E), with the Basoga tribe as the main ethnic group. The Basoga are the third largest ethnic group in Uganda with population of 2.1 million people, representing about 8.6% of Uganda's population (UBS, 2002). The communities found in these two districts are mainly of rural setting (Lamordea et al., 2010), and consume rich diversity of foods originating from local plant or animal sources through gathering or harvesting (Oryema et al., 2013; Tabuti, 2007).

### Data collection

A survey was conducted to identify foods considered to have medicinal value in Gulu and Kamuli districts in Uganda. Ethno-botanical information was obtained from informants through key informant interviews and focus group discussions. A total of 51 informants (27 in Gulu district and 24 in Kamuli district), consisting of traditional healers, health workers and elderly persons who are natives of the area were involved. Semi-structured questionnaires were used to collect information on local food plants considered to

have medicinal value. Samples of identified foods reported to have medicinal value were collected and transported to the herbarium at Makerere University for identification.

## Determination of the phenolic content and antioxidant activity of the identified foods

### Plant material

The food plant materials collected were analyzed for total phenolic and flavonoid contents including total antioxidant activity.

### Sample preparation

Different parts of the food plant (leaves and seeds) were cleaned, dried in shade at ambient temperature of about 25°C, were ground into fine powder using a stainless steel blender 7011S (Waring Commercial, Torrington, CT, USA) and sieved through a 500 µm sieve. The fine powder obtained was stored in airtight plastic containers at 4°C until further analysis, within 30 days.

### Extraction of phenolic compounds

The extraction of phenolic compounds was done according to the method described by Makkar (2000) with minor modifications. Briefly, a small quantity (0.1 g) of the powdered sample was extracted in 5 ml of solvent mixture methanol : water (50:50 v/v) for 30 min at room temperature, with intermittent shaking. The extract was cooled in a refrigerator at 4°C for 10 min and then centrifuged at 3000 x$g$ for 10 min using a centrifuge (Fisher Scientific 225, Pittsburgh, PA, USA). The supernatant was recovered and the pellet re-extracted for 45 min under the same conditions until a second supernatant was obtained. Finally, the two supernatants were pooled together and used in the determination of total phenolic and flavonoid contents and total antioxidant activity.

### Determination of total phenolic content

The total phenolic content (TPC) was determined using spectro-photometry following Makkar (2000) method. To 100 µl of sample, 400 µl of distilled water was added to make the total volume to 0.5 ml. This was followed by addition of 0.25 ml of 1 N Folin-Ciocalteu reagent and 1.25 ml of 20% sodium carbonate solution. After 40 min incubation at room temperature, absorbance was read at 725 nm on a GENESYS spectrophotometer 10UV (Thermo Electron Corporation, Madison, WI, USA) against a blank solution containing methanol instead of the sample. A calibration curve was constructed within the concentration range 0.025 - 0.225 mg ml$^{-1}$ ($R^2$ = 0.999). Mean values were calculated from three parallel analyses. The total phenolic contents were expressed in milligram gallic acid equivalents per gram of dry weight (mg GAE gDW$^{-1}$) of plant material.

### Determination of total flavonoid content

The total flavonoid content (TFC) was measured using the colo-rimetric assay procedure reported by Muanda et al. (2011). Briefly, a 250 µl of a standard solution of catechin at different con-centrations was added to 10 ml volumetric flask containing 1 ml of double distilled water (ddH$_2$O). At zero min, 75 µl of 5% NaNO$_2$ solution was added to the flask. After 5 min, 75 µl of 10% AlCl$_3$ solution was added. After 6 min, 500 µl solution of 1 N NaOH was added to the mixture. Immediately after diluting the solution by addi-

tion of 2.5 ml ddH$_2$O and thoroughly mixing, the absorbance of the pink solution mixture was measured at 510 nm in a GENESYS spectrophotometer 10UV (Thermo Electron Corporation, Madison, WI, USA) against a blank solution containing distilled water instead of sample. A calibration curve was constructed within the con-centration range 0.025 - 0.225 mg ml$^{-1}$ ($R^2$ = 0.999). Mean values were calculated from three parallel analyses. The total flavonoid contents of the samples were expressed as milligram catechin equivalents per gram dry weight (mg CE gDW$^{-1}$) of plant material.

### Determination of total antioxidant activity

The total antioxidant activity (TAC) of the methanol extracts was estimated using the 1, 1-diphenyl-2-pycrylhydrazyl (DPPH) free radical scavenging assay (Kim et al., 2002). Briefly, to a 2.95 ml solution of freshly prepared absolute methanol, 100 µM solution of DPPH, 50 µl of the sample extract or control (50% [v/v] methanol) were added.

The mixture was shaken and allowed to stand in the dark at room temperature for 30 min. The absorbance of the resulting solution was measured at 517 nm in a GENESYS spectrophotometer 10UV (Thermo Electron Corporation, Madison, WI, USA) against a blank solution of absolute methanol. The free radical scavenging activity was calculated as:

$$Scavenging\ activity\ (\%) = \left[1 - \left(\frac{absorbance\ of\ sample}{absorbance\ of\ control}\right)\right] \times 100$$

A standard solution of ascorbic acid was run using several concentrations ranging from 0.002 to 0.1 mg ml$^{-1}$. A standard calibration curve of ascorbic acid was then prepared by plotting the percentage of free radical scavenging activity against concentration ($R^2$ = 0.999). The final value was expressed as milligram ascorbic acid equivalents per gram dry weight (mg VCE gDW$^{-1}$).

## Production of juice enriched with *Tamarindus indica* L. seed powder

### Preparation of tamarind seed powder (TSP)

Fruits of *T. indica* L. were purchased from a local market and transported to the Food Chemistry laboratory at Makerere University. The tamarind fruits were soaked in cold water (seed to water ratio of 1:2 (w/v) and the seeds removed manually from the pulp. The seeds were thereafter autoclaved at 121°C for 30 min to remove astringency (Legesse and Emire, 2012). The seeds were dried under shade at ambient temperature of about 25°C, milled into fine flour using the WonderMill grain mill (Grote Molen Inc, Pocatello, ID, USA), sieved through a 200 µm sieve size and stored in airtight plastic containers at 4°C until further use.

### Preparation of mixture of tamarind pulp and seed powder juice

Tamarind pulp juice was prepared by first dissolving 295 g of tamarind fruit into one litre of cold water while stirring for about 30 min and discarding all the seeds and fibres. The resulting pulp juice (about 900 ml) was filtered through cheese cloth to obtain a volume of 600 ml of juice. The volume of juice was then diluted with an equal volume of water and 120 g of sugar added to sweeten the taste. Thereafter, the sweetened juice was further divided into four equal portions of 300 ml each. A preparation of tamarind seed powder (TSP) was added to levels of 0.5, 1 and 3% (w/v) to each of the respective first, second and third portions of the 300 ml of the sweetened juice. All the three portions were heated to boiling point to facilitate extraction of phenolic compounds from the seed powder

and the mixture was filtered through cheese cloth to obtain the final tamarind pulp and seed powder juice. The remaining fourth 300 ml portion of the sweetened juice containing no tamarind seed powder (0% TSP) was used as a control.

### Sensory evaluation of the tamarind pulp and seed powder juice

The prepared tamarind pulp and seed juice was evaluated by a panel of 30 randomly selected judges consisting of students of the Department of Food Technology and Nutrition, Makerere University Kampala. The sensory attributes evaluated included color, taste, odor, consistency and general acceptability. A nine-point hedonic scale (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely) was used (Larmond, 1977). The sensory assessors were presented with 30 ml of each sample at room temperature under normal lighting conditions. Water was provided to the assessors for mouth or palate cleansing between each sample.

### Analysis of phytochemicals in the prepared tamarind pulp and seed powder juice

#### Determination of total phenolic content

The phenolic content in the tamarind pulp and seed juice was estimated by Folin-Ciocalteu method as described by Thimmaiah (1999). Briefly, an aliquot (0.5 ml) of each juice was mixed with 2.5 ml of distilled water. To this, 0.5 ml of 1.0 N Folin-Ciocalteu reagent was added and incubated for 3 min. To each of the tube, 2 ml of 20% sodium carbonate solution was added and the tubes incubated in a boiling water bath for 1 min. The test tubes were cooled and the absorbance of each reaction mixture was read at 650 nm in a GENESYS spectrophotometer 10UV (Thermo Electron Corporation, Madison, WI, USA). A standard calibration curve of different concentrations of gallic acid (0.025 - 0.2 mg ml$^{-1}$) against 100 ml of juice were plotted ($R^2$= 0.999). The total phenolic content was expressed in milligram gallic acid equivalents per 100 ml of juice (mg GAE 100 ml$^{-1}$).

#### Determination of total flavonoid content

The total flavonoid content was determined using the method of Zhishen et al. (1999). In brief, 0.5 ml of standard solution of catechin or the sample diluted (1:2 with water) was mixed with 2 ml of deionized water and with 0.15 ml of 5% sodium nitrite. After incubation for 5 min at room temperature, 0.15 ml of 10% aluminum chloride was added, and after another 6 min, 1 ml of 1M sodium hydroxide solution was added. The total volume of the solution was adjusted to 5 ml with deionized and the absorbance was read at 510 nm in a GENESYS spectrophotometer 10UV (Thermo Electron Corporation, Madison, WI, USA). A standard calibration curve of different concentrations of catechin (0.025-0.2 mg ml$^{-1}$) versus 100 ml of juice was plotted ($R^2$ = 0.999). Total flavonoid content was expressed in milligram catechin equivalents per 100 ml of juice (mg CE 100 ml$^{-1}$).

#### Determination of total antioxidant activity

The antioxidant activity of the prepared juice sample was estimated using the 1,1-diphenyl-2-pycrylhydrazyl (DPPH) free radical scavenging assay as described by Shimada et al. (1992). In brief, to 2.9 ml of freshly prepared 100 µM of DPPH in 80% ethanol solution, 0.5 ml of the juice sample or control solution (DPPH alone)

was added. The mixture was shaken and allowed to stand in the dark at room temperature for 30 min. The absorbance of the resulting solution was measured at 515 nm using a GENESYS UV-vis spectrophotometer 10UV (Thermo Electron Corporation, USA) against a blank solution containing only 80% ethanol. The free radical scavenging activity was calculated as:

$$Scavenging\ activity\ (\%) = \left[1 - \left(\frac{absorbance\ of\ sample}{absorbance\ of\ control}\right)\right] \times 100$$

A standard solution of ascorbic acid or vitamin C was similarly run using different concentrations ranging from 0.002 to 0.1 mg ml$^{-1}$. A calibration curve of the percentage free radical scavenging activity of ascorbic acid versus its concentration per 100 ml of juice was then prepared ($R^2$ = 0.999). The final result was expressed as milligram vitamin C equivalents per 100 ml of juice (mg VCE 100 ml$^{-1}$).

### Statistical analysis

Data from three independent experiments were subjected to analysis of variance (ANOVA) at α = 0.05 and means were separated using Turkey's post hoc test. Regression analysis was performed to indicate the relationship between total phenolic or flavonoid contents and antioxidant activity. Statistical analysis was conducted using Statistical Package for the Social Sciences (SPSS), version 16 (SPSS Inc, Chicago, IL, USA).

## RESULTS AND DISCUSSIONS

### Phytochemical content and antioxidant activity of food materials

#### Total phenolic content

The total content of phenolics of the different foods ranged from 2.6 to 184.2 mg GAE gDW$^{-1}$ and was highest for *T. indica* L. seeds and lowest for *Amaranthus hypochondriacus* L. (Table 1). Based on the classification of Lai and Lim (2011), samples with TPC > 20 mg GAE gDW$^{-1}$ are classified as very high, those with TPC 10-20 mg GAE gDW$^{-1}$ are considered high, those with TPC 5-10 mg GAE gDW$^{-1}$ are moderate and samples with TPC < 5 mg GAE gDW$^{-1}$ are considered low. All the foods regarded in the communities studied as having medicinal value showed very high TPCs (> 20 mg GAE gDW$^{-1}$), except *Hyptis spicigera* Lam. which showed a high TPC (18.5 ± 0.2 mg GAE gDW$^{-1}$) and foods of the Amaranthaceae family (*A. hypochondriacus* L. and *Amaranthus cruentus* L.) with low TPCs of 2.6 ± 0.1 and 2.9 ± 0.2 mg GAE gDW$^{-1}$ respectively (Table 1).

#### Total flavonoid content

The result in Table 1 also showed variation in the total content of flavonoids from 0.3 to 162.2 mg CE gDW$^{-1}$. Total flavonoid content was highest for *T. indica* L. seeds (184.2 ± 0.2 mg CE gDW$^{-1}$) and lowest for *A. hypochondriacus* L. (2.6 ± 0.1 mg CE gDW$^{-1}$). Flavonoids

**Table 1.** Total phenolic content, total flavonoid content and total antioxidant activity of selected traditional medicinal foods identified in Kamuli and Gulu districts.

| Plant scientific name | Local names | Plant parts analyzed | TPC (mg GAE gDW$^{-1}$) | TFC (mg CE gDW$^{-1}$) | TAC (mg VCE gDW$^{-1}$) |
|---|---|---|---|---|---|
| *Tamarindus indica* L. | Enkooge | Seeds | 184.2 ± 6.4[a] | 162.2 ± 3.5[a] | 57.8 ± 0.5[a] |
| *Ipomea eriocarpa* R.Br. | Padowia kuri | Leaves | 91.9 ± 2.9[b] | 78.9 ± 2.7[b] | 57.1 ± 0.6[a] |
| *Corchorus trilocularis* L. | Otigo lum | Leaves | 76.5 ± 0.2[c] | 46.0 ± 1.4[c] | 48.7 ± 1.6[c] |
| *Ocimum suave* L. | Mujaaja | Leaves | 74.5 ± 3.6[c] | 50.1 ± 4.3[c] | 55.8 ± 1.0[a,b] |
| *Corchorus olitorius* L. | Otigo Diri | Leaves | 62.3 ± 0.9[d] | 36.2 ± 0.6[c] | 48.2 ± 0.8[c] |
| *Acalypha bipartita* Müll.Arg. | Ayuyu | Leaves | 62.0 ± 1.4[d] | 40.2 ± 0.3[c] | 49.7 ± 0.9[b] |
| *Hibiscus acetosella* Welw. ex Hiern. | Gwanya | Leaves | 51.7 ± 1.2[d,e] | 24.4 ± 0.4[e] | 36.1 ± 2.0[d] |
| *Cassia obtusifolia* L. | Oyado | Leaves | 48.1 ± 1.7[e,f] | 12.0 ± 0.2[f,g] | 20.0 ± 1.2[e,f] |
| *Solanum aethiopicum* L. | Nakati | Leaves | 45.7 ± 0.5[e,f] | 13.2 ± 0.4[f,g] | 19.8 ± 1.3[e,f] |
| *Solanum nigrum* L. | Ensugga | Leaves | 45.1 ± 0.5[e,f] | 17.5 ± 0.3[e,f] | 25.6 ± 2.5[e] |
| *Vigna unguiculata* L. | Eggobe | Leaves | 39.0 ± 1.1[f,g] | 6.1 ± 0.6[g,h] | 14.4 ± 0.9[f,g] |
| *Hibiscus cannabinus* L. | Nyarogena | Leaves | 38.4 ± 0.9[f,g] | 13.4 ± 0.2[f,g] | 22.2 ± 1.8[e] |
| *Crotalaria ochroleuca* G.Don. | Lala | Leaves | 38.3 ± 2.3[f,g] | 2.6 ± 0.3[h,i] | 8.8 ± 0.7[g,h] |
| *Cleome gynandra* L. | Ejjobyo | Leaves | 38.0 ± 0.8[f,g] | 8.7 ± 0.7[g,h] | 15.0 ± 0.7[f,g] |
| *Hibiscus sp. near H diversifolius* Jacq. | Malakwang | Leaves | 37.7 ± 2.4[f,g] | 12.9 ± 1.0[f,g] | 19.2 ± 2.1[e,f] |
| *Solanum anguivi* Lam. | Katunkuma | Fruits | 32.7 ± 1.0[f,g] | 7.2 ± 0.5[g,h] | 11.4 ± 0.6[g,h] |
| *Solanum gilo* L. | Entuula | Leaves | 25.3 ± 0.3[h,g] | 2.6 ± 0.5[h,i] | 6.4 ± 0.5[f,i] |
| *Hyptis spicigera* Lam | Lamola | Seeds | 18.5 ± 0.2[h] | 0.4 ± 0.1[i] | 1.2 ± 0.6[i] |
| *Amaranthus cruentus* L. | Doodo | Seeds | 2.9 ± 0.2[i] | 0.5 ± 0.2[i] | 0.1 ± 0.0[i] |
| *Amaranthus hypochondriacus* L. | Doodo | Seeds | 2.6 ± 0.1[i] | 0.3 ± 0.1[i] | 0.1 ± 0.1[i] |

TPC: Total phenolic content; TFC: Total flavonoid content; and TAC: Total antioxidant activity. Data are expressed as mean ± standard error from three independent experiments (n=3). Mean values in the same column with different superscript letters are significantly different ($p < 0.05$).

are a large class of polyphenolic compounds that have been attributed to nutraceutical properties of several plants. Flavonoids are potent antioxidants and metal chelators (Tapas et al., 2008) and have long been recognized to be beneficial against many chronic diseases such as cardiovascular diseases (Kris-Etherton et al., 2002), cancer (Birt et al., 2001; Middleton et al., 2000), inflammation (Manthey et al., 2001) and neuro-degenerative disorders (Lu et al., 2010; Moosmann and Behl, 2002).

### *Total antioxidant activity*

Antioxidant activity ranging from 0.1 to 57.8 mg VCE gDW$^{-1}$ was recorded for the different food materials. *T. indica* L. seeds with 57.8 ± 0.5 mg VCE gDW$^{-1}$, *Ipomea eriocarpa* R.Br. (57.1 ± 0.6 mg VCE gDW$^{-1}$), *Ocimum suave* L. (55.8 ± 1.0 mg VCE gDW$^{-1}$), *Acalypha bipartite* Müll.Arg. (49.7 ± 0.9 mg VCE gDW$^{-1}$), *Corchorus trilocularis* L. (48.7 ± 1.6 mg VCE gDW$^{-1}$), *Corchorus olitorius* L. (48.2 ± 0.8 mg VCE gDW$^{-1}$) and *Hibiscus acetosella* Welw. ex Hiern. with 36.1 ± 2.0 mg VCE gDW$^{-1}$ exhibited the highest antioxidant activities.

The total antioxidant activity of the traditional food

plants significantly correlated to both the total phenolic content ($R^2 = 0.883$; $p < 0.05$) (Figure 2) and the total flavonoid content ($R^2 = 0.965$; $p < 0.05$) (Figure 3), suggesting that antioxidant activity may be attributed to the bioactivity of the phenolic and flavonoid compounds. Abu Bakar et al. (2009) reported a strong correlation between phenolic and flavonoid content and DPPH free radical scavenging ability.

### Phytochemical content of the *Tamarindus indica* L. pulp and seed juice

Incorporation of tamarind seed powder (TSP) into tamarind pulp juice resulted into a proportionate increase in total phenolic content of the enriched juice from 24.68 ± 1.3 mg GAE 100 ml$^{-1}$ for the control (juice without TSP) to 33.78 ± 2.2, 39.14 ± 2.0 and 53.34 ± 0.6 mg GAE 100 ml$^{-1}$ for juice samples containing 0.5, 1 and 3% TSP, respectively (Table 2). The total flavonoid content (TFC) showed a similar pattern, increasing from 0.92 ± 0.0 mg CE 100 ml$^{-1}$ for the control to 9.62 ± 0.1, 11.46 ± 0.3 and 17.5 ± 0.1 mg CE 100 ml$^{-1}$ for juice samples enriched with 0.5, 1 and 3% (w/v) TSP, respectively. Similarly, total antioxidant activity increased from 8.50 ± 0.2 mg VCE ml$^{-}$
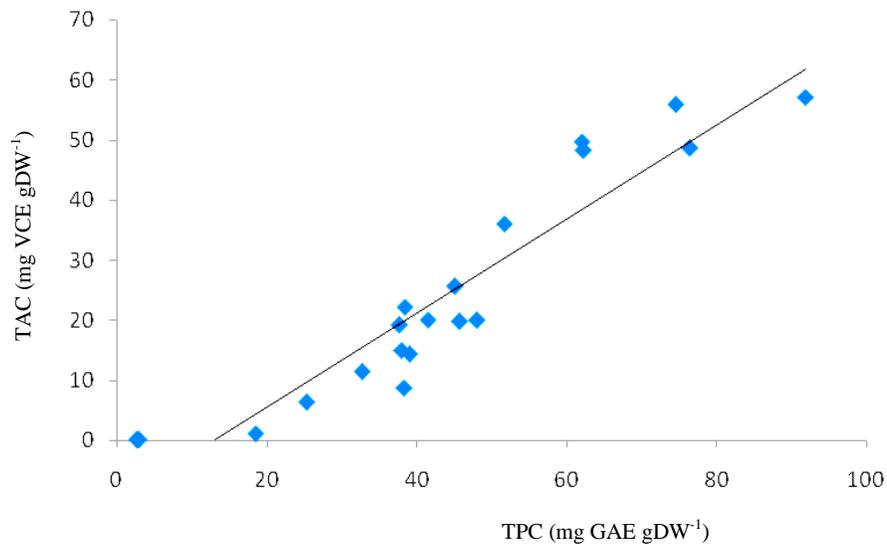
**Figure 2.** Relationship between antioxidant activities and the total phenolic contents in traditional food plants ($R^2$ = 0.883).
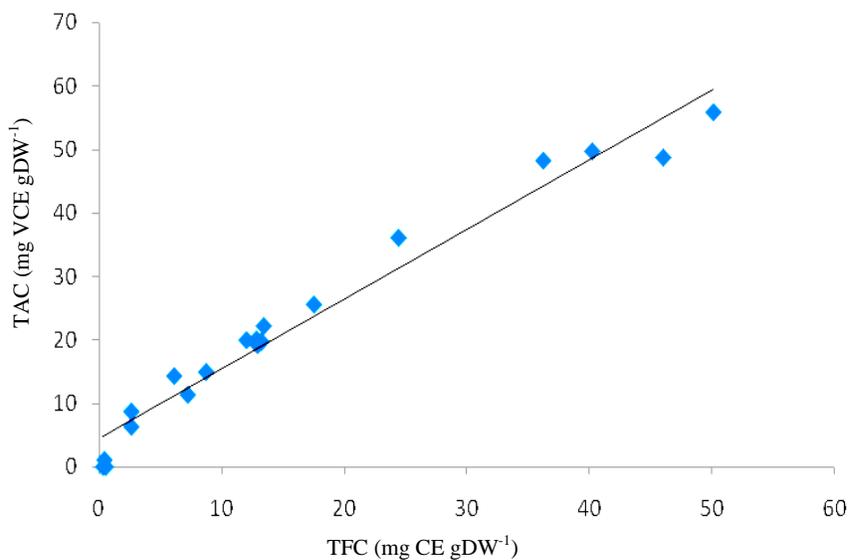


**Figure 3.** Relationship between antioxidant activities and the total flavonoid contents in traditional food plants ($R^2$ = 0.965).

**Table 2.** Total phenolic content, total flavonoid content and total antioxidant activity of tamarind pulp juices with different concentrations of tamarind seed powder.

| Tamarind seed powder concentration (%) | TPC (mg GAE 100 ml$^{-1}$) | TFC (mg CE 100 ml$^{-1}$) | TAC (mg VCE 100 ml$^{-1}$) |
|---|---|---|---|
| 0 | 24.68 ± 1.3c | 0.92 ± 0.0d | 8.50 ± 0.2d |
| 0.5 | 33.78 ± 2.2$^{b}$ | 9.62 ± 0.1c | 12.05 ± 0.3c |
| 1.0 | 39.14 ± 2.0$^{b}$ | 11.46 ± 0.3b | 15.33 ± 0.7b |
| 3.0 | 53.34 ± 0.6$^{a}$ | 17.50 ± 0.1a | 17.22 ± 0.7a |

TPC: Total phenolic content, TFC: Total flavonoid content, and TAC: Total antioxidant activity. Data are expressed as mean ± standard error from three independent experiments (n=3). Mean values in the same column with the same superscript letters are significantly different (p < 0.05).

**Table 3.** Sensory evaluation of juice prepared from tamarind pulp and seed powder.

| Tamarind seed powder concentration (%) | Colour | Aroma | Taste/flavor | Mouth feel | General acceptability |
|---|---|---|---|---|---|
| 0 | 6.70 ± 0.0[a] | 6.50 ± 0.2[a] | 6.50 ± 0.2[ab] | 6.90 ± 0.1[a] | 7.40 ± 0.4[a] |
| 0.5 | 6.45 ± 0.2[ab] | 6.80 ± 0.5[a] | 6.45 ± 0.4[ab] | 6.75 ± 0.1[a] | 6.65 ± 0.3[ab] |
| 1.0 | 6.23 ± 0.2[ab] | 5.34 ± 0.3[ab] | 6.80 ± 0.1[a] | 6.65 ± 0.2[a] | 7.01 ± 0.1[a] |
| 3.0 | 6.08 ± 0.1[b] | 4.65 ± 0.2[b] | 5.80 ± 0.1[b] | 5.30 ± 0.3[b] | 5.34 ± 0.2[b] |

Data are expressed as mean ± standard error from three independent experiments (n=3). Mean values in the same column with different superscript letters are significantly different ($p < 0.05$).

[1] for the control to 12.05 ± 0.3, 15.33 ± 0.7 and 17.22 ± 0.7 mg VCE 100 ml$^{-1}$ for the tamarind pulp juice sample containing 0.5, 1 and 3% (w/v) TSP, respectively. The phenolic contents of tamarind juice samples enriched with tamarind seed powder were comparatively higher than those of natural fresh juices made from apricot (23.75), pineapple (36.16), white grape (37.69), apple (45.38) and red grape juices (49.4), as reported by Mahdavi et al. (2010). The observed increments in total phenolic and flavonoid contents and in total antioxidant activity of the juice may be attributed to the high content of phenolic compounds and antioxidant activity in the *T. indica* L. seeds (Table 1). Previous studies have also reported high phenolic content and antioxidant activity in tamarind seeds (Siddhuraju, 2007). Overall, these results have demonstrated that incorporation of tamarind seed powder boosted the content of phenolic compounds and antioxidant activity of tamarind pulp juice. It further showed that the phenolic content and antioxidant activity increased proportionately with the amount of tamarind seed powder incorporated in the tamarind juice. However, the extent of incorporating tamarind seed powder in juice might be limited by the high content of tannins in tamarind seed coat and associated astringency (Pugalenthi et al., 2004). Thus, pre-treatment methods such as autoclaving of the seeds were found to reduce the astringency associated with tamarind seed (Legesse and Emire, 2012).

**Sensory evaluation of tamarind pulp and seed juice**

Addition of tamarind seed powder to tamarind juice to level of 1.0% (w/v) did not result in any significant difference in the perceived sensory attributes of tamarind pulp and seed juice ($p > 0.05$) as compared to the control (juice without tamarind seed powder) (Table 3). However, incorporation of tamarind seed powder at level of 3% (w/v) in the juice resulted in reduced scores in all the sensory attributes such as colour, aroma, taste and mouth feel. The largest difference in sensory scores between the control juice and juice containing 3% tamarind seed powder was in flavor and consistency, implying that the tamarind seeds disproportionately

affected these sensory attributes. Tamarind seed kernels are known to contain some polysaccharides which when mixed with water form mucilaginous dispersions, leading to increase in viscosity of the juice. Furthermore, the presence of antinutritional factors such as tannins in tamarind seed testa renders the whole seed unsuitable for consumption (Caluwé et al., 2010). Overall, the colour, aroma, flavor and consistency of 0.5 and 1% tamarind pulp and seed powder juices were well accepted and the respective general acceptability scores for these were 6.65 ± 0.3 and 7.01 ± 0.1.

**Conclusions**

Biochemical analysis of traditional food plants identified to have health benefits by local communities in Gulu and Kamuli districts of Uganda showed that most exhibited high levels of total phenolic and flavonoid contents as well as high antioxidant activity. The present study therefore provides support for continued use of these investigated traditional foods for health promotion in Uganda. In particular, the seeds of *T. indica* L. and leaves of *I. eriocarpa* R.Br., *C. trilocularis* L., *O. suave* L., *C. olitorius* L., *A. bipartita* Müll.Arg. and *Hibiscus acetosella* Welw. ex Hiern. exhibited high levels of total phenolic and flavonoid contents including high antioxidant activity. The study has further demonstrated experimentally the potential use of *T. indica* L. seed powder in boosting antioxidant activity of juices as well as enhancing levels of phenolic and flavonoid compounds.

**Conflict of interests**

The authors did not declare any conflict of interests.

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*Full Length Research Paper*

# Physical properties of dry-milled maize meals and their relationship with the texture of stiff and thin porridge

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**Selected physical properties of white maize meal, obtained by different dry-milling techniques were evaluated and correlated to the texture of stiff and thin porridge. Sifted or par-cooked maize meals had finer particles than hammer-milled maize meals. Hammer-milled maize meals had lower water absorption indices (17-38%) and higher water solubility indices (WSI, 4-5%) than sifted (41-42 and 2-3%, respectively) or par-cooked (114 and 2%, respectively) maize meals. Sifted or par-cooked maize meals had lower breakdown viscosities (0-19 BU) and higher final viscosities (818-1925 BU) than hammer-milled maize meals (89-173 BU and 530-780 BU, respectively). Stiff porridge prepared from par-cooked maize meal (34% w/v), and thin porridge from dehulled and hammer-milled maize meal (10% w/v) had the firmest textures at 80.93 and 1.28 N, respectively. There was a negative correlation (P < 0.05, r = -1.00) between the WSI and total shearing force of stiff porridge prepared from par-cooked maize meal.**

**Key words:** Maize, porridge, texture, Pearson correlation coefficient.

## INTRODUCTION

White maize (*Zea mays*) is an important source of starch, protein, fiber and a wide range of micronutrients for millions of people in sub-Saharan Africa. Maize meal, the main product obtained from white maize kernels, is used to make stiff (thick) or thin porridge. The porridges are known by different local names in different countries in the region. Stiff porridge is known as *pap* in South Africa; *ugali* in Kenya, Uganda and Tanzania; *sadza* or *isitshwala* in Zimbabwe; *nsima* in Zambia and Malawi; *phaletshe* in Botswana; *banku* or *kenkey* in Ghana; *fufu or tuwo* in the western Africa sub-region; and Mawè in

Benin and Togo. Thin porridge is known as *uji* in Kenya, Uganda and Tanzania; and also as *togwa* in Tanzania and *obushera* in Uganda; *nasha* or *hulu-mur* in Sudan; *lakh* or *fonde* in Senegal and Gambia; *mahewu* or *magou* in South Africa and Zimbabawe; *koko* or *akasa* in Ghana and Nigeria; *ogi* in Nigeria, and *poto poto* in Democratic Republic of Congo (FAO, 1995).

The main difference between stiff and thin porridge relates to the amount of maize meal that is used to prepare them, and consequently how they are eaten. Stiff maize porridge is made by adding ca. 30% w/v maize

meal to boiling water. The gruel is stirred with a flat wooden handle to make a stiff homogeneous and well-gelatinized mass that is free from lumps. The product is consumed at lunch or dinner as the main energy-giving food, preferably when it is still hot or warm. Stiff porridge has a bland taste because it is commonly prepared from unfermented maize meal without any other ingredients or additives. Thin maize porridge is prepared in a similar manner to stiff porridge but with less maize meal (ca. 10% w/v). It can also be prepared from lactic spontaneous fermented maize slurry (Afoakwa et al., 2010) or chemically-acidified maize slurry (Onyango et al., 2005). The energy density of thin maize porridge can be enhanced by adding amylase-rich cereal malt (Afoakwa et al., 2010). Thin maize porridge is commonly sweetened with sugar and is drank or eaten with a spoon as a refreshment any time of the day.

The physical quality of dry-milled maize meal used to prepare stiff and thin porridge in sub-Saharan Africa is variable. Sifted maize meal is obtained by roller milling and sifting shelled clean maize. Whole maize meal is obtained by grinding clean whole maize kernels using a hammer mill or other impact-grinding methods. Dehulled maize meal is obtained from kernels that have been dehulled, commonly using a PRL dehuller (Munck, 1995), and subsequently hammer-milled. Par-cooked maize meal is made from degerminated endosperm grits that are subsequently conditioned, steamed, flaked, dried and milled. This maize meal requires almost 10 times less time to make porridge from it than sifted or hammer-milled maize meal and can, therefore, save energy and time associated with porridge preparation. Par-cooked maize meal is a new product that has been introduced in the southern Africa market by Buehler AG (Uzwil, Switzerland) through its local subsidiary Buehler Pty Ltd. (Johannesburg, South Africa).

The transformation of maize meal into porridge is associated with gelatinization of starch in excess water. This process involves loss of starch lamellar structure with gelatinization followed by formation of complex fractal structures during starch pasting and retrogradation (Doutch et al., 2012). The behaviour of starch when heated in excess water is influenced by the quality of the flour (Bolade et al., 2009) as well as its botanical origin (Seetharaman et al., 2001; Singh et al., 2003). Bolade et al. (2009) showed that maize flour particle size influences starch functional characteristics, such as water absorption capacity, bulk density, damaged starch, and pasting behaviour; and stiff porridge texture, such as hand mouldability and mouth feel characteristics. Seetharaman et al. (2001) found that the thermal, pasting and textural properties of maize starch is affected by maize variety; and there is a strong correlation between the thermal properties and pasting and textural properties. The objective of this work was to evaluate the physical properties of maize meal and the texture of stiff and thin porridges made from them.

## MATERIALS AND METHODS

### Proximate composition of maize meal

White flint maize grain (*Zea mays* var. *indurate* Sturt), Hybrid 614, was purchased from Baraka Grain Millers, Nairobi, Kenya. It was cleaned to remove foreign matter and divided into three lots. Coarse (WM-TP) and fine (WM-SP) whole maize meal were obtained by milling the kernels in a hammer mill having 1,000 or 500 µm sieves, respectively. Coarse (DM-TP) or fine (DM-SP) dehulled maize meal were obtained by dehulling the kernels (75% extraction rate) using a PRL dehuller having 13 carborundum stones (Munck, 1995) before milling them in a hammer mill having 1,000 or 500 µm sieves, respectively. Three commercial sifted maize meals were purchased from a supermarket in Nairobi and labelled SF-T1, SF-T2 and SF-T3. Par-cooked maize meal (PC-MM) was donated by Buehler Pty Ltd. (Johannesburg, South Africa). Moisture, crude protein (N x 6.25), total ash and crude fiber contents of the maize meals were determined using AACC methods 44-40, 46-12, 08-01 and 32-10, respectively (AACC, 2005). Crude oil content was determined using AOAC method 945.16 (AOAC, 2005). Carbohydrate content (%) was calculated by subtracting protein, oil and ash contents from 100.

### Physical properties of maize meal

Particle size distribution of the maize meals was determined by sieving 50 g maize meal for 20 min in a Minor M200 electric sieve shaker (Endecotts Limited, London, UK) with 125, 180, 300, 400 and 500 µm sieves to obtain the following fractions: 125-180, 180-300, 300-400, ≥400. The different maize meal fractions were weighed and expressed as a percentage of the total weight of the maize meal.

Water absorption and solubility indices were determined by weighing maize meal (2.0 g) in pre-weighed centrifuge tubes and 20 ml distilled water added. The caps were secured and the tubes hand-shaken 10 times to suspend the maize meals. The suspensions were allowed to solvate and swell for 30 min in a water-bath at 30°C with intermittent shaking after every 10 min. The samples were centrifuged using a CN-2060 centrifuge (MRS Laboratory Equipment, Holon, Israel) at 4,000 rpm for 30 min. The supernatants were decanted into tared aluminum pans and weight gain in the gel noted. Water absorption index (WAI) was calculated as [(weight of the gel - sample weight) / (sample weight) x 100] (Inglett et al., 2009). The supernatants were evaporated to dryness at 105°C to constant weight. Water solubility index (WSI) was determined as [(weight of dried supernatant) / (dry sample weight) x 100]. Damaged starch content was determined using a Megazyme starch kit (Megazyme International Ireland Limited, Co. Wicklow, Ireland).

The pasting behavior of the maize meals was investigated in a Brabender Viscograph-E (Brabender GmbH and Co. KG, Duisburg, Germany) at 85 rpm and 700 cmg torque. Slurry, made up of ca. 40 g maize meal (adjusted to 14% moisture content) and ca. 315 ml water was poured in the Viscograph-E canister. The canister was placed in the Viscograph-E heating chamber and the instrument head lowered into it. The suspension was heated from 30 to 95°C at the rate of 1.5°C/min; held at 95°C for 15 min; cooled from 95 to 30°C at the rate of -1.5°C/min; and finally held at 30°C for 15 min. Paste temperature (°C), peak viscosity (Brabender Units, BU), time to peak viscosity (min), breakdown viscosity (BU), final viscosity (BU) and setback viscosity (BU) were determined.

**Table 1.** Proximate composition (%) of white maize meal.

| Maize meal | Moisture content | Carbohydrate | Crude protein | Crude oil | Crude fiber | Total ash |
|---|---|---|---|---|---|---|
| WM-TP | 11.09±0.01[b] | 84.81±0.24[a] | 9.30±0.26[d] | 4.73±0.03[c] | 2.27±0.80[e] | 1.17±0.07[d] |
| WM-SP | 9.13±0.12[a] | 86.07±0.13[b] | 7.51±0.04[b] | 5.08±0.04[c] | 1.73±0.01[c] | 1.33±0.07[e] |
| DM-TP | 11.52±0.37[b] | 87.83±0.04[c] | 6.75±0.09[a] | 4.53±0.11[c] | 1.44±0.02[b] | 0.89±0.07[bc] |
| DM-SP | 9.48±0.14[a] | 88.79±0.73[d] | 6.91±0.03[a] | 3.34±0.80[b] | 1.65±0.02[c] | 0.95±0.07[c] |
| SF-T1 | 12.55±0.15[c] | 85.07±0.13[a] | 11.61±0.07[e] | 2.56±0.00[b] | 1.34±0.01[ab] | 0.76±0.07[b] |
| SF-T2 | 11.17±0.08[b] | 86.80±0.21[b] | 9.08±0.22[d] | 3.25±0.01[b] | 2.20±0.02[e] | 0.87±0.02[bc] |
| SF-T3 | 12.29±0.15[c] | 90.69±0.21[e] | 7.90±0.22[bc] | 0.95±0.03[a] | 1.98±0.01[d] | 0.46±0.05[a] |
| PC-MM | 12.30±0.10[c] | 91.08±0.24[e] | 8.13±0.27[c] | 0.49±0.03[a] | 1.29±0.08[a] | 0.30±0.00[a] |

Values are means of three replicates ± standard deviation. Means followed by different superscript letters in the same column are significantly different at P < 0.05. Values are given on dry-matter basis except for moisture content. WM-TP: coarse whole hammer-milled maize meal; WM-SP: fine whole hammer-milled maize meal; DM-TP: dehulled and coarsely hammer-milled maize meal; DM-SP: dehulled and finely hammer-milled maize meal; SF-T1, SF-T2 and SF-T3 represent commercial sifted maize meal brands; PC-MM: par-cooked maize meal.

**Texture of thick and thin porridge**

Stiff porridge was made by adding 34% w/v maize meal in boiling tap water in a stainless steel cooking pan. The mixture was kneaded with a flat wooden handle for 7 min to obtain homogenous stiff paste that was devoid of lumps. The cooking pan was covered and heated further for 3 min with intermittent kneading. The stiff porridge was transferred to a clean surface and manually moulded in the shape of a dome. A block measuring 50 mm high x 40 mm wide x 72 mm long was punched out from the stiff porridge using a stainless steel die. The die was lightly oiled with edible vegetable oil on the inner surface to facilitate easy removal of the stiff porridge. The stiff porridge block was incubated in a Memmert oven (Memmert GmbH + Co. KG, Schwabach, Germany) at 55°C. A block was punched out from the residual stiff porridge and a Eutech pH510 temperature probe (Eutech Instruments Pte. Ltd., Ayer Rajah, Singapore) inserted in it before putting it in the cabinet. When the internal temperature of the residual stiff porridge block had reached 55°C, it was assumed that the analytical blocks were fully equilibrated at 55°C. This took about 10 min. A TA-XT*plus* Texture Analyzer (Stable Micro Systems, Surrey, UK) equipped with 50 kg load cell and Kramer shear cell probe attachment (HDP/KS5) was used to measure the firmness of stiff porridge at the following test conditions: height of the blades from the base of the plate was calibrated at 70 mm; pretest speed 1 mm/s; trigger force 0.05 N (the point at which the probe's lower surface was in full contact with the product); test speed 2 mm/s; distance travelled by the blades from the calibration height was 60 mm (40 mm penetration depth of blades in porridge or 80% of product height); post-test speed 5 mm/s. The force (N) versus time (s) required to cut through the porridge was recorded. The peak force (N) and total shearing force (N·s) were calculated using EXPONENT Texture Analysis software version 6.1.5.0 (Stable Micro Systems, Surrey, UK).

Thin porridge was prepared using 10% w/v maize meal in tap water. A portion of the water (ca. 40%) was initially mixed with all the maize meal to make cold slurry, which was then added to the remaining portion of boiling water in a stainless steel pan. The porridge was kept boiling for 10 min with intermittent stirring, to avoid formation of lumps, using a flat wooden handle for 10 min. The thin porridge was placed in a 50 mm diameter standard size A/BE back extrusion container (TA-XT*plus* Texture Analyzer, Stable Micro Systems, Surrey, UK) approximately 75% full (80 g) and incubated in a water bath at 60°C to allow for temperature equilibration. Porridge temperature was confirmed to have reached

60°C using a Eutech pH510 temperature probe (Eutech Instruments Pte. Ltd., Ayer Rajah, Singapore). Extrusion force was measured at the following settings: 50 kg load cell; height calibration 30 mm; disc diameter 45 mm; pretest speed 1 mm/s; test speed 1 mm/s, trigger force 0.05 N, post-test speed 10 mm/s, data acquisition rate 200 pps. When a 0.05 N surface trigger was attained (the point at which the disc's lower surface was in full contact with the product) the disc proceeded to penetrate the porridge to a depth of 30 mm after which it returned to its original position. Firmness (maximum positive force), consistency (area of the positive region of the curve), cohesiveness (maximum negative force) and work of cohesion or index of viscosity (area of the negative region of the curve) were calculated using EXPONENT Texture Analysis software version 6.1.5.0 (Stable Micro Systems, Surrey, UK).

**Experimental design and statistical analysis**

All experiments were set-up as single-factor completely randomized designs. All tests were made in triplicate and results reported as mean ± standard deviation. The data was analyzed using one-way analysis of variance and differences in treatment means evaluated using Tukey's test at 5% using Minitab Statistical Software version 13 (Minitab Inc., Pennsylvania, USA). Pearson correlation coefficients (r) between all maize meal physical properties and stiff or thin porridge texture were calculated using SPSS software version 13.0 (SPSS, Chicago, USA).

**RESULTS AND DISCUSSION**

**Proximate composition of maize meal**

Maize meals were obtained from adequately dried grains with moisture contents ranging from 9.13 to 12.55% (Table 1). The variable distribution of nutrients among the maize meals (Table 1) reflects the different dry-milling techniques that the grains were subjected to. Sifted maize meals and PC-MM maize meal had lower oil and ash contents as compared to whole hammer-milled maize meals (WM-TP and WM-SP). Dehulled and hammer-

**Table 2.** Water absorption and solubility indices, and damaged starch content of white maize meal.

| Maize meal | WAI (%) | WSI (%) | DS (g/100 g) |
|---|---|---|---|
| WM-TP | 21.73±1.32[a] | 4.43±0.18[d] | 5.98±0.88[a] |
| WM-SP | 37.94±1.60[b] | 5.35±0.36[e] | 11.44±0.75[c] |
| DM-TP | 17.06±2.66[a] | 4.23±0.25[d] | 5.95±0.04[a] |
| DM-SP | 35.98±0.33[b] | 4.02±0.18[d] | 9.33±0.18[b] |
| SF-T1 | 41.75±5.26[b] | 2.72±0.15[b] | 7.25±0.26[a] |
| SF-T2 | 42.26±3.26[b] | 3.32±0.15[c] | 7.44±0.03[a] |
| SF-T3 | 40.64±2.49[b] | 1.84±0.07[a] | 11.12±0.61[c] |
| PC-MM | 113.60±5.52[c] | 1.58±0.23[a] | 10.92±0.13[bc] |

Values are means of three replicates ± standard deviation. Means followed by different superscript letters in the same row are significantly different at P < 0.05. WM-TP: coarse whole hammer-milled maize meal; WM-SP: fine whole hammer-milled maize meal; DM-TP: dehulled and coarsely hammer-milled maize meal; DM-SP: dehulled and finely hammer-milled maize meal; SF-T1, SF-T2 and SF-T3 represent commercial sifted maize meal brands; PC-MM: par-cooked maize meal.

milled maize meals (DM-TP and DM-SP) had higher carbohydrate and lower protein, fat, ash and fiber contents than whole hammer-milled maize meals (WM-TP and WM-SP).

The proximate composition of the maize meals was compared with Kenya Standard (KS 168:2007) for dry-milled maize products. The proximate composition of whole maize meal agreed with Kenya Standard which set maximum fiber, oil, moisture and ash contents at 3.0, 5.0, 14.0 and 2.0%, respectively (KS 168:2007). Bran and germ removal in sifted and PC-MM maize meals was responsible for their lower oil and ash content as compared to whole hammer-milled maize meals (WM-TP and WM-SP). Some proximate composition parameters of sifted maize meals (SF-T1, SF-T2 and SF-T3) did not comply with Kenya Standard (KS 168:2007), which requires this type of maize meal to have maximum fiber, oil, moisture and ash contents of 0.7, 3.0, 14.0 and 0.75%, respectively. All the proximate composition parameters of PC-MM maize meal complied with Kenya Standard (KS 168:2007) for sifted maize meal.

**Physical properties of maize meal**

More than 75% of the hammer-milled maize meal particles were retained on the 400 µm sieve as compared to 55-64% of sifted maize meal particles. The PC-MM maize meal had the lowest proportion of particles that were ≥400 µm (41%) and the highest proportion of particles with sizes between 125-400 µm (59%). The PC-MM maize meal had the highest WAI (113%) and lowest WSI (1.58%, Table 2). The WAI of sifted maize meals was about 40% while their WSI ranged between 1.84 and

3.32%. Sifted maize meals had significantly lower (P < 0.05) WSI (1.58-3.32%) than hammer-milled maize meals (4.02-5.35%). Coarsely hammer-milled maize meals had significantly lower (P < 0.05) WAI (WM-TP: 21.73%; DM-TP: 17.06%) than finely hammer-milled maize meals (WM-SP: 37.94%; DM-SP: 35.98%). Water solubility index was lower in PC-MM maze meal and sifted maize meals than in hammer-milled maize meals. Nonetheless, all these values were lower than the 9-15% reported by Sandhu and Singh (2007) for maize starch. These differences appear to be associated with naturally occurring substances, such as lipids, in the maize meal matrix, which inhibit starch granule swelling and leaching of soluble polysaccharides (Tester and Morrison, 1990). Due to the high degree of refinement, maize starch granules have less interfering substances than sifted, par-cooked or hammer-milled maize meals.

The damaged starch content of the maize meals was not explicitly related with the milling technique (Table 2). Whole hammer-milled maize meal (WM-SP), one brand of the commercial sifted maize meal (SF-T3) and PC-MM maize meal had higher damaged starch contents (ca. 11%) than the other maize meals (6-9%). Finely hammer-milled maize meals had higher damaged starch contents than coarsely hammer-milled maize meals (WM-SP > WM-TP and DM-SP > DM-TP).

A major technical consequence of milling is particle size reduction and associated changes in the physical properties of starch, such as generation of damaged starch, and modification of starch pasting profile (Hossen et al., 2011; Bolade et al., 2009). In this study, particle size reduction of maize yielded coarse maize meal since more than 90% of the particles were greater than 297 µm and smaller than 638 µm (Arendt and Zannini, 2013). The different WAIs of the maize meals implied differences in the degree of availability of water binding sites. Native starch can hold up to 30% of its dry weight as moisture (Delcour and Hoseney, 2010), and this amount can be enhanced by increasing the damaged starch content (Craig and Stark, 1984) or decreasing the flour particle size (Scanlon et al., 1988). Thus, the high WAI in PC-MM maize meal could be attributed to exposure of more starch granule sites to water as a result of the more complicated processing procedure (conditioning, steaming, flaking, drying and milling) as compared to sifted or hammer-milled maize meal production. On the other hand, the high WAI of sifted maize meals could be attributed to a combination of the high damaged starch contents and small particle sizes relative to their hammer-milled counterparts. The higher WAI of finely hammer-milled maize meals as compared to the coarsely hammer-milled fractions could be attributed to the higher damaged starch content in the former.

Statistical analyses of the pasting properties of maize meals are summarized in Table 3. The onset pasting temperature of the maize meals ranged between 71.7°C

**Table 3.** Pasting properties of white maize meal.

| Maize meal | Pasting temperature (°C) | Time to peak viscosity (min) | Peak viscosity (BU) | Breakdown viscosity (BU) | Final viscosity (BU) | Setback viscosity (BU) |
|---|---|---|---|---|---|---|
| WM-TP | $80.7\pm0.2^{e}$ | $43.04\pm0.1^{ab}$ | $334.7\pm10.1^{cd}$ | $88.7\pm9.0^{c}$ (27)* | $652.0\pm23.6^{a}$ | $489.3\pm13.9^{b}$ (75)** |
| WM-SP | $71.7\pm0.2^{a}$ | $40.23\pm0.1^{a}$ | $316.7\pm5.7^{bc}$ | $131.7\pm6.4^{e}$ (42) | $530.0\pm8.2^{a}$ | $385.0\pm9.2^{a}$ (73) |
| DM-TP | $77.5\pm0.4^{c}$ | $42.59\pm0.1^{ab}$ | $398.7\pm6.0^{ef}$ | $106.3\pm2.1^{d}$ (27) | $773.0\pm8.7^{ab}$ | $568.7\pm4.6^{bc}$ (74) |
| DM-SP | $73.1\pm0.3^{b}$ | $41.75\pm0.3^{ab}$ | $431.7\pm23.8^{f}$ | $173.0\pm7.8^{f}$ (40) | $779.3\pm44.2^{b}$ | $603.3\pm32.9^{bc}$ (77) |
| SF-T1 | $81.3\pm0.3^{e}$ | $52.84\pm3.2^{c}$ | $237.7\pm2.3^{a}$ | $2.3\pm2.3^{a}$ (0) | $825.3\pm18.9^{b}$ | $572.3\pm14.7^{bc}$ (69) |
| SF-T2 | $80.9\pm0.2^{e}$ | $45.70\pm1.5^{b}$ | $297.0\pm3.6^{b}$ | $19.0\pm1.7^{b}$ (6) | $818.7\pm23.3^{b}$ | $633.3\pm28.9^{c}$ (77) |
| SF-T3 | $78.6\pm0.3^{d}$ | $50.57\pm0.2^{c}$ | $322.7\pm9.1^{bc}$ | $4.7\pm1.5^{a}$ (1) | $1605.3\pm65.2^{c}$ | $932.0\pm48.0^{d}$ (58) |
| PC-MM | $78.6\pm0.4^{d}$ | $56.97\pm1.4^{d}$ | $368.3\pm20.6^{de}$ | $0.0\pm0.0^{a}$ (0) | $1925.0\pm110.7^{d}$ | $974.0\pm70.4^{d}$ (51) |

Values are means of three replicates ± standard deviation. Means followed by different superscript letters in the same column are significantly different at $P < 0.05$. WM-TP: coarse whole hammer-milled maize meal; WM-SP: fine whole hammer-milled maize meal; DM-TP: dehulled and coarsely hammer-milled maize meal; DM-SP: dehulled and finely hammer-milled maize meal; SF-T1, SF-T2 and SF-T3 represent commercial sifted maize meal brands; PC-MM: par-cooked maize meal. Breakdown viscosity = Peak viscosity – trough viscosity. Setback viscosity = Final viscosity – trough viscosity; BU: Brabender units. *Values in parentheses indicate breakdown viscosity calculated as a percentage of peak viscosity. **Values in parentheses indicate setback viscosity calculated as a percentage of final viscosity.

for WM-SP and 81.3°C for SF-T1. These values agreed well with published reports. Ji et al. (2004) found that the onset pasting temperature of maize starch ranges between 62-87°C, while Sandhu and Singh (2007) reported a range of 76-84°C. Finely hammer-milled maize meals (WM-SP and DM-SP) had significantly lower ($P < 0.05$) onset pasting temperatures than coarsely hammer-milled maize meals (WM-TP and DM-TP), sifted maize meals and PC-MM maize meal.

The viscous character of starch paste is as a result of the suspension of swollen starch granules, mainly amylopectin, dispersed in a macromolecular solution created by amylose polymers (Alloncle and Doublier, 1991). This viscous environment is determined by several factors such as the volume fraction occupied by the swollen granules, rigidity of the swollen granules, visco-elasticity of the continuous phase, and adhesion between the starch granules and continuous phase (Eliasson and Bohlin, 1982). In this study, it appeared that maize meal quality also had an influence on the viscous character of the cooked paste. Dehulled and hammer-milled maize meals (DM-TP and DM-SP) had significantly higher peak viscosities ($P < 0.05$) than whole hammer-milled maize meals (WM-TP and WM-SP) whereas PC-MM maize meal had significantly higher peak viscosity ($P < 0.05$) than sifted maize meals.

Furthermore, we observed that hammer-milled maize meals required less time to reach peak viscosity (40.23-43.04 min) than sifted (45.70-52.84 min) or PC-MM maize meals (56.97 min). Although PC-MM maize meal is par-cooked, its peak viscosity was within the range for sifted and hammer-milled maize meals. Inglett et al. (2009) reported that pregelatinized starch shows almost instantaneous peak viscosity development when analysed in a viscograph. We did not observe this

behaviour with PC-MM maize meal probably because of its low content of pregelatinized (damaged) starch (Table 2).

The time taken by starch slurry to reach peak viscosity is an important cooking property because it is associated with energy consumption. Starch slurry that requires more time to reach peak viscosity consumes more energy than slurry that requires less time (Bolade et al., 2009). Furthermore, Ragaee and Abdel-Aal (2006) reported that flours that require more time to reach peak viscosity have lower rate of water absorption and swelling of starch granules than those that require less time. These findings do not totally agree with our results. Although PC-MM maize meal took the longest time to reach peak viscosity (Table 3), this maize meal requires almost 10 times less time to make it into porridge than sifted or hammer-milled maize meal. Also, PC-MM maize meal exhibited higher water absorption (Table 3) than the other maize meals as a result of the par-cooking treatment it was exposed to.

The viscosity of starch slurry starts to decline after reaching peak viscosity when molecules of soluble starch begin to reorient themselves in the direction of the shearing force and due to temperature- and shear-induced destruction of the swollen granules (Delcour and Hoseney, 2010; Ragaee and Abdel-Aal, 2006). The decline in paste viscosity (breakdown viscosity) appeared to be further influenced by the milling technique and meal particle size. Sifted and PC-MM maize meals exhibited lower ($P < 0.05$) breakdown viscosities (0-19 BU) than hammer-milled maize meals (88-173 BU); finely hammer-milled maize meals had higher ($P < 0.05$) breakdown viscosities than coarsely hammer-milled maize meals (WM-SP > WM-TP and DM-SP > DM-TP); and, finally, whole hammer-milled maize meals had lower ($P < 0.05$)

**Table 4.** Texture of stiff and thin porridge prepared from white maize meal.

| Maize meal | Stiff maize meal porridge[*] | | | Thin maize meal porridge[**] | | |
|---|---|---|---|---|---|---|
| | Peak force (N) | Total shearing force (N·s) | Firmness (N) | Consistency (N·s) | Cohesiveness (N) | Index of viscosity (N·s) |
| WM-TP | 42.25±3.81[a] | 409.87±41.92[a] | 0.82±0.06[c] | 22.21±1.52[c] | -0.85±0.08[cd] | -1.78±0.13[cd] |
| WM-SP | 50.49±4.51[b] | 460.07±38.90[a]b | 0.91±0.16[c] | 24.38±4.07[c] | -1.02±0.19[d] | -2.11±0.41[d] |
| DM-TP | 46.67±2.44[ab] | 454.13±21.25[ab] | 0.67±0.04[bc] | 17.78±1.02[b] | -0.67±0.07[bc] | -1.41±0.12[bc] |
| DM-SP | 58.86±4.53[b] | 540.98±45.97[b] | 1.28±0.18[d] | 34.04±3.94[d] | -1.54±0.22[e] | -3.01±0.31[e] |
| SF-T1 | 53.57±7.32[b] | 559.44±85.99[b] | 0.58±0.05[b] | 14.80±1.52[b] | -0.55±0.05[b] | -1.18±0.11[b] |
| SF-T2 | 55.01±4.99[b] | 568.47±70.55[b] | 0.91±0.11[c] | 24.92±3.34[c] | -1.03±0.12[d] | -2.13±0.26[d] |
| SF-T3 | 48.87±6.74[ab] | 527.52±89.27[ab] | 0.50±0.07[b] | 13.27±2.09[b] | -0.64±0.12[b] | -1.44±0.30[bc] |
| PC-MM | 80.93±10.45[c] | 838.99±100.61[c] | 0.24±0.04[a] | 5.58±1.19[a] | -0.23±0.06[a] | -0.34±0.28[a] |

Values are means of three replicates ± standard deviation. Means followed by different superscript letters in the same column are significantly different at P < 0.05. WM-TP: coarse whole hammer-milled maize meal; WM-SP: fine whole hammer-milled maize meal; DM-TP: dehulled and coarsely hammer-milled maize meal; DM-SP: dehulled and finely hammer-milled maize meal; SF-T1, SF-T2 and SF-T3 represent commercial sifted maize meal brands; PC-MM: par-cooked maize meal. [*]Texture of stiff porridge was measured using a Kramer shear blade of TA-XT*plus* Texture Analyser. **Texture of thin porridge was measured using a back extrusion cell of TA-XT*plus* Texture Analyser.

breakdown viscosities than dehulled and hammer-milled maize meals (DM-TP > WM-TP, and DM-SP > WM-SP). When breakdown viscosity was calculated as a percentage of peak viscosity, we noted that sifted and PC-MM maize meals had the lowest percent breakdown viscosity (0-6%) followed by coarsely hammer-milled maize meals (27%) and finely hammer-milled maize meals (40-42%). These findings imply that maize meals with lower breakdown viscosities (sifted and PC-MM maize meals) had stronger associative forces and cross-links within starch granules, which were better able to withstand shear thinning or breakdown. By contrast, maize meals with higher breakdown viscosities (hammer-milled maize meals) had less ability to withstand heating and shear stress during cooking.

Starch molecules begin to re-associate in the cooling phase leading to formation of a gel structure with higher viscosity than the hot-paste slurry. The increase in viscosity is caused by a decrease of energy in the system, which allows re-association of leached amylose molecules with each other and with gelatinized starch granules (Zhang et al., 2011; Delcour and Hoseney, 2010). Par-cooked maize meal had the highest final viscosity (1,925 BU), indicating that it had a higher degree of amylose re-association than the other maize meals. Dehulling the grains prior to milling also affected the final viscosity. Whole hammer-milled maize meals had lower final viscosities than dehulled and hammer-milled maize meals (WM-TP < DM-TP and WM-SP < DM-SP). The final viscosities of sifted maize meals could not be distinctly differentiated from those of hammer-milled maize meals. Whereas the final viscosities of SF-T1 and SF-T2 were not different (P > 0.05) from those of DM-TP and DM-SP, we observed that the final viscosity of SF-T3 was significantly different (P < 0.05) from that of hammer-

milled maize meals.

The setback viscosity (final viscosity - trough viscosity), which is also associated with reordering of soluble amylose molecules (Leelavathi et al., 1987), showed a similar pattern as the final viscosity. The par-cooked and SF-T3 maize meals had the highest setback and final viscosities whereas WM-TP and WM-SP had the lowest (Table 4). Whole hammer-milled maize meals had lower setback viscosities than dehulled and hammer-milled maize meals (WM-TP < DM-TP and WM-SP < DM-SP). The setback viscosity of SF-T1 and SF-T2 was not significantly different (P > 0.05) from that of DM-TP and DM-SP whereas the setback viscosity of SF-T3 was significantly different (P < 0.05) from that of hammer-milled maize meals. When the setback viscosity was calculated as a percentage of final viscosity it was noted that SF-T3 and PC-MM had lower values (51 and 58%, respectively) than the other maize meals whose values ranged between 69 and 77%.

**Texture of stiff and thin porridge**

The texture of maize meal porridge is determined by the relative proportions of maize meal and water used to prepare it, which in turn is determined by socio-cultural preferences of different consumer groups in sub-Saharan Africa. From the nutritional point of view, the maize meal to water ratio is also important because it determines the energy content of the porridge (Kikafunda et al., 1997). In this study, we used a maize meal to water ratio of 1:1.95 (w/w) and 1:12.5 (w/w) to make stiff and thin porridge, respectively, that would be acceptable to consumers in eastern Africa. By contrast, stiff maize porridge from western Africa is prepared using a maize meal to water

**Table 5.** Pearson correlation coefficients between the physical and textural properties of thin maize meal porridge.

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | WAI | 1 | | | | | | | | | | | |
| 2. | WSI | -0.889 | 1 | | | | | | | | | | |
| 3. | PT | -0.580 | 0.143 | 1 | | | | | | | | | |
| 4. | PV (BU) | -0.362 | -0.106 | 0.969 | 1 | | | | | | | | |
| 5. | TP (min) | 0.690 | -0.945 | 0.189 | 0.425 | 1 | | | | | | | |
| 6. | BV (BU) | -0.343 | -0.126 | 0.964 | 1.000* | 0.444 | 1 | | | | | | |
| 7. | FV (BU) | -0.389 | -0.077 | 0.976 | 1.000* | 0.399 | 0.999* | 1 | | | | | |
| 8. | SV (BU) | -0.410 | -0.053 | 0.981 | 0.999* | 0.377 | 0.997* | 1.000* | 1 | | | | |
| 9. | Firmness (N) | 0.887 | -0.577 | -0.891 | -0.752 | 0.277 | -0.738 | -0.770 | -0.785 | 1 | | | |
| 10. | Consistency (N·s) | 0.846 | -0.508 | -0.925 | -0.803 | 0.199 | -0.790 | -0.820 | -0.833 | 0.997 | 1 | | |
| 11. | Cohesiveness (N) | -0.724 | 0.327 | 0.982 | 0.905 | 0.000 | 0.896 | 0.917 | 0.926 | -0.961 | -0.980 | 1 | |
| 12. | Index of viscosity(N·s) | -0.805 | 0.443 | 0.951 | 0.845 | -0.125 | 0.834 | 0.860 | 0.872 | -0.988 | -0.997* | 0.992 | 1 |

WAI: Water absorption index; WSI: water solubility index; PT: pasting temperature (°C); PV: peak viscosity, BU; TP: time to peak viscosity, min; BV: breakdown viscosity, BU; FV: final viscosity; SV: setback viscosity, BU. *Correlation is significant at the 0.05 level (2-tailed).

ratio of 1:3.5 (w/w) (Bolade et al., 2009) whereas in southern Africa a ratio of 1:4 (w/w) and 1:6 are recommended for stiff and thin porridge, respectively (Buhler Pty Ltd., Johannesburg, South Africa).

Stiff porridge prepared from PC-MM maize meal had significantly higher (P < 0.05) peak force (80.93 N) than that prepared from sifted or hammer-milled maize meals (Table 4) whose values ranged between 42.25 N for WM-TP and 58.86 N for DM-SP. The total shearing force (the product of force and time) was also significantly higher (P < 0.05) in stiff porridge prepared from PC-MM maize meal (838.99 N·s) than from sifted or hammer-milled maize meals where the values ranged between 409.87 N·s for WM-TP and 568.47 N·s for SF-T2 (Table 4). On the other hand, thin porridge (Table 4) prepared from DM-SP maize meal was more viscous (P < 0.05) than porridge prepared from the other maize meals.

This porridge had the highest firmness (1.28 N), consistency (34.04 N·s), cohesiveness (-1.54 N) and work of cohesion (-3.01 N·s). Thin porridge prepared from PC-MM maize meal was the least viscous since it had the lowest firmness (0.24 N), consistency (5.58 N·s), cohesiveness (-0.23 N) and work of cohesion (-0.34 N·s).

**Pearson correlation coefficient analysis**

Pearson correlation coefficient (r) analysis was made for the relationships between all physical and textural properties of PC-MM (Table 5) and DM-SP (Table 6). Stiff and thin porridge prepared from these two maize meals were the only ones analyzed because they had the firmest textures. The most important kinesthetic quality of stiff porridge is a firm, cohesive and non-sticky texture. Moulding of stiff porridge with the aid of the finger

and palm is part of the preliminary actions normally carried out before it is put in the mouth (Bolade et al., 2009). Par-cooked maize meal gave the firmest stiff porridge possibly due to a combination of factors associated with physical properties of the maize meal. It had the highest WAI, damaged starch content and final and setback viscosity; and lowest breakdown viscosity. However, when this data was analyzed by Pearson correlation coefficient analysis, the only significant correlation occurred between WSI and total shearing force (P < 0.05, r = -1.00). Nonetheless, some physical properties of PC-MM maize meal showed significant correlations with each other. The time to peak viscosity was positively correlated with WAI (P < 0.05, r = 0.99); setback viscosity was positively correlated with final viscosity (P < 0.01, r = 1.00); whereas starch damage was positively correlated with time to peak viscosity (P < 0.05, r = -0.99) but negatively

**Table 6.** Pearson correlation coefficients between the physical and textural properties of stiff maize meal porridge.

|  |  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | WAI | 1 | | | | | | | | | |
| 2. | WSI | -0.760 | 1 | | | | | | | | |
| 3. | PT | -0.932 | 0.943 | 1 | | | | | | | |
| 4. | PV (BU) | 0.528 | -0.953 | -0.799 | 1 | | | | | | |
| 5. | TP (min) | -0.793 | 0.999* | 0.960 | -0.936 | 1 | | | | | |
| 6. | BV (BU) | -** | - | - | - | - | - | | | | |
| 7. | FV (BU) | 0.366 | -0.883 | -0.678 | 0.984 | -0.875 | - | 1 | | | |
| 8. | SV (BU) | 0.380 | -0.890 | -0.688 | 0.986 | 0.986 | - | 1.000* | 1 | | |
| 9. | Peak force (N) | 0.686 | -0.994 | -0.902 | 0.980 | 0.980 | - | 0.928 | 0.934 | 1 | |
| 10. | Total shearing force (N·s) | 0.703 | -0.997 | -0.913 | 0.975 | 0.975 | - | 0.919 | 0.925 | 1.000* | 1 |

WAI: Water absorption index; WSI: water solubility index; PT: pasting temperature (ºC); PV: peak viscosity, BU; TP: time to peak viscosity, min; BV: breakdown viscosity, BU; FV: final viscosity; SV: setback viscosity, BU. *Correlation is significant at the 0.05 level (2-tailed). **Not computed because breakdown viscosity values were constant.

correlated with WSI ($P < 0.01$, $r = -1.00$). Among the textural properties, the peak force of PC-MM stiff porridge showed positive correlation with total shearing force ($P < 0.05$, $r = 1.00$).

Thin porridge is drank or eaten with a spoon and, thus, mouthfeel is one of its most important sensory properties. Thin porridge must have homogeneous consistency that is free from lumps and should disperse easily in the mouth, without being chewed, prior to swallowing. It should neither be too thin nor too viscous and the maize meal particles should leave a slight sensation of grittiness in the mouth. There was no significant correlation ($P > 0.05$) between the physical properties of DM-SP maize meal and thin porridge texture but some physical properties of DM-SP maize meal correlated well with each other. Breakdown viscosity and final viscosity were positively correlated with peak viscosity ($P < 0.01$, $r = 1.00$) whereas final viscosity was positively correlated with breakdown viscosity ($P < 0.05$, $r = 1.00$). Setback viscosity was positively correlated with peak viscosity ($P < 0.05$, $r = 1.00$), breakdown viscosity ($P < 0.05$, $r = 1.00$) and final viscosity ($P < 0.01$, $r = 1.00$). Among the textural properties, the viscosity of thin DM-SP maize meal porridge was negatively correlated with its consistency ($P < 0.05$, $r = -0.99$).

## Conclusion

Maize meal is a major source of energy and protein for millions of people in sub-Saharan Africa, and is principally used to prepare stiff or thin porridge. Physical quality differences that exist among maize meals are partially influenced by the milling technique. Although we did not find statistically significant correlations between physical properties of maize meals and the textures of stiff or thin porridge texture (except for the negative

correlation between WSI and total shearing force of PC-MM stiff porridge), it was apparent that the maize meal quality differences were reflected in the texture of porridges. We therefore, recommend that additional physical, and possibly chemical, properties of maize meals that can be used to predict the textural quality of stiff and thin maize meal porridge should be identified.

## Conflict of Interests

The authors did not declare any conflict of interests.

## ACKNOWLEDGEMENT

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*Short Communication*

# Designer paneer

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**Value added paneer was prepared to improve the fibre content in the otherwise fibre deficient paneer. Cereals like wheat and finger millet at 1% level was included in the preparation of paneer. Inclusion level of more than 1% caused pasty consistency. Sensory analysis was also carried out on the designed paneer. It was found that there was no significant difference in the flavour and overall acceptability between the designer paneer and control samples. Texture analysis was carried out for various attributes. It was found that wheat based paneer was equal to control samples in terms of adhesiveness which may be due to the pasty nature of the wheat flour. However, this designed paneer had an overall acceptability and could be used as a value added product.**

**Key words:** Paneer, millet based paneer, fibre enriched paneer.

## INTODUCTION

Paneer is a type of soft, unaged, acid-set, non-melting curd cheese made by curdling heated milk with lemon juice, vinegar or any other food acid. It is native to India, although it is used in some parts of Middle East as well. It is one of the most common cheeses used in Indian cuisine, and it has a simple, fresh, versatile flavor which makes it highly useful in an assortment of recipes.

As it is made with whole milk, it is also very high in protein, making it a sound addition to a vegetarian diet. Paneer is marble white spongy in nature and has a sweetish nutty flavour (Agarwal, 2007). Paneer contains 53-55% moisture, 23-25% fat, 17-18% proteins, 2-2.5% lactose and 1.5-2.0% minerals (Arora et al., 1996). However, since milk is devoid of dietary fibre, paneer has no fibre content. In the present study, value addition of

paneer with millet or cereal as a source of dietary fibre was attempted.

Cereals are rich in complex carbohydrates that provide ample energy, proteins, fats, lipids, minerals, vitamins and enzymes. Finger millet (ragi) is also a rich source of calcium, iron, protein, fiber and other minerals. In the present study, paneer was designed to contain fibre. The paneer thus designed was subjected to sensory and texture analysis

A texture study was devised to augment human sensory evaluation as a tool to evaluate food texture. It can be regarded as a manifestation of the rheological properties of a food (Karadbhajne and Bhoyarkar, 2010). Characterization of food texture commonly falls into two main groups, based on sensory and instrumental

**Table 1.** The textural calculation.

| Textural variable | Description | Unit |
|---|---|---|
| Hardness | Height of the peak during the first compression | g |
| Cohesiveness | Ratio of positive area under second peak to that of the first peak | |
| Springiness | Distance that the sample covered during the time that elapsed between the first bite and the start of the second bite | mm |
| Gumminess | Product of hardness and cohesiveness | g |
| Chewiness | Product of gumminess and springiness | G x mm |

**Table 2.** Comparison of sensory evaluation of control paneer and designer paneer 9 point Hedonic scale[#].

| Sensory parameter | C | W 1% | R 1% | F value |
|---|---|---|---|---|
| Colour and appearance | 8.00[b]± 0.000 | 7.16[a]± 0.200 | 8.16[b]± 0.2 00 | 4.97* |
| Body and Texture | 8.00[b]± 0.000 | 7.00[a]± 0.001 | 6.66[a] ±0.244 | 19.81** |
| Flavour | 7.66± 0.024 | 7.50 ± 0.244 | 7.33 ± 0.244 | NS |
| Overall acceptability | 7.86± 0.200 | 7.20 ± 0.250 | 7.33 ± 0.350 | NS |

[#]Average of six trails. NS: Not significant; *statistically significant ($P \leq 0.05$); **statistically highly significant ($P \leq 0.01$); [a-b]different superscripts in a row indicate significant differences.

methods of analysis. Sensory analysis includes use of the senses of smell, taste, sound and touch. Evaluation of food texture by touch includes the use of the fingers, as well as lips, tongue, palate and teeth in the mouth. As would be expected, sensory methods of analysis are subject to wide variability, though this variability can be reduced by using trained assessors.

It is sometimes preferable to use instrumental methods of assessing food texture rather than sensory analysis because they can be carried out under more strictly defined and controlled conditions (Peleg, 1983). In the present study, only sensory evaluation was conducted for acceptance of the product.

**MATERIALS AND METHODS**

**Preparation of paneer**

Paneer was prepared from cow milk (3.5% fat and 8.5% SNF) supplied to the dairy plant at the Department of Dairy Science, Madras Veterinary college, Chennai, India. Milk was boiled to 80°C and 2% solution of citric acid was added as a coagulant. The coagulum was then strained and pressed to obtain paneer.

**Preparation of designer paneer**

Two lots of designer paneer was prepared by the incorporation of 1% wheat flour and 1% ragi flour to milk and boiled. 2% solution of citric acid was added as a coagulant. The coagulum was then strained and pressed to obtain designer paneer. This study evaluated only the sensory attributes and textural properties of value enhanced designer paneer.

**Sensory evaluation**

These designer paneers were subjected to sensory evaluation using nine point hedonic scale. Sensory attributes were evaluated by a 10 trained panel of six replications for appearance, flavour, body and texture and over all acceptability.

**Texture analysis**

Texture analysis was carried out using Texture Analyser from Stable Micro Systems, U.K. with Powder Flow Analyser Assembly fitted with 2 kg load cell and aluminium blunt compression probe. Control and the two designer paneer samples were cut using sampler and were placed centrally beneath the probe. A crosshead speed of 5.0 mm/s with a trigger force of 20 g was used to compress the cores to 80% of their original height. Each sample was compressed twice in a reciprocating motion to give a two-bite texture profile curve. The graphs obtained were analysed for hardness, springiness, cohesiveness, gumminess and chewiness using the Texture Expert Exceed software supplied along with the instrument. The textural calculation is represented in Table 1.

**Statistical analysis**

The data obtained were analyzed statistically as per the procedure of Snedecor and Cochran (1980). Completely randomized design was used for comparing and studying the textural properties of the designer paneer

**RESULTS**

The results of the sensory analysis using 9 point hedonic scale for appearance, flavour, body and texture and over all acceptability are presented in (Table 2).

**Table 3.** Textural analysis of designer paneer[#].

| Textural analysis | Control | Ragi 1% | Wheat 1% | F value |
|---|---|---|---|---|
| Hardness | 3283.95[b]±760.078 | 1041.13[a] ±45.702 | 597.50[a]±89.336 | 37.91** |
| Adhesiveness | -20.40[a]±2.950 | -44.09[b] ±18.156 | -16.13[a]±3.770 | 4.51* |
| Springiness | 0.8438[b]±0.011 | 0.5806[a]±0.009 | 0.5164[a]±0.039 | 75.32** |
| Cohesiveness | 0.616[b]±0.047 | 0.399[a]±0.027 | 0.488[a]±0.086 | 4.40* |
| Gumminess | 2098.29[b]±626.466 | 414.25[a]±24.728 | 274.66[a]±20.986 | 20.01** |
| Chewiness | 1759.139[b]±512.351 | 240.73[a]±14.230 | 143.66[a]±20.862 | 21.93** |
| Resilience | 0.2790[b]±0.037 | 0.1056[a]±0.010 | 0.1590[a]±0.054 | 33.89** |

[#]Average of six trails. NS: Not significant; *statistically significant ($P \leq 0.05$); **statistically highly significant ($P \leq 0.01$); [a-b]different superscripts in a row indicate significant differences.

The scores for colour and appearance were higher for control samples and ragi paneer. The scores for body and texture was more for control samples than for either of the designer paneer. The flavour attribute showed no significant difference between any of the paneer. Overall, there was no significant difference in the sensory attributes between either of the paneer.

In the texture scores, control paneer samples had higher scores for hardness, springiness, cohesiveness, gumminess, chewiness and resilience (Table 3). The cereal and millet based paneer did not have similar characteristics as that of control samples. The adhesiveness nature of wheat based paneer and control were similar. However, an attempt was made in the preparation of cereal and millet based paneer which had adequate flavour and overall acceptability scores.

## DISCUSSION

The scores for colour and appearance were higher for control samples and ragi paneer. This could be due to the mottling appearance of paneer with ragi flour which was more appealing than the color for wheat paneer which appeared to look doughy. The scores for body and texture was more for control samples than for either of the designer paneer as they were more pasty in consistency. The yield of control paneer was 17% whereas for both designer paneer, the yield was 18%.

The flavour attribute showed no significant difference between any of the paneer. Flavour is one of the important attribute for consumer acceptance. Hence, this designer paneer could be used as a means of improved fibre content in the otherwise deficient milk paneer. Overall, it showed no significant difference in the sensory attributes between either of the paneer.

Control paneer samples had higher scores for hardness, springiness, cohesiveness, gumminess, chewiness and resilience which are characteristics of an ideal paneer or soft cheese. However, the adhesiveness

nature of wheat based paneer and control were similar and this might be due to the pastiness of the cereal based paneer. However, due to the improved adhesiveness nature, wheat based paneer can also be considered as a value added paneer. In the sensory analysis, the flavour scores and overall acceptability of of ragi based paneer was on par with control samples and hence these two varieties of value added designer paneer can be used in cuisine as an innovative culinary attempt.

## Conflict of Interests

The authors did not declare any conflict of interests.

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*Full Length Research Paper*

# Physico-chemical and microbiological characteristics of dried Waragashi

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In this study, effects of drying temperature on the physico-chemical and microbiological characteristics of Waragashi were investigated. Three types of produced Waragashi were investigated. The first one (V1) was obtained after coagulation of milk, the second called coloured Waragashi (V2), was obtained by boiling V1 for three minutes in aqueous solution of *Sorghum vulgaris* panicle (15 g/L) and the third one, refined Waragashi (V3) was obtained by boiling for three minutes, the Waragashi V1 into aqueous solution of *S. vulgaris* panicle (15 g/L), salt (10 g/L) and potash (3 g/L). The three fresh Waragashis contained 64.26, 57.34 and 54.04% moisture, respectively, for white Waragashi (V1), colored Waragashi (V2) and refined Waragashi (V3), while dried samples varied between 12.28 and 15.57%. The corresponding dried Waragashi were firmer than the fresh ones. Moreover, the colored cheeses (V2) were firmer (60.08 ± 5.40N and 206.4 ± 13.70 N) than the refined one (38.57 ± 3.10 N and 55.89 ± 5.89 N). After 48 h of drying, all samples showed a decrease of micro-organisms (mesophilic total bacteria, lactic bacteria, yeasts and moulds, *Enterobacteria* and *Staphylococcus* spp.) counts. However, drying at 45°C preserve more the physico-chemical characteristics of Waragashi.

**Key words:** Waragashi, cheese, drying, physico-chemical characteristics, microbiological quality.

## INTRODUCTION

Due to the difficult conservation of the fresh cow's milk in developing countries, attempts of technological approaches were developed to transform it into added value products (Dossou et al., 2006). The production of cheeses provides a useful service by extending the shelf life of a valuable human foodstuff-milk.

Waragashi is Benin local dairy product that is widely consumed in rural as well as suburban and urban zones. The lack of standard processing methods explains the variations observed in quality of Waragashi in the different areas of production (Turkoglu et al., 1987, Belewu, 2004).

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Waragashi is an excellent source of protein, fat and minerals (calcium, iron and phosphorous), vitamins and essential amino acids. It is often consumed as a substitution to the meat and fish in various dishes (Kees, 1996).

In spite of its economic and nutritional importance, it is difficult to preserve Waragashi for a long time. Common traditional methods applied for preservation did not extend efficiently its shelf life. In order to increase the shelf life of Waragashi, a study on the preservation of this product by evaluating the effect of drying and vacuum packaging was carried out (Sacramento, 2008). As a result, Waragashi can be preserved during two months. However, the cost of the product makes the implementation of developed process difficult in economical point of view. Another work in Nigeria on wara cheese conservation focused on the effect of short-term frozen storage on the chemical composition and coliforms microflora was reported (Alalade and Adeneye, 2006).

It is well known, that the drying foods, preserves the shelf life of the products as well as the nutritive value and produces a new flavor (Shikha and Usha, 2012). In spite of the popularity of Waragashi in West Africa areas, few studies on appreciation of physico-chemical, technological and microbiological characteristics of dried Waragashi produced in Benin were investigated. The aim of present work was to evaluate the effect of temperature on physico-chemical, technological and microbiological characteristics of Waragashi cheese after drying.

## MATERIALS AND METHODS

### Preparation and drying of Waragashi

The milk used for the manufacture of Waragashi was collected at a Peulh camp at Abomey-Calavi. The collected cow milk was stored immediately in isothermical box containing ice in order to suppress the increase of microorganism population. The Waragashi was prepared according to the process developed by Dossou et al. (2006). The *Calotropis procera* leaves obtained from the University of Abomey-Calavi grounds were used to prepare the coagulant solution as describe by Dossou et al. (2006). The *Sorghum vulgaris* panicle, the potash and salt were bought in a market of Godomey (Benin) and were used to color Waragashi. The produced Waragashi was drained and shaped on cylindrical box. The weight of the matrix was determined before and after 30 min of drainage with a portable electronic balance (Acculab Sartorius Group, Edgewood, NY, USA). Each Waragashi drained was cut into four parts and regrouped into three batches: the main one (Variety 1: V1), the coloured Waragashi (Variety 2: V2) and the refined Waragashi (Variety 3: V3).

The Waragashi V2 was obtained by boiling for three minutes the Waragashi V1 into aqueous solution of *S. vulgaris* panicle (15g/L) while the Waragashi V3 was obtained by boiling for three minutes the Waragashi V1 into aqueous solution of *S. vulgaris* panicle (15 g/L), salt (10 g/L) and potash (3 g/L). The three batches of Waragashi were closely followed during drying into electric oven (D 06060, model 400; MEMMERT, W 8540 Schwarbach, Gmbh + CoKCT). The thermic drying at hot air was made into two temperatures during 48 h: 45°C (temperature 1: T1) and 60°C (Temperature 2: T2) through two batches. In total, 20 samples of

of Waragashi by batch were analyzed for physico-chemical, physical and microbiological analysis before and after 48 h of drying. The kinetics of the water loss vs. time was determined by the "drying characteristic curve" (DCC), which represents the drying rate V(t) as a function of reduced water content $\phi$. These two variables are defined as described by (Ahouannou et al., 2000):

$$\phi = \frac{X(t) - X_{eq}}{X_{cr} - X_{eq}} \quad \text{and} \quad V(t) = -\frac{dX}{dt}$$

X(t): Dry basis product water content at time t, $X_{eq}$: dry basis product water content when equilibrium between air and product is reached, $X_{cr}$: dry basis product water content at the head of the first drying phase (initial phase with constant drying rate), V(t): drying rate of product at time t, $V_0$: drying rate of product during the first drying phase.

A mathematical expression of DCC for a product with specific initial dimensions is sought through the analysis of experimental drying curves obtained with different conditions of temperature, humidity and air drying speed. A representative example of such curves is shown in Figure 3, for the case of biological products. According to Jannot et al. (2002), the phase of raising temperature phase is most often negligible, especially if the difference between the air temperature and the product is low; and if the dimensions of the product are also low. Our experimental results are in concordance with this hypothesis.

The experimental curves X(t) have been derived to obtain the estimated curves $V(t) = \left(-dX/dt\right)(t)$. Such analysis gives a mean value of the critical water content $X_{cr}$ as shown in Figure 3. Several authors, among them, Desmorieux and Moyne (1992) have considered that for biological products it is difficult to identify a critical water content different from the initial water content $X_0$. Thus, it is assumed equal, $X_{cr} = X_0$.

### Physico-chemical analysis

Total solids content, ash and acidity were determined according to the methods described by AOAC (1990). Total sugar was determined according to the methods described by Dubois et al. (1959). Cheese samples were analyzed for pH values by using a digital pH-meter (Hanna Instruments, Model HI 98129, Singapore). The fat was extracted from cheese in a Soxhlet extractor with petroleum ether according to the methods described by Bligh and Dyer (1959) and Hubbard et al. (1977). The fat content was gravimetrically measured after the remotion of the solvent by rotary evaporation under vacuum (Bligh and Dyer, 1959). All physico-chemical analysis tests were conducted in triplicate.

### Physical analysis

The texture was determined using a Stevens LFRA Texture Analyser (TA Instruments, USA) (Cayot et al., 2009) and the color through a chromameter Minolta Chroma CR-210 b (Minolta Camera Co. Ltd, Osaka, Japan). Analyses were done in triplicate.

### Microbiological analysis of milk and various types of Waragashi

The analyses were achieved both on the milk and Waragashi. The count of mesophilic total bacteria, lactic bacteria, coliform bacteria, yeasts and moulds, and *Staphylococcus* spp. were evaluated. The plate count agar was used for the mesophilic total bacteria count

**Table 1.** Physico-chemical characteristics of milk.

| Parameter | x ± SD[a] |
|-----------|-----------|
| Relative density | 1.02 ± 0.01 |
| Moisture (%)[b] | 87.60 ± 0.37 |
| pH | 6.78 ± 0.05 |
| Total acidity (%)[c] | 0.19 ± 0.02 |

[a]Means of three independent trials ± standard deviation (SD). [b]The water content was given in three repetitions according to the thermogravimetric method for the determination of the water content in food according to standard AOAC (1990). [c]Total acidity by titration with NaOH 0.05 N until the indicator turn pink.

(Multon et al., 1994). The Man Rogosa Sharp medium (ISO 15214: 1998 (F)) was used for the lactic bacterias, the Malt Extract Agar (ISO 7954: 1987 (F)) for yeasts and moulds, the Violet Red Bile Agar for coliform bacterias count, according to the V 08-020 (1994)/ISO 7251 and V 08-021 (1993)/ISO 7402 norms (Bourgeois et al., 1989) and the Baird-Parker agar was used for *Staphylococcus* spp. count. For all microbiological analyses, samples of 25 g were taken from the cheese, transferred into 225 ml of the peptone water (%, w/v) and homogenized. From the initial dilution, appropriate decimal dilutions were prepared and aliquots were plated in duplicate in the different mediums.

### Mesophilic total bacteria count

One milliliter of each dilution was placed into each box after homogenization. 15 to 20 ml of the Plate Count Agar were smoothly added and kept at 45°C ± 0.5 (Multon et al., 1994). After the solidification, the box was turned over and incubated at 30°C during 72 h.

### Lactic bacteria count

One milliliter of each dilution was inoculates into each Man Rogosa Sharpe box (ISO 15214: 1998 (F)). The incubation was done at 30°C for three days.

### Yeasts and moulds count

The selectivity of the malt extract agar was changed with the addition of lactic acid at 10% (sterilized previously at 121°C during 15 min) using a relationship of 100:2 ml. The incubation was done at 25 ± 1°C for 5 days (ISO 7954: 1987 (F)).

### Coliform bacteria counts

From the decimal dilutions taken from $10^{-1}$ to $10^{-3}$, 1 ml in two boxes was introduced aseptically in two. About 20 ml of the Violet Red Bile Agar was added to the content of each boxes, melted and cooled at 45 ± 1°C. The mixture was homogenized by a circular movement. A range of box was incubated at 30°C during 24 - 48 h so as to detect the total coliform and a second range is incubated at

44°C during 24 - 48 h to detected the fecal coliforms (Bourgeois et al., 1989).

The boxes incubated at 44°C were recovered; the germ that they contained was streaked onto Eosine Methylene Blue (EMB) and incubated at 37°C during 24 h in order to detect E. *coli*.

### *Staphylococcus* spp. *count*

0.1 ml of each dilution was inoculated onto the surface of the Baird-Parker agar, incubated at 37°C in aerobic conditions and the examination was achieved after 24 and 48 h.

### Statistical analysis

The data obtained from these studies were analyzed using Statistical Analysis Software (SAS) and SPSS 17 (SPSS Inc. 233 South Wacker Drive, 11th Floor Chicago, IL 60606-641). The statistical analyses carried out were mean, standard deviation and analysis of variance (ANOVA) (Ogbeibu, 2005).

## RESULTS

### Physicochemical and microbiological characteristic of milk

Tables 1 and 2 show, respectively, the physicochemical and microbiological characteristic of the milk. The relative density (1.02 ± 0.01), the moisture (87.60 % ± 0.37), the pH (6.78 ± 0.05) and the total acidity (0.19 % ± 0.02) are very close to that listed in NF ISO 11816-1 (87%, 1.020 and 6.78, respectively for moisture, relative density and pH). The average of lactic bacteria, yeasts and moulds, Enterobacterias and *Staphylococcus* spp. counts for milk were determined and were, respectively, 5.30, 6.30, 1 and 0 log (CFU/ml), respectively, whereas the value of the mesophilic total bacteria count was lower (2.47 log CFU/ml) (Table 2). The mesophilic total bacteria and Enterobacterias counts were in concordance with the Institut Sénégalais de Normalisation (ISN) (1988) and Agence Française de Normalisation (AFNOR) (1976) criteria. However, the value of mesophilic total bacterias count found was higher than that advisable for commercialized milks by ISN (1988). Moreover, the negative results obtained from both tests of white side and blue methylene may confirm the good quality of the milk for the production of Waragashi.

### Physico-chemical characteristics of Waragashi

Tables 3 and 4 show the characteristics of Waragashi produced (V1, V2 and V3) and the drying one (V1T1, V2T1, V3T1, V1T2, V2T2 and V3T2). The moisture content of Waragashi produced was 64.26, 57.34 and 54.04% respectively, for white Waragashi, coloured Waragashi and refined Waragashi (Table 3). Dried samples varied between 12.28 and 15.57%. As observed, during the analysis, the total sugar content of all Waragashi was statistically invariable and showed

**Table 2.** Microbiological quality (log CFU/ml)[a] of milk.

| Microbiological quality | Mesophilic total bacterial count | Lactic bacteria count | Yeasts and moulds count | Enterobacte rial count | *Staphyloco ccus* spp. count | White side test | Blue methylene test |
|---|---|---|---|---|---|---|---|
| Milk used | < 2.47 | 5.30 ± 0.34 | 6.30 ± 0.34 | 1.00±0.00 | Absence | - | - |
| Microbiological criteria (ISN, 1988) | 4.30 | * | * | 2 | Absence | * | * |
| Conformity | *Conform* | - | - | *conform* | *conform* | - | - |

*Absence of criterion; -: Disregarded; [a]Means of two independent trials (log CFU/ml) ± standard deviation (SD). I.S.N. (Institut Sénégalais de Normalisation).

**Table 3.** Physico-chemical characteristics* of Waragashi.

| Waragashi | Dry matter (%)[1] | pH | Total Acidity (%db)[2] | Total Sugar (%db)[3] | Ash (%db)[4] | Lipid (%db)[5] |
|---|---|---|---|---|---|---|
| V1 | 35.74±0.11[a] | 6.70±0.00[d] | 0.12±0.00[b] | 5.75±0.11[a] | 2.06±0.04[a] | 47.26±0.001[f] |
| V2 | 42.66±0.60[b] | 6.65±0.07[c] | 0.14±0.00[d] | 5.10±0.09[a] | 2.09±0.03[a] | 35.77±0.002[a] |
| V3 | 45.96±0.05[c] | 6.60±0.14[b] | 0.15±0.00[b] | 5.64±0.08[a] | 2.17±0.06[a] | 36.88±0.001[b] |
| V1 T1 | 85.64±0.01[d] | 6.15±0.07[a] | 0.20±0.04[c] | 5.40±0.27[a] | 3.75±0.01[b] | 50.86±0.09[h] |
| V2 T1 | 87.72±0.47[f] | 6.35±0.07[b] | 0.16±0.02[d] | 5.42±0.23[a] | 3.78±0.13[b] | 43.80±0.067[e] |
| V3 T1 | 87.53±0.87[f] | 6.45±0.07[b] | 0.14±0.05[b] | 3.25±0.27[a] | 4.09±0.13[b] | 48.30±0.42[g] |
| V1 T2 | 86.42±0.75[e] | 6.50±0.00[b] | 0.05±0.01[a] | 5.81±0.17[a] | 4.66±0.12[c] | 42.61±0.28[d] |
| V2 T2 | 85.04±0.51[d] | 6.40±0.00[b] | 0.07±0.00[a] | 2.57±0.10[a] | 4.54±0.08[c] | 39.35±0.39[c] |
| V3 T2 | 84.43±0.98[d] | 6.45±0.07[b] | 0.13±0.00[a] | 2.60±0.14[a] | 4.67±0.05[c] | 50.09±0.71[h] |

*Means of three independent trials ± standard deviation (SD). 1, 2, 3, … 5. Expressed as … V1: White Waragashi, V2: Coloured Waragashi, V3: Refine Waragashi , V1T1: White Waragashi dry at 45 °C, V2T1= Coloured Waragashi dry at 45°C, V3T1: Refine Waragashi dray at 45°C, V1T2: White Waragashi dry at 60°C, V2T2 = Coloured Waragashi dry at 60°C, V3T2: Refine Waragashi dray at 60°C. The mean values followed by same letter in the same column are not significantly different (p< 0.05).

**Table 4.** Physical characteristics of Waragashi.

| Waragashi | L* (brightness) | a* (red indicator) | b* (yellow indicator) | Strength (N) |
|---|---|---|---|---|
| V1 | 88.79±0.88 | -1.98±0.042 | 13.12±0.49 | 7.01±0.48 |
| V2 | 43.78±1.82 | 42.24±0.39 | 31.39±1.18 | 10.38±1.31 |
| V3 | 37.49±1.19 | 32.98±0.72 | 19.63±0.73 | 10.2±0.13 |
| V1T1 | 61.46±0.87 | -2.89±0.22 | 13.63±1.15 | 43.58±4.92 |
| V2T1 | 31.21±1.24 | 26,10±0.78 | 14.23±1.35 | 60.08±5.40 |
| V3T1 | 26.41±0.815 | 17.03±0.76 | 4.03±0.84 | 38.57±3.10 |
| V1T2 | 59.63±0.91 | 4.10±0.56 | 34.22±0.74 | 181.33±15.2 |
| V2T2 | 31.22±0.75 | 25.10±1.18 | 20.78±0.43 | 206.4±13.70 |
| V3T2 | 26.57±0.59 | 19.23±0.23 | 5.11±0.45 | 55.89±5.89 |
| P | *** | *** | *** | *** |

V1: White Waragashi, V2: Coloured Waragashi, V3: Refine Waragashi, V1T1: White Waragashi dry at 45°C, V2T1: Coloured Waragashi dry at 45°C, V3T1: Refine Waragashi dray at 45°C, V1T2: White Waragashi dry at 60°C, V2T2: Coloured Waragashi dry at 60°C, V3T2: Refine Waragashi dray at 60°C, P: Probability; (***) = great variability (level: 1%) in the same column.

values between 2.57 and 5.75% (db) (dry base).

On the other hand, the total acidity content of the produced Waragashi (V1, V2 and V3) was statistically equal to that of the dried sample at 60°C. The total acidity content of the Waragashi varied between 0.12 and 0.15 % (db), the samples dried at 60°C varied between 0.05
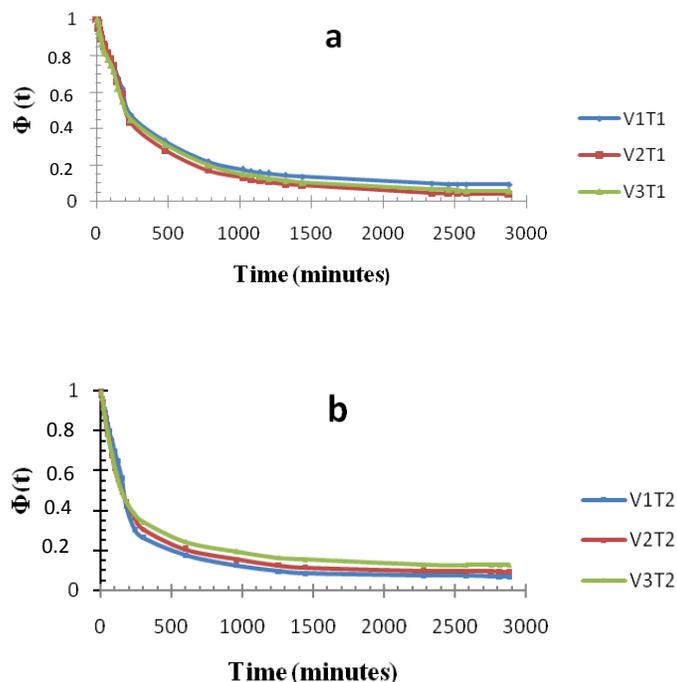
**Figure 1.** Kinetics of the reduced moisture of Waragashi (white, colored and refined) dried at 45 (a) and 60°C (b).
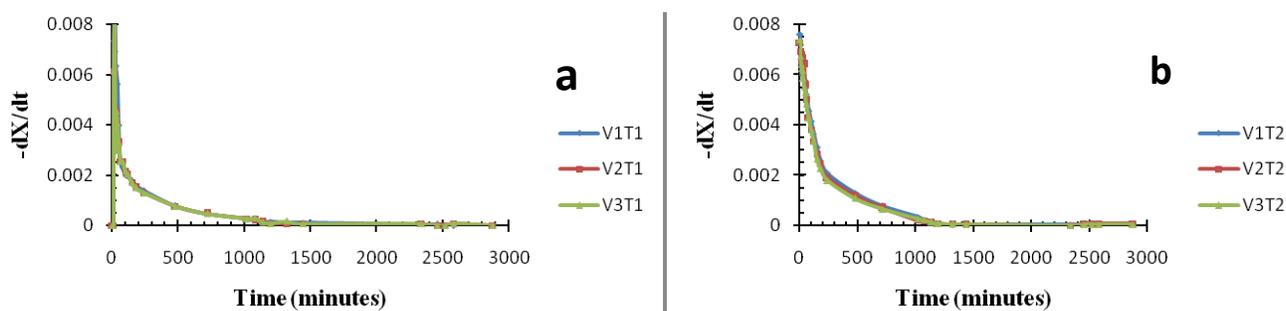


**Figure 2.** Kinetics of drying speed of waragashi (white, colored and refined) at 45°C (a) and 60°C (b).

and 0.13% (db). The ash content of the Waragashi (V1, V2 and V3) was statistically equal and varied between 2.06 and 2.17% (db). Likewise, the ash content of the dried samples were statistically equal and varied between 3.75 and 4.09% (db) and between 4.54 and 4.67% (db) for the dried samples at 45 and 60°C, respectively. As clearly shown in Table 3, the lipid content of Waragashi varies accordingly with the variety and the drying temperature.

The results of the texture and the color characteristics of fresh and dried Waragashi (Table 4) showed a great variability (level: 1 %) between the two types of cheese. Meanwhile, Waragashi V1 had a great brightness (88.79±0.88), low red indicator (-1.98±0.042) and low yellow indicator (13.12±0.49). This cheese is less firm (7.01 N) than the colored (10.38 N) and the refine (10.2 N) ones. The colored sample was redder (42.24±0.39)

and lighter (43.78±1.82) than the refined sample (32.98±0.72, 37.49±1.19, respectively for red indicator and brightness).

The brightness of dried sample varied between 59.63 and 61.46 for Waragashi V1 dried at 45 and 60°C, while those from V2 and V3 varied between 26.41 and 31.22. On the other hand, the dried cheeses were firmer than the fresh one.

Moreover, colored cheeses (V2) were firmer (60.08±5.40 and 206.4±13.70) than that of the refined one (38.57±3.10 and 55.89±5.89) at 45 or 60°C.

## Kinetic of drying

Figures 1, 2 and 3 present the kinetic of reduced moisture of Waragashi, the kinetic of drying speed of
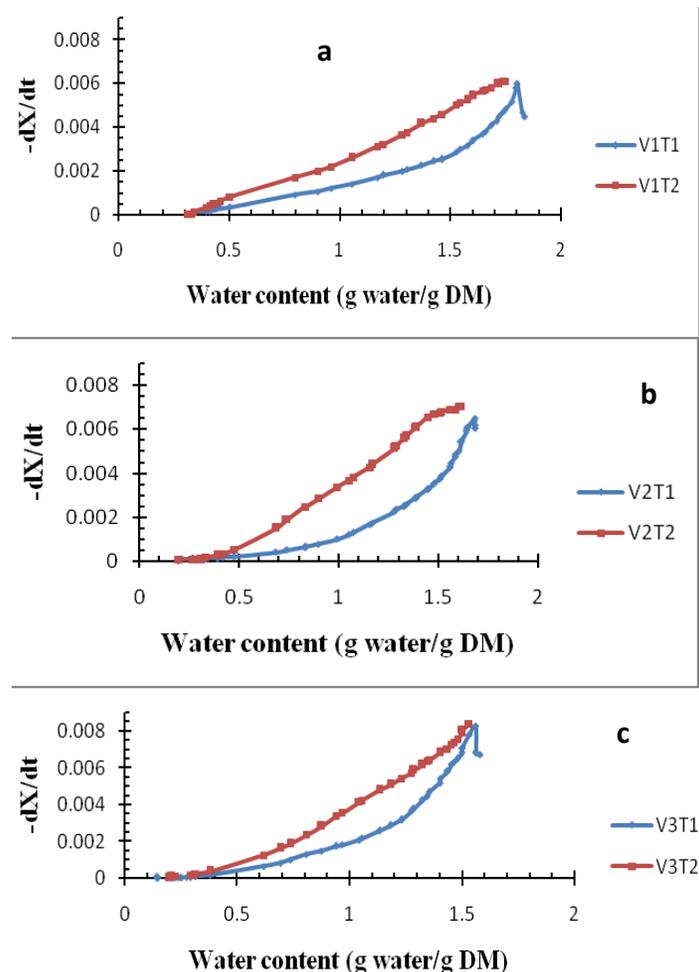
**Figure 3.** Kinetics of drying speed of samples (white (a), colored (b) and refined (c)) at 45 and 60°C.

Waragashi and kinetic of evolution of drying speed of samples.

From the results of the Figures 1a and b, the plots are leading as typically observed on biologic product like food. The plot present two principal phases, in the first part, corresponding to the 24 h of drying, the moisture decreased rapidly followed by a less fast decreased phase. The average moisture of fresh samples and dried one at 45°C (Figure 1a), respectively, were 1.837 and 0.147 g water/g DM (db) for white cheese, 1.684 and 0.129 g. water/g DM (db) for colored cheese and 1.595 and 0.132 g water/g DM (db) for the refine one. However, statistically there is the significant difference (5%) between the declines of moisture of all samples from the eighteenth to forty-eighth hour at the end of drying. The average moisture of fresh samples and dried one at 60°C (Figure 1b) were 1.897 and 0.167 g water/g DM (db) for white cheese, 1.642 and 0.176 g water/g DM (db) for the colored cheese and 1.579 and 0.201 g water/g DM (db) for the refine sample.

Static analyses showed a significant difference (5 %) between the declines of moisture of all samples from 16 to 48 h by the end of drying. Figure 2a and b show the evolution of the drying speed at 45 and 60°C of white, colored and refined Waragashi. During the 10 first min of drying at 45°C, no evolution was observed (Figure 2a) but after twenty minutes of drying, there was a maximal average of 0.0086 g water/g DM.min$^{-1}$ for Waragashi V1 and speed of 0.0080 g water/g DM.min$^{-1}$ for Waragashi V2 and V3. Later on, the speed began to decrease until the end of the process.

However, the analysis of variance did not show a significant difference between the drying' speeds of the three cheeses from the beginning to the end of the process. The evolution of speed of each variety of cheeses was also investigated (Figures 3a, b and c). From the results of Figure 3, the average speed of the elimination of water was 0.006 g water/g DM x min$^{-1}$ for drying at 45°C and was 0.007 g water/g DM x min$^{-1}$ for 60°C.

**Table 5.** Microbiological quality (CFU/g)[a] of Waragashi fresh with respect to the Food Safety Consult (F.S.C.) (2005) criteria.

| Waragashi | Mesophilic total bacteria count | Lactic bacteria count | Yeasts and moulds count | Enterobacterias count | *Staphylococcus* spp. count |
|---|---|---|---|---|---|
| V1 | 2.30 ± 0.21 | 3.30 ± 0.24 | 2.60 ± 0.24 | 1 | < 1 |
| V2 | 1.78 ± 0.07 | 1.70 ± 0.18 | 1.30 ± 0.00 | < 1 | < 1 |
| V3 | 1.70 ± 0.08 | 1.30 ± 0.24 | 1.48 ± 0.36 | < 1 | < 1 |
| Microbiological criteria | * | * | * | < 2 | < 2 |
| Level of conformity of the samples (%) | - | - | - | 100 | 100 |

V1: White Waragashi, V2: Coloured Waragashi, V3: Refine Waragashi *: Absence of criterion; -: Disregarded; [a]Means of two independent trials (log CFU/ml) ± standard deviation (SD).

**Table 6.** Microbiological quality (CFU/g)[a] of Waragashi dried with respect to the AFNOR criteria (1994).

| Waragashi | Mesophilic total bacterial count | Lactic bacterial count | Yeasts and moulds count | Enterobacterias count | *Staphylococcus* spp. count |
|---|---|---|---|---|---|
| V1 T1 | < 1.30 | 1.60 ± 0.11 | 1.70 ± 0.49 | < 1 | < 1 |
| V2 T1 | < 1.30 | 1.60 ± 0.24 | 1.30 ± 0.24 | < 1 | < 1 |
| V3 T1 | < 1.30 | 1.48 ± 0.17 | 1.00 ± 0.00 | < 1 | < 1 |
| V1 T2 | < 1.30 | 1.48 ± 0.36 | 1.48 ± 0.24 | < 1 | < 1 |
| V2 T2 | < 1.30 | 1.60 ± 0.43 | 1.00 ± 0.00 | < 1 | < 1 |
| V3 T2 | < 1.30 | 1.48 ± 0.00 | 1.48 ± 0.36 | < 1 | < 1 |
| Microbiological criteria | 3 | 2 | 2 | 2 | 2 |
| Level of conformity of the sample (%) | 100 | 100 | 100 | 100 | 100 |
| | 100 | 100 | 100 | 100 | 100 |

V1T1: White Waragashi dry at 45°C, V2T1: Coloured Waragashi dry at 45°C, V3T1: Refine Waragashi dray at 45°C, V1T2: White Waragashi dry at 60°C, V2T2: Coloured Waragashi dry at 60°C, V3T2: Refine Waragashi dray at 60°C. [a]Means of two independent trials (log CFU/ml).

### *Microbiological quality of Waragashi*

Table 5 shows the microbiological quality of the produced Waragashi. The average of mesophilic total bacteria, lactic bacteria, enterobacteria and yeasts and moulds counts for white Waragashi (V1) were 2.30, 3.30, 1 and 2.60 log(CFU/g), respectively, while *Staphylococcus* spp. counts were lower (1 log(CFU/g). Roughly, there is a decimal decreasing of micro-organism in pre-treated cheeses (V2 and V3) and, after 48 h of drying, all the samples present a decrease of micro-organisms and were in concordance with AFNOR (1994) criterias (Table 6).

### DISCUSSION

It is well known that during drying of foods, bio-chemical changes such as glycolysis, proteolysis, and lipolysis take place by the modification of composition of the product and enhancing the characteristics features to foods, especially the texture and flavour. The analysis of variance (Table 4) shows that the colored and the refined Waragashi (V2 and V3) were firmer (10.38 and 10.2 N, respectively) than the white cheese (p <0.05). The variations observed may be due to the infiltration of the molecules of color in white cheese during coloration and refining.

In fact, the infiltration of these may form a film of solution which decreases the moisture content of white cheese from 64.26 to 57.34% after coloration by a gradient of density (Table 3). The use of salt and potash may concentrate coloring molecule and increase gradient of density. This observation justifies the decrease of moisture of refined cheese (54.04 %).

The decrease of moisture is physically expressed by the firmness of Waragashi as reported in several works in the literature (Mazou et al., 2012). Mazou et al. (2012), Sacramento (2008) and Kora (2005) suggested that the use of salt and potash favored the decrease of moisture and increase the texture of the cheeses.

The colored cheeses had great red and yellow indicator

(a*= 42.24, b*=31.39) comparatively to the refined cheese (a*= 32.98, b*=19.63, p <0.05) whereas the white cheeses had low red and yellow indicator (a*= -1.98; b*= 13.12,p <0.05) (Table 4). In this result, the sorghum panicle colors the cheese red while potash concentrates the red coloration and turn slightly on black as reported by Sacramento (2008) and Mazou et al. (2012). The dried white cheese had statistically a comparable brightness (L* = 61.46 and L* = 59.63, respectively for 45 and 60°C). This result showed that the temperature of 45 and 60°C did not influence the brightness of Waragashi. However, the temperature of 60°C browned the white cheeses and draws the lipid up.

On the other hand, the interval between 45 and 60°C did not affect the red indicator and the brightness of colored cheese. The brightness, the red and the yellow indicators of the refined cheeses after drying drop significantly when the temperature increases from 45 to 60°C (Table 4). Generally, the dried cheeses are firm when the temperature increases. This result confirms those of Sacramento (2008) who reported that fresh Waragashi were less firm and more friable than the dried one and were easily transportable and marketable (Shaffer, 1998).

The dry matter, pH, total acidity, sugar and ash Waragashi were similar to those reported by Mazou et al. (2012). But the sugar content is lower than that observed by Kora (2005). Ultimately, the statistical results of the three varieties of Waragashi, showed that pH, total acidity, sugar content and the ash content of the three varieties of Waragashi are similar. This shows that the pretreatment does not affect the physicochemical characteristics of colorful and refined Waragashi over the white Waragashi.

The lipid contents of colored and refined Waragashi (35.77 and 36.88%, respectively) are lower than those from white Waragashi (47.26%). This reduction in the lipid content of pretreated Waragashi can be explained by the loss of lipid during cooking and coloring operations.

The dry matter of white Waragashi dried at 45 and at 60°C (85.64 and 86.42%, respectively) does not exceed 5% of difference and the moisture contents (14.36 and 13.58%, respectively) were closed to the equilibrium moisture content values (15%) of dried foods (BIT, 1990). The temperature range 45 - 60°C therefore does not affect the moisture content of the dried white Waragashi and reaches the equilibrium of water content (15%) in dried foods. However, the apparent acidity and the ash content varied significantly between fresh white samples dried Waragashi at 45 and dried at 60°C. The significant change in the ash content due to the degradation of the material justifies the significant variation in the pH of white Waragashi. There was a certainly production of acid by lactic bacteria during the drying of white Waragashi. In general, the temperature range 45 - 60°C is suitable for drying white Waragashi. Statistical analysis showed that the dry matter content and sugar content of

white Waragashi dried at 45°C and those dried at 60°C are similar. However, pH and ash content of Waragashi samples dried at 45°C respectively, 6.15 and 3.75% are lower than that of the dried samples at 60°C, respectively, 6.50 and 4.66%. The best drying temperature (45°C) for dried white Waragashi was similar to that of fresh white Waragashi. Meanwhile, the temperature of 60°C does not contribute to preserve pH and ash content of Waragashi but decreases the lipid content.

The plot of instantaneous evolution of reduced moisture of Waragashi (Figure 1), confirms the assertion that the infiltration of colored molecules on the colored and refined cheese decreases the moisture content and the facility of evaporation of water by drying at 60°C (Figure 1b). In fact, the reduction of moisture increases with the increase of temperature as observed by Silva et al. (2011). The required drying time to reduce the moisture of white Waragashi at an inferior or equal value of 0.20 g water/g DM were between 44 and 48 h, respectively of thermic drying for the samples dried at 60 and 45°C. The decrease of drying time with the augmentation of the temperature of drying was also observed by Lagunas (2007). However, the pre-treatment of white Waragashi did not affect the speed of drying (Figure 2). As shown in Tables 5 and 6, the produced Waragashi showed lower mesophilic total bacteria and *Staphylococcus* spp. counts than those detected in the milk. This finding confirms the destruction of the micro-organisms by heat. On the other hand, the count of yeasts and moulds in Waragashi were higher than that recommended by the AFNOR (1976) norms, which maintain these counts at a level than 1 log(CFU/ml) (Guiraud and Galzy, 1980). During samples drying, significant decrease of germs was observed, as reported also by Sacramento (2008) on Waragashi drying. The increase of temperature inhibited the development of most germs, as observed in this work during storage of Waragashi.

## Conclusion

The effect of the drying temperature (45 and 60°C) preserves the physico-chemical characteristics of Waragashi, and the multiplication of microorganisms. However, drying at 45°C preserve more the physico-chemical characteristics of Waragashi due to the fact that it maintains the physico-chemical and technological characteristics of Waragashi close to the fresh one.

## Conflict of interests

The authors did not declare any conflict of interests.

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