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Evaluation of some thin layer drying models and effective moisture diffusivity of yam (*Dioscorea rotundata*) slices

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ABSTRACT

In this study, thin layer drying characteristics of yam were investigated experimentally in a convective dryer. The drying experiments were conducted at drying air temperatures of 35, 40 and 45 °C and at an airflow rate of 0.60 m/s. Seven thin layer drying models available in the literature were fitted to the experimental data. The fit quality of the proposed models was evaluated by using the determination of coefficient (r^2), reduced chi-square (χ^2), root mean square error (RMSE) and sum of residuals. The modified Page, Page and Weibull models showed a better fit to experimental drying data as compared to other models. The effective moisture diffusivity of the yam is found to increase with the drying air temperatures and ranged between $10.08x10^{-10}$ and $11.21x10^{-10}$ m^2/s. Using the effective diffusivity and an Arrhenius type relationship, it is found that yam activation energy is 28.76 kJ/mol.

Keywords: Yam; thin layer drying; Effective moisture diffusivity; Activation energy.

INTRODUCTION

Yam belongs to the genus Dioscorea (family Dioscoreaceae). It is an important source of carbohydrate for lot of people in the world. Therefore, it can be found in Africa, the West Indies and parts of Asia, south and Central America and Oceania. Yams are cultivated through the tropics and in parts of the sub-tropics and temperate zones. In West Africa and New Guinea yam is a primary agricultural commodity. Yam is also used as health foods and herbal medicinal ingredients in Asia countries including Korea, China and Japan (Kim et al., 2005). It must be noticed that yam contributes more than 200 dietary calories per capita daily for more than 150 million people in West Africa and serves as an important source of income to the farmers (Badaleye, 2003). Moreover, it should be noted that yam plays an important role in Central and West Africa such as food security, social and cultural role. Although yam is sought as a staple food or health food, yam tubers have high water content (approximately 70% wet basis) at harvest and therefore cannot be preserved for a long time under ambient conditions (Gikuru & Olwal, 2005). This is a disability for its storage and its international marketing. Therefore many research works are done in the world to find the mean of its preservation and to reduce the post harvest loss which is in the range of 25 to 60% according to Girardin (1996).Yam post harvest loss in Côte d'Ivoire is 40% which is consistent with the previous values given. This high post harvest loss percentage constitutes a big loss for farmers which are confronted with the problem of preserving their harvested crops to prevent spoilage during storage. Drying is probably the oldest and the most important method of food preservation practiced by humans. This process improves the food stability, since it reduces considerably the water and microbiological activity of the material and minimizes physical and chemical changes during its storage. Drying is a complex thermal process involving simultaneous heat and mass transfer (Yilbas et al., 2003). The materials are dried by thin layer drying due to faster drying with minimum loss of nutrients. In order to improve drying behaviour of yam slices, it is important to develop a better understanding of the controlling parameters of this complex process. Mathematical models are used for enabling simulation of the drying process under different conditions. Many mathematical models have proposed to describe the drying process, of them, thin layer drying models have been widely in use. These models can be categorized as theoretical, semi-theoretical and empirical (McMinn, 2006). Many studies have emphasized drying kinetics and thin layer drying models for fruits and vegetables – cassava, ginger and okra (Ahouannou et al., 2000), banana and mango (Talla et al., 2001; Koua et al., 2009), apricot (Doymaz, 2004), banana (Jannot et al., 2004), peach (Kingsly et al., 2007), mulberry (Akpinar, 2008) apple (Goyal et al., 2008), carrot (Kaya et al., 2009), leaves of inga edulis (Silva et al., 2011), pumpkin and green pepper (Guiné and Barroca, 2012). Some works have been published concerning processing attributes of yam. Montes et al. (2008) observed the effect of drying air temperature on the drying time and calculated the effective diffusivity and activation energy for drying of yam. Fioreze and Morini (2000) studied the drying characteristics of yam and simulated the drying as a function of the drying air

conditions and of the initial and final water content of yam. Falade et al. (2007) determined the effect of pre-treatment and of drying air temperature on the drying of yam. However, study on thin layer drying models of yam has not been reported yet. In this study, the thin layer drying behaviour of yam in a convective dryer is investigated and a mathematical modelling by using the thin layer drying models is performed. Additionally, the effective diffusivity and the activation energy of yam slices are computed.

MATERIAL AND METHODS

Material

Fresh yam was procured from local market in Abidjan, Côte d'Ivoire. After thorough cleaning and washing, the yams were peeled and sliced into 20 x 10 x 25 mm (width x thickness x length) using a mechanical slicer. The initial water content was determined by drying in an oven at 103 °C a sample of yam until constant weight and amounted to 2.29 kg water/kg dry basis. The analysis was carried out in triplicate.

Experimental apparatus

The drying apparatus was a convective dryer. It consists of an electrical fan providing the desired air velocity, a heating unit and a drying chamber. The heating control unit has an electrical heater (3 kW) placed inside an air-duct. The drying chamber was made of wood covered with metallic sheets. The air passed from heating unit and heated to the desired temperature and then channelled to the drying chamber. The samples were uniformly arranged in a thin layer on a steel perforated tray. The drying air temperature in the drying chamber was measured directly using 0.001 m diameter chromel/alumel thermocouples with measurement precision of ± 1 °C. The required air flow rate for drying was kept at the desired level at about 3 cm just above the tray surface using a hot wire anemometer with discrete probe. Weight loss of samples was recorded by using a digital display electronic balance (type Metter PL 1200) with a sensitivity of 0.01 g.

Experimental procedure

Drying experiments were performed at drying temperatures varying from 35 to 45 °C, with 5 °C increment, and a constant air velocity of 0.60 ± 0.01 m/s for all circumstances. The weight of tray with the samples was measured with a digital balance and recorded at 15 min interval for all temperature range selected for the study. For measuring the weight of the samples during the experimentation, the tray with samples was taken out of the drying chamber, weighed on the digital top pan balance and placed back on the drying chamber. The digital balance was very close to the drying unit. The drying procedure was preceded until no appreciable variation of the weight of the samples was recorded. All drying experiments were conducted in triplicate and the average of the water content at each value was used for the drawing of the drying curves. The weight of the sample was converted to water content (X) on dry basis using the standard formula. Where W is the weight of sample and W_d is the weight of dry material in the sample.

$$X = \frac{W - W_d}{W_d} \quad (1)$$

Theoretical formulations

Mathematical modelling of drying curves

The data for the water content obtained for the drying temperatures 35, 40 and 45 °C were converted into moisture ratio, X_r. The dimensionless moisture ratio of the sample was defined according to Eq. (2) where X_0 represents the product initial water content and X_{eq} is the equilibrium product water content given by the sorption isotherms and depending on drying air conditions.

$$X_r = \frac{X - X_{eq}}{X_0 - X_{eq}} \quad (2)$$

However, the reduced water content was simplified according to Akgun and Doymaz (2005) and Thakor et al. (2005), since the X_{eq} values are relatively smaller when compared to X and X_0, for long drying times and one can write:

$$X_r = \frac{X}{X_0} \quad (3)$$

In this study, the drying rate (DR) of yam slices was calculated by the application of the following formulas (Jannot et al., 2004):

At initial time t_0, the drying rate, DR_0, is calculated as:

$$DR_0 = (-\frac{dX}{dt})_0 = \frac{X_0 - X_1}{t_1 - t_0} \quad (4)$$

At each time t_i, i varying from 1 to f – 1, the drying rate is calculated as:

$$DR_i = (-\frac{dX}{dt})_i = \frac{1}{2}(\frac{X_{i-1} - X_i}{t_i - t_{i-1}} + \frac{X_i - X_{i+1}}{t_{i+1} - t_i}) \quad (5)$$

At the final time t_f, the drying rate, DR_f, is calculated as:

$$DR_f = (-\frac{dX}{dt})_f = \frac{X_{f-1} - X_f}{t_f - t_{f-1}} \quad (6)$$

The experimental data of moisture ratio versus drying time were fitted to the most important semi-theoretical models, which are widely used in the scientific literature to describe the kinetics of the drying process. In this study, the experimental drying data of yam slices at different temperatures were fitted into seven (7) commonly used thin layer drying models, listed in Table (1).

Table 1: Mathematical models of the experimental drying

Non-linear regression analyses are done by using the Statistical routine. The coefficient of determination (r^2), reduced chi-square (χ^2), root mean square error (RMSE) and sum of residuals were calculated for each model in order to test their accuracy in reproducing the

Model number	Model name	Equation	References
1	Henderson and Pabis	$X_r = a \exp(- kt)$	Henderson and Pabis (1961)
2	Lewis	$X_r = \exp(- kt)$	Bruce (1985)
3	Midilli et al.	$X_r = a \exp(- kt^n) + bt$	Midilli et al. (2002)
4	Modified Page	$X_r = \exp(- (kt)^n)$	Overhults et al. (1973)
5	Page	$X_r = \exp(- kt^n)$	Page (1949)
6	Two term	$X_r = a \exp(- kt) + b \exp(- k_0 t)$	Henderson (1974)
7	Weibull	$X_r = \exp(-(\frac{t}{\beta})^{\alpha})$	Corzo et al. (2008)

experimental data. The higher values of the coefficient of determination (r^2) and the lower values of the reduced chi-square (χ^2), RMSE and sum of residuals were chosen for goodness of fit (Midilli and Kucuk, 2003; Celma et al., 2012). These parameters can be calculated as:

$$\chi^2 = \frac{\sum_{i=1}^{N} (X_{rth,i} - X_{r\,exp,i})^2}{N - n} \quad (7)$$

$$Residuals = \sum_{i=1}^{N} (X_{r\,exp,i} - X_{rth,i}) \quad (8)$$

$$RMSE = \left[\frac{1}{N} \sum_{i=1}^{N} (X_{rth,i} - X_{r\,exp,i})^2 \right]^{1/2} \quad (9)$$

where $X_{rexp,i}$ is the ith experimental moisture ratio, $X_{rth,i}$ is the ith predicted moisture ratio, N and n are the number of observations and constants, respectively. The best model obtained in this stage was used to predict the duration of drying time necessary to achieve the required equilibrium water content.

Effective moisture diffusivity

The effective moisture diffusivity is an important transport property in food and other materials drying processes modelling, being a function of temperature and water content in material (Liu et al., 2009; Doymaz, 2012). Fick's second law of diffusion equation, symbolized as a mass diffusion equation for drying agricultural products in a falling rate period, is shown in the following equation:

$$\frac{\partial X_r}{\partial t} = D_{eff} \nabla^2 X_r \quad (10)$$

where D_{eff} is the effective moisture diffusivity (m^2/s) and t is the drying time (s). An analytical solution of the second law diffusion (Eq. (10)), developed by Crank (1975) under the assumption of a one dimensional moisture movement with no volume change, constant diffusivity, uniform initial water content distribution and negligible external resistance was assumed. Where L is the thickness of the product sample (m)

$$X_r = \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left[-(2n+1)^2 \frac{\pi^2 D_{eff}}{L^2} t \right] \quad (11)$$

For long drying times, $X_r < 0.6$, Eq. (11) can be further simplified to retain only the first term of the series allowing rewriting Eq. (11) in a logarithmic form as follows (Ramesh et al., 2001; Schössler et al., 2012).

$$\ln(X_r) = \ln(\frac{8}{\pi^2}) - \frac{\pi^2 D_{eff}}{L^2} t \quad (12)$$

Based on this equation, the moisture diffusivity D_{eff} can be determined applying the method of slopes (Mazutti et al., 2010; Schössler et al., 2012). Diffusivities were typically determined by plotting experimental drying data in terms of $\ln(X_r)$ versus drying time in Eq. (12), providing a straight line with the slope given by:

$$Slope = \frac{\pi^2 D_{eff}}{L^2} \quad (13)$$

The dependence of the effective moisture diffusivity on temperature may be described by an Arrhenius-type relationship as follows (Doymaz, 2004; Rafiee et al., 2010; Xiao et al., 2010):

$$D_{eff} = D_0 \exp(-\frac{E_a}{RT}) \quad (14)$$

where E_a is the activation energy (kJ/mol), D_0 is the Arrhenius pre-exponential factor of Arrhenius equation (m^2/s), R is the universal gas constant (R = 8.314J/molK) and T is the absolute drying air temperature (K). From the slope of the straight line of $\ln(D_{eff})$ versus reciprocal of T, described by the Arrhenius equation, the activation energy, E_a, could be calculated.

RESULTS AND DISCUSSION

Drying characteristics

The yam slices were dried at 35, 40 and 45 °C and at an airflow rate of 0.60 m/s in a forced convective dryer in thin layer with thickness of 10 mm. The initial water content of yam was found to be around 2.29 kg water/kg dry basis and the final water content was 0.12 kg water/kg dry basis. The characteristics of drying curves for yam slices are shown in Figs. 1 and 2. It is clear that the water content and moisture ratio of yam decrease continuously with drying time. Decrease in water content and moisture ratio indicates that diffusion has governed the internal mass transfer. Drying curves exhibited behaviour typical for drying food (Zielinska and Markowski, 2007 and 2010) and indicated an initial high rate of water removal. The total drying time required to reach the final water content of yam slices were 555, 465 and 390 min at the drying air temperatures of 35, 40 and 45 °C, respectively. The decrease in drying time with an increase in the drying air temperature has been reported for many agricultural products (Rafiee et al., 2010; Falade and Solademi, 2010, Zielinska and Markowski, 2010; Doymaz, 2012). The Figure (3,4) showed the drying rate (DR) versus drying time and the variations of drying rate with water content of the yam slices at drying air temperatures of 35, 40 and 45 °C and at an airflow rate of 0.60 m/s. It was also observed that the drying rates were higher at elevated drying air temperatures. As seen in Figs. 3 and 4, two falling rate periods for convective drying of yam slices were observed in all the drying processes. Separation of first and second falling rate periods was taken to be at about the moisture ratio of 0.28. Two falling rate periods are typically observed for drying of hygroscopic products (Schössler et al., 2012). Theoretically, the drying process starts with a period of constant drying rate, where the drying rate is controlled by external conditions when moisture transfer to the product surface is sufficient to keep it constantly wet. This period is rarely observed in food drying studies and several authors reported that no constant rate period occurred during drying of different agricultural products (Krokida et al., 2003; Togrul, 2006; Mazutti et al., 2010; Schössler et al., 2012). It can be seen that at higher water content, the increase in temperature has more considerable effect on the drying rates than at lower water content, which was almost negligible at the end. It was further observed that water loss was faster at the start of drying than at the end. The reduction in the drying is mainly due to reduction in water content as drying advances. The rate of migration of water from inner surface to outer surface decreases at the final stage of drying and hence lower drying rates (Rajkumar et al., 2007; Kumar et al., 2012).

Mathematical modelling of the drying curves

The water content data obtained at different drying air temperatures were converted to dimensionless moisture ratio (Eq. (1)) and then fitted to seven thin layer drying models (Table 1). Nonlinear regression analysis was used to estimate the parameters of those seven models. The statistical results from models are summarized in Table 2. The best model describing the thin layer drying characteristics of yam slices was chosen as the one with the highest r^2 values and the lowest χ^2, RMSE and sum of residual values. For all models, r^2 values were > 0.98, χ^2 < 0.00299, RMSE < 0.05172 and sum of residuals < 0.77404. Of all the models tested, the modified Page, Page and Weibull models give the highest values of r^2 and the lowest values of χ^2, RSME and sum of residuals. Accordingly, the modified Page, Page and Weibull models were selected as the suitable models to represent the thin layer drying characteristics of yam slices. Figures (5-7) compare experimental data with those predicted with the modified Page, Page and Weibull models for yam slices at 35, 40 and 45 °C. As shown in Figs. 5 - 7, the prediction using the models showed X_r values banded along a straight line, which showed the suitability of these models in describing the drying characteristics of yam slices. To take into account the effect of drying temperature on the model parameters and attempting to generalise the model, a regression analysis was applied to set up the relationship between these parameters and the drying temperature for the yam slices at the airflow rate of 0.60 m/s. The regression equations of these parameters against drying temperature for each experimental condition are presented in Table 3.

Determination of effective moisture diffusivity

The values of effective moisture diffusivity (D_{eff}) of yam slices for the two falling rate periods were calculated using Eq. (13) and are presented in Table 4. The values of D_{eff} in the first falling rate period ranged from $8.28x10^{-10}$ to $11.83x10^{-10}$ m^2/s, whereas for the second period the variation was from $9.30x10^{-10}$ to $13.18x10^{-10}$ m^2/s. The values of D_{eff} in overall falling rate period ranged from $8.70x10^{-10}$ to $12.51x10^{-10}$ m^2/s. The average effective moisture diffusivity for first, second and overall falling rate periods were $10.08x10^{-10}$, $11.21x10^{-10}$ and $10.62x10^{-10}$ m^2/s, respectively. The effective moisture diffusivities in the second falling rate period were greater than those found for the first falling rate period. Similar results have been reported by Wang et al. (2007) and Kumar et al. (2012). As expected, the values of D_{eff} increased greatly with increasing drying air temperature, which is in accordance with the previous studies (Mohapatra and Rao, 2005; Chandrashekar, 2008; Thorat, 2012). When yam

Table 2: Result of statistical analysis on the mathematical models for yam slices

T (°C)	Model number	Model coefficients	Drying constants	r^2	χ^2	RMSE	Sum of residuals
	1	a = 1.0398	k = 0.0052	0.9998	0.000148	0.011853	0.354482
	2		k = 0.0051	0.9995	0.000096	0.009646	0.281162
	3	a = 1.00288; n = 1.1898					
35		b = - 0.00000675	k = 0.00178	0.9935	0.000798	0.026773	0.113679
	4	n = 1.0201	k = 0.00496	0.9999	0.000009	0.003059	0.035116
	5	n = 1.0201	k = 0.00446	0.9999	0.000009	0.003065	0.035577
	6	a = - 17.2239	k = 0.00998	0.9823	0.002989	0.051719	0.184125
		b = 18.2279	k_0 = 0.00949				
	7	α = 1.0201; β = 201.4139		0.9999	0.000009	0.003056	0.034908
	1	a = 1.0418	k = 0.0063	0.9987	0.000197	0.013606	0.360828
	2		k =0.0061	0.9997	0.000049	0.006938	0.131304
	3	a = 0.99878; n = 1.1998					
40		b = - 0.00000653	k =0.00189	0.9955	0.001532	0.036613	0.774042
	4	n = 1.0214	k = 0.00601	0.9999	0.000010	0.003125	0.029968
	5	n = 1.0214	k = 0.00539	0.9999	0.000010	0.003124	0.029925
	6	a = - 17.2449	k = 0.00999	0.9957	0.000514	0.021216	0.006876
		b = 18.2309	k_0 = 0.00966				
	7	α = 1.0214; β = 166.4256		0.9999	0.000010	0.003117	0.029521
	1	a = 1.0399	k = 0.0074	0.9988	0.000179	0.012876	0.285659
	2		k =0.0072	0.9997	0.000055	0.007276	0.124566
	3	a = 0.98978; n = 1.2987					
45		b = - 0.00000547	k =0.00139	0.9911	0.002187	0.043163	0.549355
	4	n = 1.0216	k = 0.00708	0.9999	0.000010	0.003047	0.024879
	5	n = 1.0216	k = 0.00636	0.9999	0.000010	0.003045	0.024782
	6	a = - 17.3447	k = 0.00979	0.9997	0.000031	0.005176	0.067451
		b = 18.3309	k_0 = 0.00965				
	7	α = 1.0216; β = 141.2551		0.9999	0.000010	0.003055	0.025284

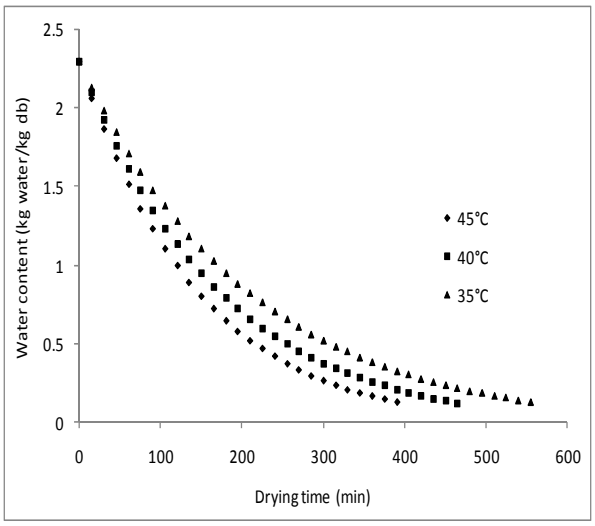

Fig. 1: Thin layer drying curves of yam slices at different temperatures.

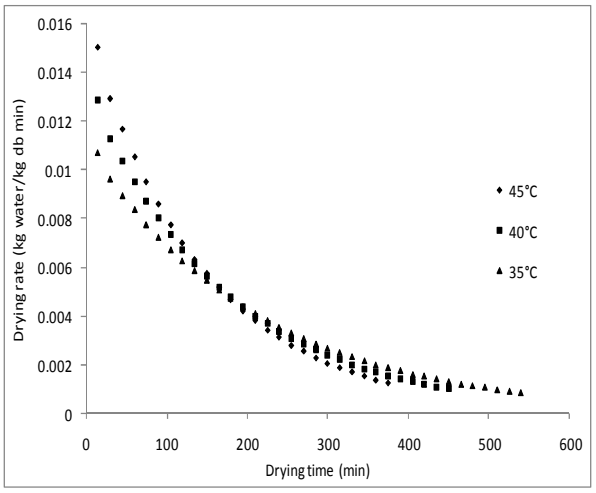

Fig. 3: Drying rate versus drying time at different temperatures

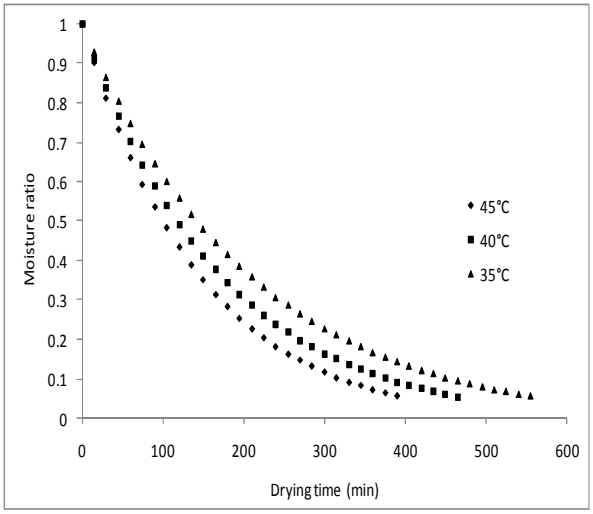

Fig. 2: Moisture ratio versus drying time at different temperatures

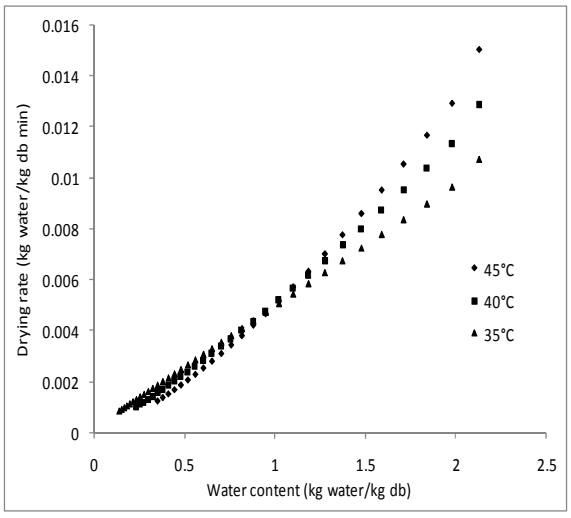

Fig. 4: Drying rate versus water content at different temperatures.

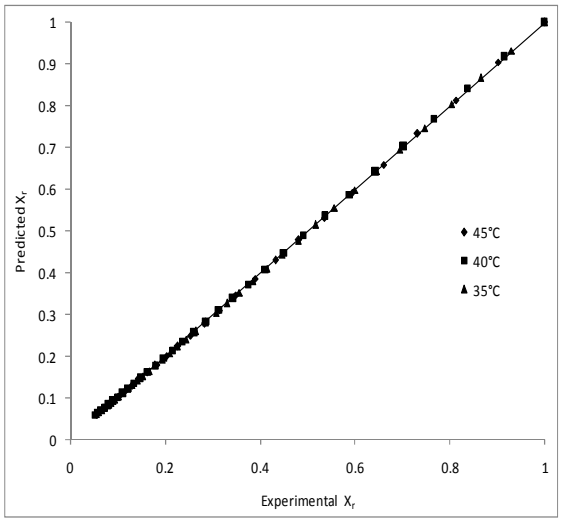

Fig. 5: Comparison of experimental and predicted moisture ratio by the modified Page model

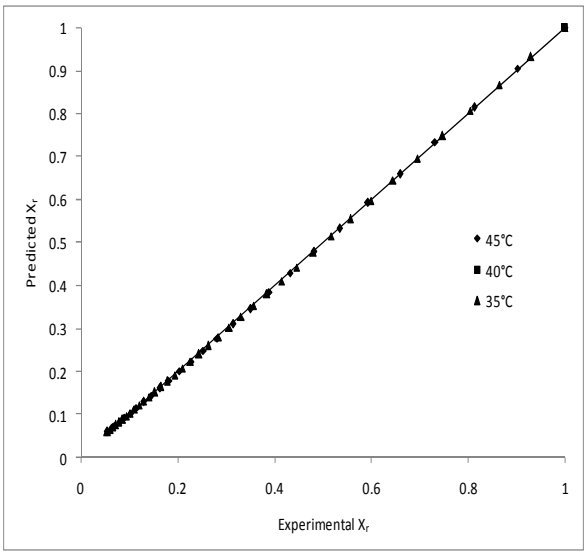

Fig. 7: Comparison of experimental and predicted moisture ratio by the Weibull model.

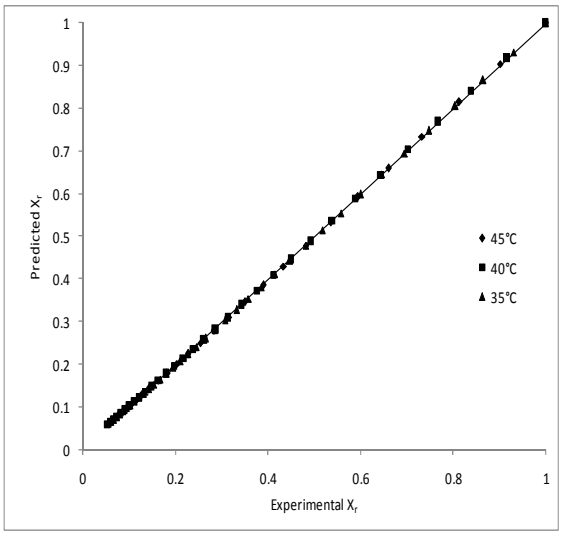

Fig. 6: Comparison of experimental and predicted moisture ratio by the Page model

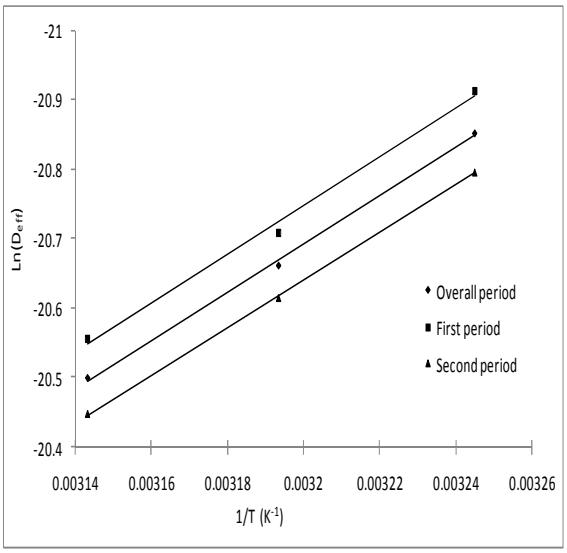

Fig. 8: Arrhenius-type relationship between effective moisture diffusivity and reciprocal absolute temperature.

molecules leading to higher moisture diffusivity (Xiao et al., 2010). The values of D_{eff} obtained from this study lie within in general range 10^{-11} – 10^{-9} m^2/s for drying of food materials (Wang et al., 2006).

Determination of activation energy

The activation energy can be determined from the slope of Arrhenius plot, $\ln(D_{eff})$ versus reciprocal of absolute temperature. The $\ln(D_{eff})$ as a function of 1/T was plotted in Fig. 8. The slope of the line is ($- E_a/R$) and the intercept equals to $\ln(D_0)$. The results show a linear relationship due to Arrhenius type dependence as described in Eqs. (15), (16) and (17).

First falling rate period

$$D_{eff} = 7.15x10^{-5} \exp(-\frac{3500}{T}) \ (r^2 = 0.995) \quad (15)$$

Second falling rate period

$$D_{eff} = 6.13x10^{-5} \exp(-\frac{3418}{T}) \ (r^2 = 0.999) \quad (16)$$

Overall falling rate period

$$D_{eff} = 6.65x10^{-5} \exp(-\frac{3460}{T}) \ (r^2 = 0.998) \quad (17)$$

The calculated values of activation energy for yam were 29.09, 28.42 and 28.77 kJ/mol for first, second and overall falling rate periods respectively. These values are similar to those proposed in the literature by Falade et al. (2007) for different varieties of yam e.g. between 25.26 and 72.47 kJ/mol. The values of activation energy were within the general range of 12.7 - 110 kJ/mol for various food materials (Zogzas et al., 1996).

Conclusion

In this study, drying kinetics of yam was investigated as a function of drying conditions. Drying of yam slices occurred in falling rate period; no constant rate period of drying was observed for the present study, which implies that moisture removal from the material was governed by diffusion phenomenon. Increasing the temperature of drying air decreases the drying time. According to statistical analysis applied to seven thin layer drying models, the modified Page, Page and Weibull models gave the best representation of drying data under all experimental conditions. The effective moisture diffusivity of yam was computed from Fick's second law, the values of which varied between $10.08x10^{-10}$ and $11.21x10^{-10}$ m^2/s over the temperature range of 35 – 45 °C. The average value of activation energy of yam was found to be 28.76 kJ/mol.

References

1. Ahouannou, C., Jannot, Y., Lips, B. and Lallemand, A. 2000. Caractérisation et modélisation du séchage de trois produits tropicaux : manioc, gingembre et gombo. Sci. Aliments. 20: 413-432.
2. Akgun, C.A. and Doymaz, I. 2005. Modeling of olive cake thin layer drying process. J. Food Eng. 68: 455-461.
3. Akpinar, E.K. 2008. Mathematical modeling and experimental investigation on sun and solar drying of White mulberry. J. Mech. Sci. Technol. 22: 1544-1553.
4. Badaleye, T. 2003. Raising the status of the yam, a major food crop in West Africa. ANB-BIA Supplement issue/Edition, 463: 1-3.
5. Bruce, D.M. 1985. Exposed layer barley drying three models fitted to new data up to 150 °C. J. Agric. Eng. Res. 32: 337-347.
6. Celma, A.R., Cuadros, F. and Lopez-Rodriguez, F. 2012. Convective drying characteristics of sludge from treatment plants in tomato processing industries. Food and Bioproducts Processing 90: 224-234.
7. Chandrashebar, S. 2008. Modeling drying kinetics of mustard in fluidized bed. Inter. J. Food Eng. 4(3): 1-14.
8. Corzo, O., Bracho, N., Pereira, A. and Väsquez, A. 2008. Weibull distribution for modelling air drying of coroba slices. LWT- Food Sci. Technol. 41: 2023-2028.
9. Crank, J. 1975. The mathematics of diffusion. 2nd ed. London: Oxford University Press.
10. Doymaz, I. 2004. Convective air drying characteristics of thin layer carrots. J. Food Eng. 61 (3): 359-364.
11. Doymaz, I. 2012. Evaluation of some thin layer drying models of persimmon slices (Diospyros kaki L.). Energy Convers. Manage. 56: 199-205.
12. Falade, K., Olurin, T., Ike, E. and Aworh, O. 2007. Effect of pre-treatment and temperature on air-drying of Dioscorea alata and Dioscorea rotundata slices. J. Food Eng. 80 (4): 1002-1010.
13. Falade, K.O. and Solademi, J.S. 2010. Modelling of air drying of fresh and blanched sweet potato slices. Inter. J. Food Sci. Technol. 45: 278-288.
14. Fioreze, R. and Morini, B. 2000. Yam (Dioscorea sp.) drying with different cuts and temperatures: experimental and simulated results. Cienc. Tecnol. Alimentos 20 (2): 262-266.
15. Gikuru, M. and Olwal, J.O. 2005. The drying kinetics of kale (Brassica oleracea) in a convective hot air dryer. J. Food Eng. 71(4) : 373-378.
16. Girardin, O. 1996. Technologie après récolte de l'igname: étude de l'amélioration du stockage traditionnel en Côte d'Ivoire. Thèse de doctorat en sciences techniques. Ecole polytechnique fédérale de Zurich, Suisse.
17. Goyal, R.K., Mujjeb, O. and Bhargava, V.K. 2008. Mathematical modeling of thin layer drying kinetics of apple in tunnel dryer. Inter. J. Food Eng. 4(8): Article 8.
18. Guiné, R.P.F. and Barroca, M.J. 2012. Effect of drying treatments on texture and color of vegetables (pumpkin and green pepper). Food and Bioproducts Processing 90: 58-63.
19. Henderson, S.M. (1974). Progress in developing the thin layer drying equation. Transaction of the ASAE, 17(6): 1167-1168
20. Henderson, S.M. and pabis, S. 1961. Grain drying theory. II. Temperature effects on drying coefficients. J. Agric. Eng. Res. 6(3): 169-174.
21. Jannot, Y., Talla, A., Nganhou, J. and Puiggali, J-R. 2004. Modeling of banana convective drying by the

drying characteristic curve (DCC) method. Drying Technol. 22 (8): 1949-1968.
22. Kaya, A., Aydin, O. and Demirtas, C. 2009. Experimental and theoretical analysis of drying carrots. Desalination 237: 285-295
23. Kim, S.S., Koh, K. H., Mee, S. and Oh, M. S. 2005. Preparation and quality of dried yam chip snack coated with ascorbic acid cocrystallized sucrose. Food Sci. Biotechnol. 4 (5)/ 661-666.
24. Kingsly, R.P.,Goyal, R.P., Manikantan, M.R. and Ilyas, S.M. 2007. Effect of pretreatments and drying air temperature on drying behaviour of peach slice. Inter. J. Food Sci. Technol. 42: 65-69.
25. Koua, K.B., Fassinou, W.F., Gbaha, P. and Toure, S. 2009. Mathematical modelling of the thin layer solar drying of banana, mango and cassava. Energy 34: 1594-1602.
26. Krokoda, M.K., Karathanos, V.T., Maroulis, Z.B. and Marinos-Kouris, D. 2003. Drying kinetics of some vegetables. J. Food Eng. 59(4): 391-403.
27. Kumar, N., Sarkar, B.C. and Sharma, H.K. 2012. Mathematical modelling of thin layer hot air drying of carrot pomace. J. Food Sci. Technol. 49: 33-41.
28. Liu, X., Qju, Z., Wang, L., Cheng, Y., Qu, H. and Chen, Y. 2009. Mathematical modeling for thin layer vacuum belt drying of panax notoginseng extract. Energy Convers. Manage. 50: 928-932.
29. Mazutti, M.A., Zabot, G., Boni, G., Skovronski, A., Oliveira, D.D., Lucio, M.D., Oliveira, J.V. et al. 2010. Mathematical modeling of thin layer drying of fermented and non-fermented sugarcane bagasse. Biomass and Bioenergy 34: 780-786.
30. McMinn, W.A.M. 2006. Thin layer modeling of the convective, microwave, microwave-convective and microwave-vacuum drying of lactose powder. J. Food Eng. 72: 113-123.
31. Midilli, A. and Kucuk, H. 2003. Mathematical modelling of thin layer drying of pistachio by using solar energy. Energy Convers. Manage. 44(7): 1111-1122.
32. Midilli, A. and Kucuk, H. And Yapar, Z. 2002. A new model for single layer drying. Drying Technol. 20: 1503-1513.
33. Mohapatra, D. and Rao, P.S. 2005. A thin layer drying model of parboiled wheat. J. Food Eng. 66: 513-518.
34. Montes, E. J., Tores, R. G, Andrade, R. D. P., Pérez, O.A.S., Marimon. J. L. E and Meza, I.I.H. 2008. Modelado de la cinética de secado de ñame (Dioscorea rotundata) en capa delgada. Revista Ingeniería E Investigación 28 (2): 45-52.
35. Overhults, D.D., White,G.M., Hamilton, M.E. and Ross, I.J. 1973. Drying soybeans with heated air. Transaction of the ASAE 16: 195-200.
36. Page, G.E. 1946. Factors influencing the máximum rates of air drying shelled corn in thin layers. Department of Mechanical Engineering, Purdue University, Purdue, USA, M.S. Thesis
37. Rafiee, S., Sharifi, M., Keyhani, A., Omid, M., Jafari, A., Mohtasebi, S.S., et al. 2010. Modeling effective moisture diffusivity of orange slices (Thompson Cv.). Inter. J. Food Properties 13:32-40.
38. Rajkumar, P., Kaailappan, R., Viswanathan, R. and Raghavan, G.S.V. 2007. Drying characteristics of foamed alphonso mango pulp in a continuous type foam mat dryer. J. Food Eng. 79: 1452-1459.
39. Ramesh, M.N., Wolf, W., Tevini, D. and Jung, G. 2001. Influence of processing parameters on the drying of spice paprika. J. Food Eng. 49: 63-72.
40. Schössler, K., Jäger, H. and Knorr, D. 2012. Effect of continuous and intermittent ultrasound on drying time and effective diffusivity during convective drying of Apple and red bell pepper. J. Food Eng. 108: 103-110.
41. Silva, E.M., Da Silva, J.S., Pena, R.S. and Rogez, H. 2011. A combined approach to optimize the drying process of flavonoid-rich leaves (Inga edulis) using experimental design and mathematical modelling. Food Bioproducts Processing 89: 39-46.
42. Talla, A., Jannot, Y., Kapseu, C. and Nganhou, J. 2001. Etude expérimentale et modélisation de la cinétique de séchage des fruits tropicaux : Application à la banane et à la mangue. Sci. Aliments 21(5) : 499-518.
43. Thakor, N.J., Sokhansanj, S., Sosulski. F.W. and Yannacopoulos, S. 2005. Mass and dimensional changes of single canola kernels during drying. J. Food Eng. 40: 153-160.
44. Thorat, I.D., Mohapatra, D., Sutar, R.F., Kapdi, S.S. and Jagtap, D.D. 2012. Mathematical modeling and experimental study on thin layer vacuum drying of ginger (Zingiber officinale R.) slices. Food and Bioprocess Technology 5(4): 1379-1383.
45. Togrul, H. 2006. Suitable drying model for infrared drying of carrot. J. Food Eng.77(3): 610-619.
46. Wang, Z., Junhong, S., Chen, F., Xiaojun, L. and Xiaosong, H. 2007. Mathematical modelling on thin layer microwave drying of apple pomace with and without hot air predrying. J. Food Eng. 80: 536-544.
47. Wang, Z., Sun, J., Liao, X., Chen, F., Zhao, G., Wu, J. and Hu, X. 2006. Mathematical modelling on hot air drying of thin layer apple pomace. Food Res. Inter. 40(1): 39-46.
48. Xiao, H.W., Pang, C.L., Wang, L.H., Bai, J.W., Yang, W.X. and Gao, Z.J. 2010. Drying kinetics and quality of monukka seedless grapes dried in an air-impingement jet dryer. Biosystem Eng.105: 233-240.
49. Yilbas, B.S., Hussain, M.N. and Dincer, I. 2003. Heat and moisture diffusion in slab products to convective boundary. Heat and Mass Transfer 39: 471-476.
50. Zielinska, M. and Markowski, M. 2010. Air drying characteristics and moisture diffusivity of carrots. Chemical Engineering Processing: Process Intensification 49: 212-218.
51. Zielinska, M. and Markowski, M. 2007. Drying behavior of carrots dried in a spout-fluidized bed dryer. Drying Technol. 25: 261-270.
52. Zogzas, N.P., Maroulis, Z.B. and Marinos-Kouris, D. 1996. Moisture diffusivity data compilation in foodstuffs. Drying Technol. 14: 2225-2253.

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Functional properties and in vitro protein digestibility of fermented sorghum and broad bean (*Visia faba* L. Major) blended flour

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ABSTRACT

The fermented sorghum flour paste (*ajena*), is one of the most common traditional cereal based food products in Africa, especially in Sudan and Ethiopia. It is used as a main ingredient or starting material for other food products. This traditional fermentation biotechnology has recently caught the attention of food scientists trying to improve various properties of the product. In this study, the functional and protein quality properties of naturally fermented blended flour made up of 20% native broad bean and 80% sorghum flours were compared with conventionally fermented sorghum flour. The blended flour increased in protein composition by about 5% from 11.46 to 16.37 after fermentation. The protein quality in terms of *in vitro* protein digestibility (IVPD) increased from 58.53 % in fermented sorghum flour to 71.93 % in the blended flour. The increased protein, improved ($p \leq 0.05$) the functionality of the blended flour as reflected in its better water-absorption capacity, compared to value obtained from fermented sorghum for this functional property. Bulk density and swelling power of the two flours showed no difference. The Mixolab results indicated lower dough stability, elasticity and consistency in the blended flour as compared to non-blended sorghum flour samples. Thus, *ajena*, with improved nutrition in terms of protein and less elasticity, like those used in cereal based drinks can be processed by blending sorghum and broad bean flours before fermentation.

Keywords: Fermented sorghum flour, blended flour, broad bean, functional properties, protein digestibility.

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench), which belongs to the *Gramineae* family of crops and the *Andropogoneae* tribe, is the world's fourth and Africa's second major cereal crop. It is mostly grown as a subsistence dry land crop by resource limited farmers under traditional management conditions in semiarid tropic (SAT) regions of the Africa, Asia and Latin America, which are frequently drought-prone and characterized by fragile environments (Reddy et al., 2006, 2008). India grows the largest acreage of sorghum in the world followed by Nigeria and Sudan, and produces the second largest tonnage after the United States of America. In most of the regions of India, it is cultivated both as a rainy- and post rainy-season crop (FAO, 1995). The main industries currently using sorghum are the poultry feed, animal feed, alcohol distilleries and human food.

Despite its low in many nutritional values, sorghum in Africa is processed into a very wide variety of attractive and nutritious traditional foods, such as semi-leavened bread, couscous, dumplings and fermented and non-fermented porridges. It is the grain of choice for brewing traditional African beers. Sorghum is also the grain of 21st century Africa (Taylor, 2003). New products such as instant soft porridge and malt extracts are great successes. In the competitive environment of multinational enterprises, sorghum has been proven to be the best alternative to barley for lager beer brewing.

Sorghum plays a big role in lives of the human and livestock in Africa, especially the northern region, around which it is believed to have originated (Mann et al., 1983; Wendorf et al, 1992). Fermented sorghum flour paste product, locally known as *injera* in Ethiopia (Belton and Taylor, 2004) and *ajena* in Sudan, has been one of the major staple foods in these two countries since time in memorial. The *Ajena* is normally used to make different kinds of human food products such as thin layer bread (*kisra)*, *nesha* and *aseda*, just to mention a few.

Lately, fermented sorghum products have caught interest of food scientists in an attempt to develop food products with improved safety, longevity, nutritional and functional properties. In this study therefore, attempt was made to produce an *ajana* with enhanced nutritional and functional properties by blending sorghum and broad bean (*Visia faba*, L. Major) flours as starting material. Broad bean is one of the major sources of traditional protein dish among Sudanese and Egyptian local people (Saxena and Nassib,

1989), but very little, if any, has been reported on its use as an ingredient in other food products, especially fermented sorghum. Broad bean, better known as the faba bean, fava bean, horse bean, field bean, tick bean, or open mouth nut, is probably a native to North Africa and Southwest Asia. Broad bean is rich in protein and fibre, and contains notable levels of vitamin A and C, as well as phosphorous and iron. The lipid content of broad bean is very low (1.5%) compared to soybean (21%) (Whittaker and Tannenbaum, 1977). Broad beans contain L-dopa, which is used as a treatment for Parkinson's disease (Carlsson, 1993). Depending on the cultivar, broad bean may contain up to 35% protein (dry basis), the quality of which is comparable to that of soy proteins, except for the tryptophan, methionine and cystine contents. Additionally, it contains fewer anti-nutritional and flatulence factors than soybean (Zee et al., 1988; Marquardt et al., 1975).

The upgrading of traditional foods through the inclusion of protein seed meals or other protein rich sources in their preparations has been reported (Ekpenyoung et al., 1977; Plahar et al., 1983; Plahar and Leung, 1983). These extenders have to possess the desirable properties to make them compatible in food formulations (Kinsella, 1976). In the earlier studies (Ahmed et al., 1987; Ahmed and Ramanatham, 1987, 1988), the addition of edible groundnut flour to sorghum meal in a co-fermentation process considerably improved the nutritive value of Sudanese staple thin layer bread. The fermentation process was shown to degrade proteins (Ahmed et al., 1987a) and carbohydrates (Ahmed and Ramanatham, 1984) of sorghum meal as well as of the composite flour. It is based on its attributes, couples with its availability in SAT region of Africa, including Sudan, that broad bean was preferred for this study than soybean.

MATERIALS AND METHODS

Procurement of materials

The white sorghum (*Sorghum bicolor,* L. Moench) and broad bean (*Vicia faba* L. Major var. *minuta*) were obtained from a super market and local market respectively, in Wuxi, Jiangsu province, China. All the chemicals used were of analytical grade.

Sample preparation

Well sorted and cleaned sorghum and dehalled broad beans grains were separately ground into flour to pass through an 80 µm mesh size sieve. Then, samples of 100 g native sorghum (NSF) and a mixture of 80 g NSF and 20 g native broad bean flour (NBF) were each mixed with 100 ml of distilled water and incubated at 37 °C for 24 h, and dried at 40° C in an open air oven. The fermented samples were then ground again into flour to pass through an 80 µm mesh sieve, to make fermented sorghum flour (FSF) and fermented sorghum-broad bean blended flour (FSBF). The two flours were separately packed in polythene bags and stored at ambient temperature of around 25±2 °C until further analysis.

Proximate composition

Proximate analysis of four different samples was conducted as follows: Crude protein was determined by micro-Kjeldahl method with the common conversion factor of N x 6.25 (AOAC, 2000). Crude oil was extracted by the traditional Soxhlet extraction apparatus and determined according to AOAC (2000) official method. Total ash was analyzed using the conventional method by dry-ashing in muffle furnace at 550 °C. Moisture content was calculated by drying weighed samples for 3 h in an oven at 120 °C (Smith et al., 1966).

Bulk density

Determination of bulk density was conducted according to the method of Okaka and Potter (1979). A 50 g flour sample was put into a 100 ml measuring cylinder. The cylinder was tapped continuously until a constant volume was obtained. The bulk density was calculated as weight of flour (g) divided by its volume (cm^3).

Water absorption capacity

Water absorption capacity of the flour samples were determined by Beuchat (1977) methods. One gram of the flour was mixed with 10 ml of water in a centrifuge tube and allowed to stand at ambient temperature (30±2.00 °C) for 1 h. It was then centrifuged at 10000 rpm, 20 °C for 20 min. The volume of water captured in the sediment was measured by subtracting the volume of water above the sediments from the original volume added to the flour. Water absorption capacity was calculated as ml of water per gram of flour.

Swelling power

The swelling power was determined by the method described by Leach et al. (1959) with modification for small samples. One gram of the flour sample was mixed with 10 ml distilled water in a centrifuge tube and heated at 80 °C for 30 min, continually shaken during the heating period and allowed to cool. The suspension was then centrifuged at 10000 rpm, 25 °C for 20 min. The supernatant was decanted and the weight of the paste taken. The swelling power was calculated as the weight of the paste divided by the original weight of the dry flour.

Dough Proprety Mixolab

The Mixolab (Chopin, Tripette and Renaud, Villeneuve-la-Garenne, France) was used to study the properties of dough

made from the each of three flour samples (NSF, FSF and FSBF). A flour sample with known weight and moisture content was placed into the Mixolab analyzer bowl and mixed to obtain dough of 75 g. The water required for the dough to produce a torque of 1.1 nm (C1) was added automatically by the Mixolab system. According to manufacturer instructions, the obtained curve is typically divided into five stages as shown in figure 1. In the first stage (C1), dough-mixing characteristics such as stability, elasticity, and water absorption can be determined. During this stage, an increase in the torque is observed until a maximum is reached. Consistency of the dough decreases with amount of mixing, which is an indication of protein weakening (stage 2, C2). As the temperature of the dough increases, as a result of the increase in block temperature, first a decrease and then an increase in consistency is observed and is attributed to starch gelatinization (stage 3, C3). In stage 4, consistency decreases, attributed to amylolytic activity (C4). Finally, in stage 5, the decrease in temperature causes an increase in the consistency, attributed to gel formation. This stage is also related to the retrogradation of starch.

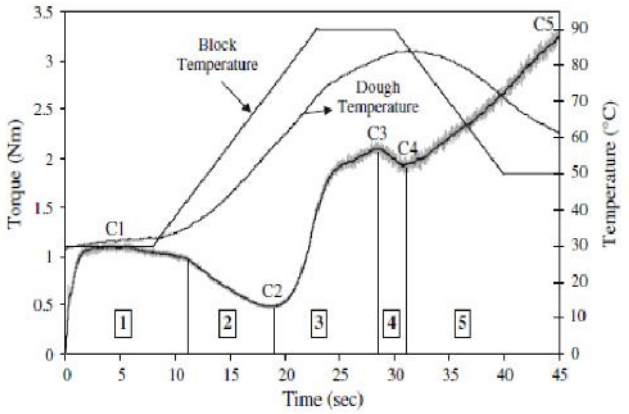

Figure 1: The typical Mixolab^{TM} standard curve

In vitro protein digestibility

In vitro protein digestibility (IVPD) was determined by the method of Saunders et al. (1973) with some modifications. A sample (0.2 g) was placed in a 50 ml centrifuge tube, 15 ml of 0.1 M HCl containing 1.5 mg pepsin were added, and incubated at 37 °C for 2 h. The suspension was then neutralized with 3 ml 0.5 M NaOH and treated with pancreatin solution [4.0 mg in 7.5 ml 0.2 M phosphate buffer pH 8.0 containing Thymol (25 mg per 250 ml phosphate buffer)]. The mixture was then incubated at 37 °C in a gently shaken water bath for 24 h. After incubation, the reaction was stopped with 10 % trichloroacetic acid (10 ml) and centrifuged at 5000 rpm for 20 min at room temperature. Nitrogen in the supernatant was determined by micro Kjeldahl method (AOAC, 2000). Digestibility was calculated using the formula: Protein digestibility (%) = N in supernatant - enzyme N / N in sample.

Statistical analysis

The triplicate sample data were analyzed and the figures averaged. Differences were assessed for significance at $p \leq 0.05$ by analysis of variance (ANOVA) using Duncan's multiple-range test, by SAS analytical software (SAS Institute Inc., Cary, NC, USA), version 8.1.

RESULT AND DISCUSSION

Proximate analysis

The proximate composition of the four different samples (NSF, NBF, FSF and FSBF) in terms of crude protein, ash, moisture and crude oil were determined and the results are presented in table 1. From the results it can be observed that comparatively, all compositions, except moisture were relatively higher in fermented sorghum-broad bean blended flour (FSBF) sample than in the initial fermented sorghum flour (FSF). Crude protein was higher by about 42.84 %, ash (57.89 %) and oil (14.52 %), while moisture was 47.60 % lower in FSBF than in FSF. This is not surprising, considering the similar situation observed when comparing the initial starting material (NSF and NBF). Thus, the variation is attributed to the presence of broad bean flour. This scenario is very likely to be affected by the ratio of sorghum to broad bean flours. Although the moisture and the rest of the compositions are affected by the extent of sample drying, the fact that both flours were dried under the same conditions clears the doubt. The decrease in the moisture composition may have a significant bearing on water absorptions capacity, resulting into more water needed in order to produce dough of a particular thickness and consistency.

Bulking density

The bulk density and the pasting properties of the both flour were studied. The Bulk density is a measure of heaviness of a flour sample. The results showed no significant difference ($p \leq 0.05$) between the fermented blended flour (0.65 ± 0.01 g/ml^3) and the fermented sorghum flour (0.64 ± 0.00 g/ml^3) in this property. The bulk density is very important in preparation of food for special people such as manual workers and infants. Fermentation has been reported as a very useful traditional method for the preparation of low-bulk weaning foods (Desikachar, 1980). The similarity in low-bulk density values between the two samples indicates the possibility of using the new flour product even for the preparation of weaning food, just as it is currently the case with fermented sorghum flour.

Table 1: Proximate composition for FSF and FSBF

	Proxmate composition*			
Sample	**Protein (%)**	**Ash (%)**	**Moisture (%)**	**Oil (%)**
NSF	13.25 ± 0.19	0.58 ± 0.16	12.00 ± 0.24	1.64 ± 0.03
NBF	29.30 ± 0.72	2.88 ± 0.03	11.69 ± 0.10	2.23 ± 0.96
FSF	11.46 ± 1.08	0.57 ± 0.06	12.00 ± 0.22	1.24 ± 0.27
FSBF	16.37 ± 0.67	0.90 ± 0.07	8.13 ± 0.19	1.42 ± 0.12

*Values are means of triplicate tests.

Table 2: Mixolab results for FSF and FSBF

Mixolab stages	**Time (min)**		**Torque (nm)**		**Dough temp. (°C)**	
	FSF	FSBF	FSF	FSBF	FSF	FSBF
C1	1.63	22.02	0.27	0.42	29.00	66.70
C2	15.10	23.18	0.04	0.21	45.10	71.50
C3	17.77	29.72	0.74	3.48	51.50	83.30
C4	21.20	31.73	0.32	3.37	64.80	84.70
C5	45.05	45.03	4.26	4.43	61.90	62.00
C4 to C5 torque increase (%)			1231.25	31.45		

*Values are means of triplicate tests.

Water absorption capacity

The addition of broad bean flour significantly increased ($p \leq 0.05$) the water absorption capacity from 1.07 ± 0.02 g/g in FSF to 1.21 ± 0.05 g/g in FSBF, representing 13.08 % increase. These findings are similar to those reports by Ahmed and Ramanatham (1988), where an increase was also observed in fermented sorghum and groundnut composite meal. The water absorption capacity is very important in determining the amount of water needed for a specific extent of gelatinization and dough thickness. Higher absorption capacity is desirable for making thicker gruels. Generally, sorghum flour has a higher water binding capacity than flours of higher protein materials such as raw fluted pumpkin seed (Giami and Bekebain, 1992).

Swelling power

The swelling power correlates with gruel solid content. The results for both fermented flours were compared and there was no significant difference between them (FSF = 4.37 ± 0.69; FSBF = 4.91 ± 0.16). The sight increase in swelling power in FSBF indicates a slightly higher content of amorphous material than in FSF. It is noted that the water absorption capacity is directly proportional to swelling power, when the water absorption capacity increases, the swelling power also increases.

Dough property by Mixolab

Mixolab determines a comprehensive qualitative profile of the flour and plots, in real time, the torque (expressed in nm) produced by the passage of the dough between two kneading arms when submitted to both shear stress and a temperature constraint (Dhaka et al., 2012). In this study the results given by the Mixolab, as pasting properties of the flour samples, are shown in tables 1. At almost the same moisture content of 26.16±22 for FSF and 25.23±19 for FSBF, stability and viscosity of the two doughs differed widely. Stability dropped from 1.33 min. in FSF to 0.63 min. in FSBF, while viscosity declined from 6.00 to 1.00, respectively, where as amylase showed the same value (9.00) in both fermented flour samples. In normal situation (standard curve) at stage C5, the decrease in dough temperature, results in the increase in torque (figure 1), signifying increase in dough consistency due to gel formation. From table 1, it can be observed that from C4 to C5 the FSF showed great amount of increase in torque (by 1231.25%), indicating much higher dough consistency compared to the 31.45% observed in FSBF.

The differences in dough stability, viscosity and consistency observed between the two fermented flour samples, may be attributed to the relatively reduced starch composition in the FSBF, which is responsible for gelatinization when it retrogrades. The similarity in the amylase values indicates that amylolytic activity may not be the major reason for the differences.

Mixolab results

In vitro protein digestibility

In vitro protein digestibility (IVPD) has been reported to closely relate to true digestibility, and is normally used as a more quick and convenient alternative. In this study, after natural fermentation of the two samples, IVPD increased from 58.53 ± 0.33 % in sorghum flour to 71.93 ± 2.49 % in the blended flour. These results are considerably higher compared to those reported by Fadlallah et al. (2010) in the similar study, where they supplemented the sorghum flour with chickpea flour. The differences in IVPD in fermentation studies between low and high tannin sorghum cultivars have been reported (Romo-Parada et al. 1985; Cummins, 1971). Thus, the difference with those reported, may be attributed to the difference in the cultivars and legume used. The increased IVPD indicates that the protein in the FSBF may be more available for the body's nourishment than that of FSF.

Conclusion

An *Ajena* with improved protein composition and digestibility can be prepared by blending the native sorghum and broad bean flours before the fermentation process. Some functional properties associated with this kind of *ajena* product do not differ much with the conventional one. Those that differ have other applications in different the preparation of other food products. With regard to the nutritional problems, which is pandemic to most of the developing countries, such as those in many African and Asian regions, it is imperative for food scientist to actively engage in studying even the seemingly basic traditional foods, which form integral part of the local people's diet, regardless of whether yields industrial economic benefit or not, in order to improve the nutritional status of the people. Use of supplementation of sorghum flour with broad bean may be a more convenient and cheaper option in countries like Sudan, Egypt and Ethiopia, and other Asian countries, where it is readily available and is already an integral part of their local population's diet.

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REFERENCES

1. Ahmed, A.R. and Ramanatham G. 1987. Essential amino acid composition and m vitro digestibility of

proteins of sorghum bread "Kisra" enriched with edible defatted groundnut flour. Nutr. Rep. Int. 35(3): 487.

2. Ahmed, A.R. and G. Ramanatham. 1988. Effect of Natural Fermentation on the Functional Properties of Protein-Enriched Composite Flour. J. Food Sci. 53(1): 218-221.
3. Ahmed, A.R. and G. Ramanatham. 1984. Oilseeds Technology Discipline. Unpublished data. CFTRI, Mysore-13, India.
4. Ahmed, A.R., A.G. Appu Rao and G. Ramanatham. 1987. Effect of autofermentation on the hysicochemical properties of proteins of sorghumgroundnut composite flour. J. Agric. Food Chem. 36(4): 90–694.
5. Ahmed, A.R., H.N. Chandasekhara and G. Ramanatham. 1987. The protein quality of sorghum bread "Kisra" enriched with edible defatted groundnut flour. Nutr. Rep. Int. 35(l): 205.
6. AOAC. 2000. Official method of analysis, 17th ed.) Association of Official Analytical Chemists, Inc. Maryland, USA.
7. Belton, P.S. and J.R.N. Taylor. 2004. Sorghum and millet: protein sources for Africa. Trends Food Sci. Tech. 15(2): 94-98.
8. Beuchat, L.R. 1977. Functional and electrophoretic characteristics of succiylated peanut flour proteins. J. Agric. Food Chem. 25: 258-262.
9. Carlsson, A. 1993. Thirty years of dopamine research. Adv. Neurol. 60: 1-10.
10. Cummins, D.G. 1971. Relationships between tannin content and forage digestibility in sorghum. Agron. J. 63: 501-502.
11. Desikachar, H.S.R. 1980. Development of weaning foods with high caloric density and low paste viscosity using traditional technologies. Food Nutr. Bull. 4: 57–59.
12. Dhaka, V., N. Gulia and B.S. Khatkar. 2012. Application of Mixolab to Assess the Bread Making Quality of Wheat Varieties. 1: 183. doi:10.4172/scientificreports.183.
13. Ekpenyoung, T.E., B.L. Fetuga and V.A. Oyenuga. 1977. Fortification of maize flour based diets with blends of cashewnut meal, African locust bean meal and sesame oil meal. J. Sci. Food Agric. 28: 710.
14. Fadlallah, O.E., A.H. El Tinay and E.E. Babiker. 2010. Biochemical characteristics of sorghum flour fermented and/or supplemented with chickpea flour. Int. J. Biol. Life Sci. 6: 21-25.
15. FAO.1995.Sorghum and Millets in Human Nutrition. FAO Food and Nutrition Series; No. 27, ISBN 92-5-103381-1.
16. Giami, S.Y. and D.A. Bekebain. 1992. Proximate composition and functional properties of raw and processed fullfat fluted pumpkin seed (*Telfairia occidentalis*) flour. J. Sci. Food Agric. 59: 321-325.
17. Kinsella, J.E. 1976. Functional properties of proteins in foods. Crit. Rev. Food Sci. Nutr. 7: 219-280.
18. Leach, H.W., L.D. McCowen and T.J. Schoch. 1959. Swelling power and solubility of granular starches. Cereal Chem. 36: 534-544.
19. Mann, J.A., C.T. Kimber and F.R. Miller. 1983. The origin and early cultivation of sorghums in Africa. Tex. Agric. Exp. Stn. Bull. 1454.
20. Marquardt, R.R., J.A. McKirdy, T. Ward, L.D. Campbell. 1975. Amino acid, hemagglutinin and trypsin inhibitor levels and proximate analyses of faba beans *(Vicia faba* L.) and faba bean fractions. Can. J. Anim. Sci. 55: 421-429.
21. Okaka, J.C. and N.N. Potter. 1979. Physicochemical and Functional properties of cowpea powders processed to reduce beany flavours. J. Food Sci. 44: 1235–1240.
22. Plahar, W.A. and Leung, H.K. 1983. Composition of Ghanaian fermented maize meal and the effect of soy fortification on sensory properties. J. Sci. Food Agric. 34: 407-411.
23. Plahar, W.A., Leung, H.K., and C.N. Coon, 1983. Effects of dehydration and soy fortification on physicochemical, nutritional and sensory properties of Ghanaian fermented maize meal. J. Food Sci. 48: 1255-1259.
24. Reddy, B.V.S., S. Ramesh, K.A. Ashok and C.L.L. Gowda (eds.). 2008. Sorghum improvement in the new millennium. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Andhra Pradesh.
25. Reddy, B.V.S., S. Ramesh, P.S. Reddy. 2006. Sorghum genetic resources, cytogenesis, and improvement. Taylor and Francis Group, LLC, New York.
26. Romo-Parade, M.L., R.E. Simard and S.S. Larrea-Renoso. 1985. Infuence of germination, hixtamalization and fermentation on nutritional value of sorghum protein. Micro. Almer Nutr. 3: 125-132.
27. Saunders, R.M., M.A. Connor, A.N. Booth, E.N. Bickoff and C.O. Kohler. 1973. Measurement of digestibility of alfalfa protein concentration by in vitro and in vivo methods. J Nutr. 103: 530–535.
28. Saxena, M.C. and A.M. Nassib. 1989. Relevance of the ICARDA [International Center for Agricultural Research in Dry Areas] Nile valley project of faba beans for rice-based cropping systems in Egypt. International Symposium on Rice Farming Systems, Sakha Egypt, 31 Jan - 3 Feb 1987; IRRI, Los Banos, Laguna, Philippines.
29. Smith, A.K., J.J. Rackis, P. Isnardi, J.L. Cartter and O.A. Krober. 1966. Nitrogen solubility index, isolated protein yield, and whey nitrogen content of several soybean strains. Cereal Chem. 43:261-270.

30. Taylor, J.R.N. 2003. Overview: Importance of sorghum in Africa. Paper 1 in: AFRIPRO, workshop on the protein of sorghum and Millet: Enhancing nutritional and functional properties for Africa. P.S. Belton and J.R.N. Taylor, eds. Online: http://www.afripro.org.uk/papers/Paper01Taylor.pdf. Accessed February 15 2013.

31. Wendorf, F., A.E. Close, R. Schild, K. Wasylikowa, R.A. Housley, R.A. Harlan and H. Krolik. 1992. Saharan exploitation of plants 8000 years B.P. Nature. 356:721-724.

32. Whittaker, J.R. and S.R. Tannenbaum. 1977. Food proteins. AVI Publishing Company, Inc., Westport.

33. Zee, J.A., A. Boudreau, M. Bourgeois and R. Breton. 1988. Chemical composition and nutritional quality of faba bean (*Vicia faba L. Minor*) based tofu. J. Food Sci. 53(6): 1772-1774.

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Microencapsulation by complex coacervation of fish oil using gelatin/SDS/NaCMC

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ABSTRACT

Microencapsulation of fish oil was investigated using gelatin/SDS/NaCMC as the coating materials. The microencapsulation efficiency (MEE) of microencapsulated fish oil (MFO) was investigated with respect to three variables including the Concentration of Wall Material (CWM), Ratio of Core and Wall Material (RCW) and the pH. The response surface model for MEE showed a high coefficient of determination and a non-significant lack of fit ($p < 0.05$). The optimum MEEs were 75.2 ± 0.73% during spray drying and 53.2 ± 0.39% during freeze drying. Compared to MFO, the peroxide value (POV) of the total oil from the MFO under the optimized conditions was significantly lower at 60 ± 1 °C after 30 days, which indicates a promising feature as functional food ingredients.

Key words: Complex coacervation, Response surface methodology; Microencapsulation; fish oil; Lipid oxidation

Introduction

The current intake of omega-3 fatty acids in a typical western diet is lower than the recommended level and the intake of polyunsaturated fatty acids (PUFAs) consists primarily of omega-6 fatty acids (Gebauer et al., 2006, Simopoulos, 2008). The low intake of omega-3 fatty acids and increasing scientific evidence of the beneficial effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has led to introduction of omega-3 fatty acids enriched foods in the market (Jacobsen, 2010). Currently, functional foods containing omega-3 lipids is one of the fastest growing food product categories with around 1300 omega-3 fatty acids enriched products launched in 2007 in the USA and Europe.

Complex coacervation is a term to describe the spontaneous liquid–liquid phase separation that can occur when oppositely charged polyelectrolytes are mixed in aqueous media. Not all polyelectrolytes exhibit this phenomenon, which depends on several conditions such as pH, charge density on the polymers, colloid concentration, ionic strength of the medium, temperature, etc (Thimma and Tammishetti, 2003); Microencapsulation by coacervation has been defined by Gouin as the phase separation of one or many hydrocolloids from the initial solution and the subsequent deposition of the newly-formed coacervate phase around the active ingredient suspended or emulsified in the same reaction media (Gouin, 2004). The microencapsulation technology has been used to encapsulate flavor oil (de Kruif et al., 2004, Weinbreck et al., 2004, Leclercq et al., 2009), vitamins (Junyaprasert et al., 2001), casein hydrolysate (Mendanha et al., 2009), and drugs (Huang et al., 2007) to retard or avoid the oxidation of these oils has been reported and it has drawn considerable attention in the food industry (Partanen et al., 2002, Velasco et al., 2006, Sanguansri, 2007, Ahn et al., 2008a, Klaypradit and Huang, 2008)

The efficiency of the protection or controlled release mainly depends on the composition and structure of the established wall. This wall could act as a barrier and it may protect against oxygen, water, light or could avoid contact with other ingredients or control diffusion. Walls material for microencapsulation of oil by spray-drying must have emulsifying properties, high water solubility,

low viscosity, and drying properties (Young et al., 1993, Bae and Lee, 2008).

In this paper, we have studied the stability of fish oil with gelatin/SDS/NaCMC during 4 weeks. Fatty acid composition has been evaluated. Furthermore, oxidative stability (OSI) has been studied during the microencapsulation process in order to evaluate the fish oil attitude to the spray-drying and freeze drying processing. Different aspects of microencapsulation process such as wall material composition, ratio of core–wall material (RCW) and microencapsulation efficiency (EE) were evaluated, and a suitable system for microencapsulation was achieved.

Materials and methods

Materials

A type gelatin, sodium carboxymethylcellulose (NaCMC), sodium phosphate was purchased from Sinopharm Chemical Reagent, Shanghai Chemical Reagent Corporation (Shanghai, China). Commercial microbial transglutaminase (TG) was obtained from Yiming Biological Products Company (Taixing, China), with a nominal enzymatic activity of 60 U/g, measured by hydroxamate method. Fish oil was granted from Wuxi Xunda Chemicals Company. Sodium hydroxide and acetic acid were purchased from Sinopharm Chemical Reagent, Shanghai Chemical Reagent Corporation (Shanghai, China).

Microencapsulation process

Designs of single factor experiments

Single factor experiments were performed to determine optimal conditions for single factors by analyzing their impact to microencapsulation effect, which were concentration of wall materials (CWM) (gelatin/NaCMC, ratio 9:1), ratio of core material to wall (RCW) and pH value. We have used Design Expert 8.0 for studying CWM effects on encapsulation. Wall materials were prepared into 0.5% (w/w), 1.0% (w/w), 1.5% (w/w), 2.0% (w/w) and 2.5% (w/w) solutions. The pH range was around 4.4 to 4.8.

Preparation of emulsion

Fish oil (3 g) was mixed into 150ml of gelatin solution at 50C using a three-bladed Teflon overhead stirrer. It was allowed to stir for 30min for stabilization of oil droplets; 150ml of NaCMC solution (also at 50℃) was then added to the above solution in the ratio 9:1. The mixture was stirred at 12000 rpm for 3 min using a homogenizer (FJ 200-S, Shanghai Specimen Model factory, China). Fish oil was added to form an emulsion and the homogenization was maintained at this rate for 3 min. This level of mixing was found to be sufficient, confirming the findings of Chen (Chen and Tao, 2005) as the emulsion was stable even after a month (data not shown). Using 10 % (w/w) aqueous acetic acid, the pH of the emulsion was adjusted to 4.4–4.8 at the stirring speed of 400 rpm by overhead stirrer (RW 20 digital n, IKA, Germany). After 15 min, the system was then slowly allowed to cool to room temperature. The rate of cooling was found to be crucial for successful encapsulation and should not be more than 1℃ per min. Once the system attained room temperature, it was cooled, using an external ice bath, to 10℃. The nascent capsules were then cross-linked by adjusting the pH to 6.0 while transglutaminase (15U/g gelatin) was added to harden the microcapsule walls under agitation during 12 hours.

Emulsion Morphology

The coacervate-rich lower layer was visualized as a function of homogenization rates and total biopolymer concentration. The morphology of moist microcapsules suspended in the water was observed under a light microscope (OLYMPUS BX 51) under an objective magnification of 20x equipped with a camera.

Spray-drying

The emulsions prepared after 12 h of hardening, the microcapsule suspension was fed to a spray drier equipped with a centrifugal wheel atomizer (QZ-5, Wuxi Linzhou Drying Equipment Co. Ltd., China) to get dried coacervate microcapsules (Wampler, 1998), operating at an inlet and outlet air temperature of 190 ± 3℃ and 90 ± 3 ℃, respectively, at an evaporative capacity of 6 kg/h.

Freeze drying

The freeze-dried microcapsules were produced according to the protocol described by Parris (Parris, 2005), with modifications, using a lyophilizer (LGJ-10 Freeze Dryer, Beijing, China). The freeze-dried microcapsules were recovered and stored at -18 ℃ for further analysis.

Water activity

The water activity of each sample (500 mg) was measured by an FA-st lab system (GBX Water Activity Meter, Romans, France). Each analysis was repeated three times.

Moisture content

The moisture content of each sample (500 mg) was determined by loss of weight in an oven (DHG – 9076A, Shanghai, China) at 105℃, and figured out by the

formula as follows (Mendanha et al., 2009); each analysis was repeated three times.

$$C\ (\%) = \frac{W1 - W2}{W2} \times 100 \quad (1)$$

Where C is the water content rate, w_1 is the weight of products before dry treatment, and w_2 is the weight after that operation.

Size distribution of microcapsules

The analysis of powder particle size was performed using the laser light scattering method by an analyzer with a batch cell unit (Mastersizer, Malvern Instruments, UK). Encapsulated powders were dispersed in propan-2-ol for the particle size analysis and then assayed for size distribution by Mastersizer 2000 laser particle analyzer. The average particle diameter formula is as follows:

$$Dn = \frac{\sum_{i=1}^{n} ni \times di}{\sum_{n=1}^{n} ni} \quad (2)$$

Where D_n represents average diameter, n_i refers to the number of microcapsules and d_i is diameter of single microcapsule.

Particle yield

The particle yield for each experimental assay was calculated using Eq. (2) as reported by Zhong (Zhong et al., 2009a):

$$Yield = \frac{Mass\ of\ collected\ product}{Non - solvent\ mass\ in\ the\ feed} \times 100 \quad (3)$$

Microencapsulation efficiency (MEE)

The MEE is defined as the ratio of core material in the final dried microcapsules to that in the original emulsion (Zilberboim et al., 1986) and was calculated according to Eq. (3), as provided elsewhere (Pauletti and Amestoy, 1999, Velasco et al., 2006, Ahn et al., 2008a, Ahn et al., 2008b).

$$MEE(\%) = \frac{(Total\ oil - Surface\ oil)}{Total\ oil} \times 100 \quad (4)$$

Total oil

The method described by Wanasundara (Wanasundara and Shahidi, 1995) was used to determine the ME of MMO and MSO. The total oil (TO) (%) of microencapsulated fish oils contained both encapsulated and surface oils.

Surface oil

The surface oil, also known as the non-encapsulated oil fraction, was determined according to Velasco (Velasco et al., 2006) with modifications.

Flowing properties

The bulk (ρB) and tapped (ρT) densities were determined in a 25 ml glass graduated cylinder as described by Chinta (Chinta et al., 2009**).** The powder flowability was evaluated using the Carr's Index or "percent compressibility" (C) and the Hausner Ratio (HR) (Turchiuli et al., 2005). The Carr's Index and the Hausner Ratio were calculated using the following equations, respectively:

$$C = \frac{\rho t - \rho b}{\rho t} \times 100 \quad (5) \qquad HR = \frac{\rho t}{\rho b} \quad (6)$$

Peroxide Value, Anisidine Value, Total Oxidation, Free Fatty Acids, Color and Moisture of microcapsules

The peroxide value (PV), anisidine value (AV), and free fatty acids (FFA) were determined following American Oil Chemists Society (AOCS) Official Methods (A.O.C.S, 1997b). The PV of oils was calculated as indicated in Eq. (7) in which V_S is the volume (mL) of $Na_2S_2O_3$ used to titrate the sample, V_B is the volume (mL) of $Na_2S_2O_3$ used to titrate the blank, M is the molar concentration of the $Na_2S_2O_3$ solution (N), and W is the weight of the sample in grams.

$$PV = \frac{(Vs - Vb) * M}{W} \times 1000 \quad (7)$$

AV is given by Eq. (8) in which Ab is the absorbance of the solution at 350 nm, as is the absorbance of the sample, m is the weight of the oil sample:

$$AV = \frac{25 * (1.2As - Ab)}{m} \quad (8)$$

TOTOX values were calculated as described by Wai (Wai et al., 2009).

$$TOTOX = 2PV + AV \quad (9)$$

Free fatty acids were measured in PFO, and MFO as described by AOCS method Ca 5a-40 (A.O.C.S, 1997a), with slight modifications. The percentage FFA expressed as oleic acid was calculated as follows:

$$\text{FFA (\%)} = \frac{NaOH\ (mL) * N * 28.2}{Mass\ (g)} \quad (10)$$

N stands for the normality of the NaOH and mass (g) refers to the mass of sample used.

Hygroscopicity

For hygroscopicity determination, we use the method described by Cai with little modifications. (Cai and Corke, 2000).

Scanning electron microscopy (SEM)

The SEM analysis of the microspheres was carried out by using S-4800 scanning electronic microscope (Hitachi, Japan). The samples were sprinkled on one side of double-side adhesive stuck on the stub and then was coated with gold. The microspheres were observed at an accelerating voltage of 10 kV.

Thermal behavior

The thermal behavior was determined by differential scanning calorimetry (DSC) according to Mendanha (Mendanha et al., 2009), using a DSC PerkinElmer device controlled by Pyris 1 DSC (PerkinElmer, Wellesley, MA, USA). The dried samples were added into the DSC sample box with the blank as reference (pans are sealed), and heated from 25 ℃ to 110 ℃ at the rate of 5 ℃/min.

Infrared atlas analysis of microcapsules and wall materials

Bromide potassium was grinded with gelatin, NaCMC, sodium polyphosphates and dried products, respectively, to obtain different powders. The plates were scanned by infrared instruments (FT-IR SPECTROMETER, NICOLET NEXUS).

The storage stability of microcapsules

The prepared microcapsules were initially put in a desiccator for 48 h and then sealed in hyaline plastic bags and kept in different thermal conditions. The content of fish oil in microcapsules was tested each week. The retention percentage, which was defined as the ratio between the content of fish oil that retained in the microcapsule after some time and the original rate of fish oil in the microencapsulate, was used to evaluate the storage stability of fish oil microcapsules.

Statistical Analysis

The entire experiment was replicated three times and means and standard deviations were reported. Statistical Package for the Social Sciences (SPSS Version 19,IBM) software was used to conduct analyses of variance to determine differences among treatment mean.

Results and discussion

Process optimization

The experiments were arranged according to Box-Benhnken's central composite design. The single factor experiments were as follows: CWM 0.5 - 1.5%, RCW 1:2 - 2:1 and pH value 4.4-4.8. On the basis of these results, taking encapsulation efficiency (EE) as response value of CWM (A), RCW (B) and pH value (C).

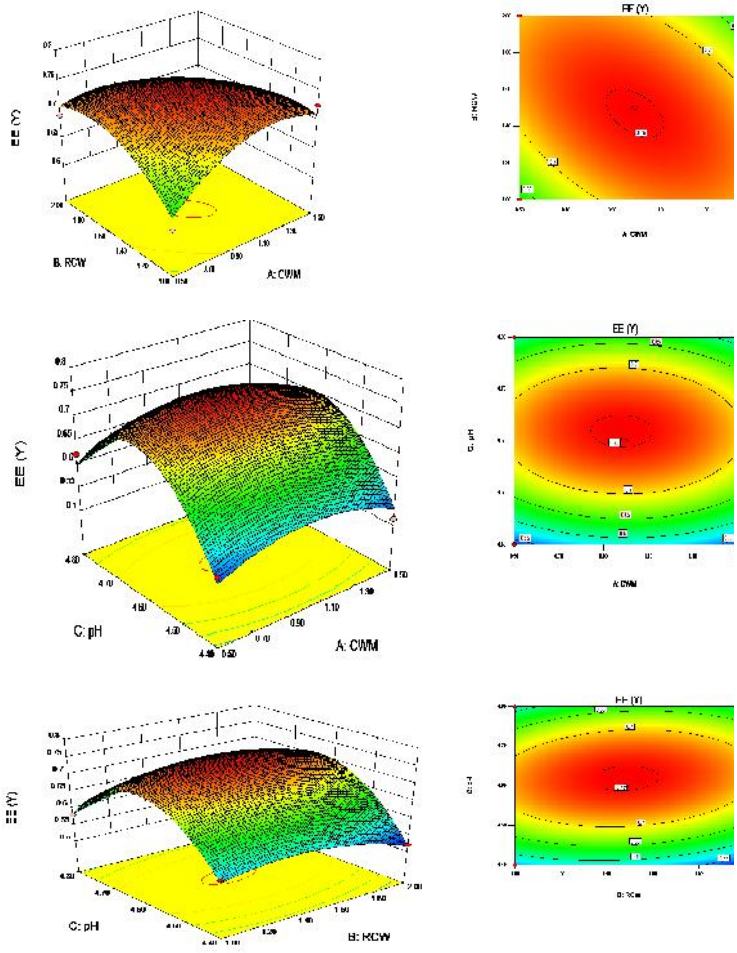


Figure 1: The contour plots of response surface methodology. (a) The response surface and contour plot of the effect of the wall material concentration and wall core/material material ratio; (b) the response surface and contour plot of the effect of the wall material concentration and the pH value; (c) the response surface and contour plot of the effect of the wall core/material material ratio and the pH value.

The experimental results were analyzed by Design Expert 8.0, the regression equation is:

EE (%) = 0.75 -3.125E-003A - 5.000E-003B +0.028C - 0.041AB - 4.750E-003AC +0.017BC - $0.045A^2$ - $0.041B^2$ - $0.14C^2$

Influence of surfactant

In order to improve the encapsulation efficiency and yield of the process, we studied the effect of adding an oppositely charged surfactant in GE solution at pH 4.6 at 45°C. The figure 2.1 shows the amount of NaCMC adsorbed on oil/water interface increases with the addition of SDS. Further increase of SDS concentration from 1.3 to 2.5mM leads to the decrease of the yield. At 2.5mM, the yield decreases dramatically. The low yield of the microcapsules could be attributed to the weak interaction between gelatin and NaCMC. It is reported that adding small molecular surfactant into the gelatin and NaCMC system could improve the adsorption of NaCMC on the oil/water interface.(Sovilj and Petrović, 2006, Li et al., 2009, Dai et al., 2010).

There is a maximum yield at 1.3mM, after which the yield decreases rapidly. This is in agreement with results using sodium dodecylbenzyl sulfonate. and with dioctyl sulfosuccinate sodium (Dai et al., 2010).

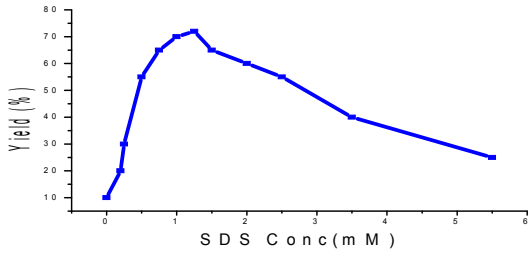


Figure 2.1 Effect of concentration of SDS on microencapsulation yield

In order to understand the interactions between gelatin and SDS, we studied the surface tension of the system using a pendant drop apparatus (Dataphysics). According to the results, as shown in Fig 2.2, Gelatin concentration was maintained at 1% by weight, and the SDS concentration was varied from 0.01 to 100mM. The pH was maintained at 4.6 and the temperature at 45°C. These results are qualitatively similar to the results of Knox et al. (Knox Jr and Parshall, 1970, Mayya et al., 2003). On addition of gelatin, there is a lowering of surface tension to 58 for SDS concentrations, as low as 0.01mM. This indicates the complex formation between SDS and GE at the surface which has a strong surface activity. The surface tension decreases with increasing SDS up to about 0.2mM, after which, it remains constant. This can be described as the critical aggregation concentration (CAC). Above this concentration, complexes are formed mainly in the bulk and are found to be insoluble in water, though, they are formed of SDS and GE, which are soluble. (Asnacios et al., 1998)

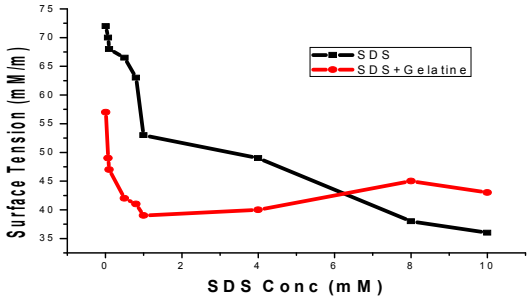


Figure 2.2 Surface Tension of Gelatin-SDS System

Particle size distribution of microcapsules

According to the result got from Mastersizer®, it is shown that the microcapsules are spherical in shape and optical transparent. It is found that under the same stirring speed, the size distribution of the microcapsules becomes narrow and the average diameter descends with increasing the concentration of SDS. The broad distribution in the size range had the biggest distributional proportion in the range of 10-20 mm, indicating homogeneous microcapsules produced under the optimum condition. Their mean diameter was 8.18 mm.

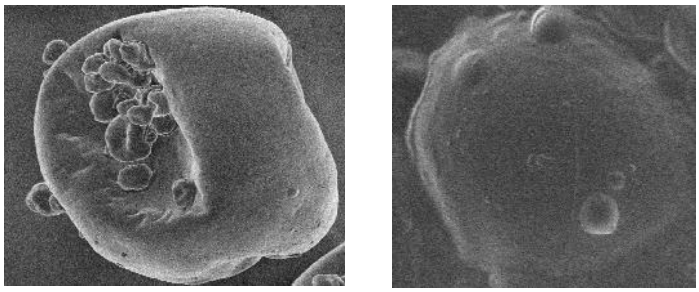
Zeta Potential

Complex coacervation is a pH-sensitive process, because the charge and charge density of polymers vary with pH. The effects of pH on production of the NC were investigated. NC yields were highest at pH = 4.6 and then dropped with the increasing pH. The pH of maximum coacervate yield is believed to correspond to the electrical equivalence pH (EPP), where both polymers carry equal but opposite charges (Papadopoulou-Mourkidou, 1993). At the EPP, attracting forces between the charged components neutralize each other, leading to strong binding and the highest coacervation yield.

Scanning electron microscopy

SEM images of the fish oil microcapsules after spray and freeze drying are shown in Fig. 3. The surface morphology of microcapsules showed that the particles are irregularly shaped with an internal porous network

Fig. 3. This observation is similar to that presented by Zhong (Zhong et al., 2009b).



a) Spray drying 20 µm b) freeze drying 50µm

Figure 3: Scanning electron microscopic images

Microcapsules prepared by freeze drying resulted in small spheres. Furthermore, the particles appear to be agglomerated microcapsules. This behavior was also observed by Parris (Parris, 2005). Tan, (Tan et al., 2009)observed that microspheres with excessive amounts of surface oil promoted significant agglomeration with reduced microencapsulation efficiency. It is reported that NaCMC/SDS complexes formed due to hydrophobic interaction between NaCMC and SDS help to increase the charge density and amount of polysaccharide in coacervate droplets (Li et al., 2009). Moreover, SDS can interact with positively charged gelatin to form complexes with stronger surface activity. Consequently, charge density of protein and polysaccharide increased together, which is propitious to rearrange the soluble complexes into the capsule wall. (Mayya et al., 2003)

MEE and Encapsulation Yield (EY)

The results of Total oil (TO) and surface oil (SO) are listed in Table 1. These results are comparable to Drusch (Drusch and Berg, 2008) and J.D Estrada (Estrada et al., 2011) results which indicated that depending on the extraction method used, oil load and spray drying conditions, the SO of fish oil microcapsules ranges from 0.99 ± 0.03 to 13.5 %.

Flowing properties

The flowing properties for fish oil microcapsules are listed in Table 1. According to the Turchiuli's classification (Turchiuli et al., 2005) for powder flowability, microcapsules prepared by spray- and freeze-drying had poor or very poor handling properties. The microcapsules prepared in this study were very compressible compared to other oil microcapsules prepared by spray drying as reported in the literature (Onwulata et al., 1996, Turchiuli et al., 2005, Fuchs et al., 2006). The higher Hausner ratio means that the powder is more cohesive and less able to flow freely. The same result was observed for microcapsules produced by spray or freeze drying, despite the decrease of the Hausner ratio and Carr index (Fuchs et al., 2006). Fuchs and Turchiuli (Fuchs et al., 2006) reported similar results of poor flowability for oil microencapsulated. The bulk densities found in this study were typical of encapsulated powders (Onwulata et al., 1996).

Table1: Physicochemical characteristics of microencapsulated fish oil.

Item	MFO
PV, mEq/Kg of oil	2.98 ± 0.12
AV	3.92 ± 0.04
TOTOX	9.88 ± 0.06
FFA, %	0.84 ± 0.03%
Moisture, %	4.8 ± 1.6
Water activity	0.13 ± 0.01
Hygroscopicity, %	30 ± 0.6
Carr's Index, %	1.44 ± 0.2
Hausner Ratio	30.02 ± 1.6
TO, %	10.86 ± 0.33%
SO, %	2.69 ± 0.89%
MEE, %	75.2 ± 0.73%
EY, %	72.68 ± 0.51%
Color L*	73.07 ± 0.02
Color a*	6.35 ± 0.02
Color b*	8.76 ± 0.01

The EY and MEE calculated for our study were 72.68 ± 0.51% and 75.2 ± 0.73% (Table 1). Weigang Li (Li et al., 2009) showed the yield of the complex coacervation as a function of the SDS concentration. It was found that the yield of the system without SDS is very low and the addition of SDS enhances the yield remarkably.

Peroxide Value, Anisidine Value, Total Oxidation, Free Fatty Acids and Color of microcapsules

All those values are listed in Table 1 and in accordance with the prescriptions. According to Gracey (Gracey, 1999), oil with a PV below 5 meq/ kg can be considered fresh oil or one in which hydroperoxides have degraded into secondary oxidation products. The Council for responsible nutrition set a fish oil quality standard of an AV less than or equal to 20. The FFA content of MFO may be influenced by hydrolysis promoted by higher moisture content (Table 1) in MFO or in the encapsulated PFO. FFA content greater than 3% is considered inedible (Gracey, 1999). Yin and Sathivel (Yin and Sathivel, 2010) concluded that an increase in FFA content during storage

may be influenced by hydrolysis promoted by the initial moisture content of the oil. We compared our results to a previous one done by Estrada (Estrada et al., 2011) on microencapsulated salmon oil (MSO) and found that MSO had a lighter color than MFO. Results for a* indicated that MFO had a redder color compared to MSO; MSO had significantly higher b* values (more yellowness) which may be attributed to the presence of FO around the microcapsules.

Differential scanning calorimetry

DSC was used for studying thermal transitions occurring in the course of heating under an inert atmosphere (figure 4). According to the integral procedural decomposition temperature (IPDT) values calculated based on the TGA Thermograms by Zohuriaan (Zohuriaan and Shokrolahi, 2004).

The gradual weight loss, not more than 20%, of microcapsules is found below the degradation temperature, due to desorption of small molecules such as H_2O as well as the release of core material. The transitions associated with loss of water (2–10 wt %) correspond to the hydrophilic nature of functional groups of the respective polymer.

Furthermore, the particle size of the samples affects this transition to some extent. On the other hand, no glass transition temperature (T_g) was recorded. The reason may be attributed to interference of the T_g transition by the moisture endothermic peak. The glass transitions may also lie at temperatures lower than the starting temperature of the DSC analysis, i.e. 27 °C.(Zohuriaan and Shokrolahi, 2004).

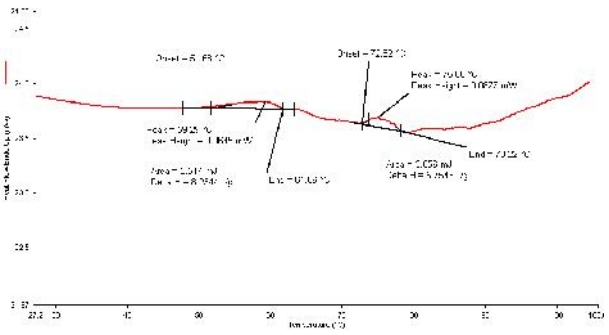
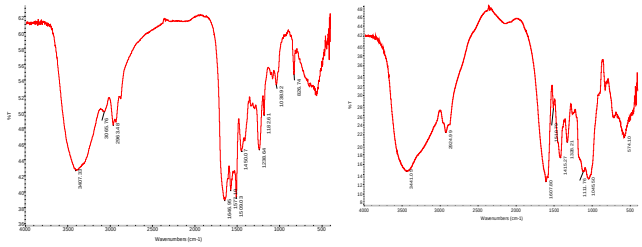


Figure 4: Differential Scanning electron

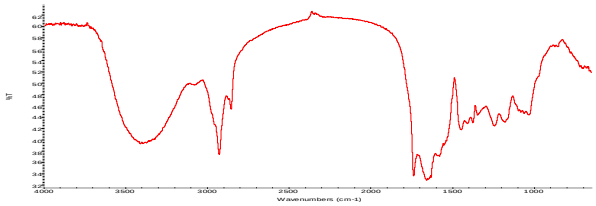
Infrared Spectrum analysis of gums and microcapsules

The infrared spectrums of gelatin, sodium CMC and fish oil loaded microcapsule were shown in Figure 5 below**.** The peak values of particles from complex coacervation approximated those peak values of gelatin existed in products as the peak values are approximate at the wave numbers of 3407.33, 3065.76, 2963.48, 1646.95; also, NaCMC at the wave numbers of 3441.05, 2924.99, 1607.80, 1510.79, 1111.76, 1045.50, respectively, showing sodium CMC present in capsules.



NaCMC

gelatin



Microencapsulated fish oil

Figure 5: Infrared spectroscopy analysis.

No generation of new chemical bond evidenced as no specific peak value found between spectrums of microcapsules and wall materials, further confirms the formation of complexes promoted by electrostatic interaction rather than chemical reactions (Liu et al., 2007, Qv et al., 2011) By comparison, it is verified that the interaction between gelatin and NaCMC was based on electrostatic force.

CONCLUSION

Complex coacervates for carrying and protecting EPA and DHA in fish oil were designed to yield a free flowing powder that acted to inhibit the production of primary and secondary oxidative products during 4 weeks of storage at room temperature, relative to the bulk oil. Using the Response surface Model, it was demonstrated that particle yield and microencapsulation efficiency were significantly affected by the pH during the process. Capsules formed by complex coacervation had sufficient stability through electrostatic attraction to maintain their structure. Furthermore, the statistical model in this study

predicted successfully the microencapsulation efficiency. On the other hand, despite the high particle yield, freeze-dried microcapsules showed low microencapsulation efficiency. The use of this encapsulation design could lead to increased utilization of fish oil in aqueous food systems, so as to contribute to the health and well-being of consumers.

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REFERENCES

1. A.O.C.S. 1997a. Sampling and Analysis of Commercial Fats and Oils. Official Method Pages Ca 5a-40 in Free Fatty Acids. Reapproved 1997. American Oil Chemists Society.
2. A.O.C.S. 1997b. Sampling and Analysis of Commercial Fats and Oils. Official Method Cd 8-53, . American Oil Chemists Society Peroxide Value Acetic Acid-Chloroform Method, Reapproved 1997.
3. Ahn, J.-H., Y.-P. Kim, Y.-M. Lee, E.-M. Seo, K.-W. Lee, and H.-S. Kim. 2008a. Optimization of microencapsulation of seed oil by response surface methodology. Food Chemistry 107(1):98-105.
4. Ahn, J.-H., Y.-P. Kim, E.-M. Seo, Y.-K. Choi, and H.-S. Kim. 2008b. Antioxidant effect of natural plant extracts on the microencapsulated high oleic sunflower oil. Journal of Food Engineering 84(2):327-334.
5. Asnacios, A., D. Langevin, and J. F. Argillier. 1998. Mixed monolayers of cationic surfactants and anionic polymers at the air-water interface: Surface tension and ellipsometry studies. The European Physical Journal B - Condensed Matter and Complex Systems 5(4):905-911.
6. Bae, E. K. and S. J. Lee. 2008. Microencapsulation of avocado oil by spray drying using whey protein and maltodextrin. Journal of Microencapsulation 25(8):549-560.
7. Cai, Y. Z. and H. Corke. 2000. Production and Properties of Spray-dried Amaranthus Betacyanin Pigments. Journal of Food Science 65(7):1248-1252.
8. Chen, G. and D. Tao. 2005. An experimental study of stability of oil–water emulsion. Fuel Processing Technology 86(5):499-508.
9. Chinta, D. D., R. A. Graves, S. Pamujula, N. Praetorius, L. A. Bostanian, and T. K. Mandal. 2009. Spray-Dried Chitosan as a Direct Compression Tableting Excipient. Drug Development and Industrial Pharmacy 35(1):43-48.
10. Dai, R., G. Wu, W. Li, Q. Zhou, X. Li, and H. Chen. 2010. Gelatin/carboxymethylcellulose/dioctyl sulfosuccinate sodium microcapsule by complex coacervation and its application for electrophoretic display. Colloids and Surfaces A: Physicochemical and Engineering Aspects 362(1–3):84-89.
11. de Kruif, C. G., F. Weinbreck, and R. de Vries. 2004. Complex coacervation of proteins and anionic polysaccharides. Current Opinion in Colloid & Interface Science 9(5):340-349.
12. Drusch, S. and S. Berg. 2008. Extractable oil in microcapsules prepared by spray-drying: Localisation, determination and impact on oxidative stability. Food Chemistry 109(1):17-24.
13. Estrada, J. D., C. Boeneke, P. Bechtel, and S. Sathivel. 2011. Developing a strawberry yogurt fortified with marine fish oil. Journal of Dairy Science 94(12):5760-5769.
14. Fuchs, M., C. Turchiuli, M. Bohin, M. E. Cuvelier, C. Ordonnaud, M. N. Peyrat-Maillard, and E. Dumoulin. 2006. Encapsulation of oil in powder using spray drying and fluidised bed agglomeration. Journal of Food Engineering 75(1):27-35.
15. Gebauer, S. K., T. L. Psota, W. S. Harris, and P. M. Kris-Etherton. 2006. n−3 Fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. The American Journal of Clinical Nutrition 83(6):S1526-1535S.
16. Gouin, S. 2004. Microencapsulation: industrial appraisal of existing technologies and trends. Trends in Food Science & Technology 15(7–8):330-347.
17. Gracey, J. F., D. S. Collins, and R. Huey. 1999. Fat Rancidity. Harcourt Brace and Co. Ltd, UK 10th ed.:407 in Meat hygiene.
18. Huang, C.-Y., C.-M. Chen, and Y.-D. Lee. 2007. Synthesis of high loading and encapsulation efficient paclitaxel-loaded poly(n-butyl cyanoacrylate) nanoparticles via miniemulsion. International Journal of Pharmaceutics 338(1–2):267-275.
19. Jacobsen, C. 2010. Enrichment of foods with omega-3 fatty acids: a multidisciplinary challenge. Annals of the New York Academy of Sciences 1190(1):141-150.
20. Junyaprasert, V. B., A. Mitrevej, N. Sinchaipanid, P. Boonme, and D. E. Wurster. 2001. Effect of Process Variables on the Microencapsulation of Vitamin A Palmitate by Gelatin-Acacia Coacervation. Drug Development and Industrial Pharmacy 27(6):561-566.
21. Klaypradit, W. and Y.-W. Huang. 2008. Fish oil encapsulation with chitosan using ultrasonic atomizer. LWT - Food Science and Technology 41(6):1133-1139.
22. Knox Jr, W. J. and T. O. Parshall. 1970. The interaction of sodium dodecyl sulfate with gelatin. Journal of Colloid and Interface Science 33(1):16-23.
23. Leclercq, S., K. R. Harlander, and G. A. Reineccius. 2009. Formation and characterization of microcapsules by complex coacervation with liquid or solid aroma cores. Flavour and Fragrance Journal 24(1):17-24.
24. Li, W., G. Wu, H. Chen, and M. Wang. 2009. Preparation and characterization of gelatin/SDS/NaCMC microcapsules with compact wall structure by complex coacervation. Colloids and

Surfaces A: Physicochemical and Engineering Aspects 333(1–3):133-137.
25. Liu, Y.-F., K.-L. Huang, D.-M. Peng, P. Ding, and G.-Y. Li. 2007. Preparation and characterization of glutaraldehyde cross-linked O-carboxymethylchitosan microspheres for controlled delivery of pazufloxacin mesilate. International Journal of Biological Macromolecules 41(1):87-93.
26. Mayya, K. S., A. Bhattacharyya, and J. F. Argillier. 2003. Micro-encapsulation by complex coacervation: influence of surfactant. Polymer International 52(4):644-647.
27. Mendanha, D. V., S. E. Molina Ortiz, C. S. Favaro-Trindade, A. Mauri, E. S. Monterrey-Quintero, and M. Thomazini. 2009. Microencapsulation of casein hydrolysate by complex coacervation with SPI/pectin. Food Research International 42(8):1099-1104.
28. Onwulata, C. I., R. P. Konstance, and V. H. Holsinger. 1996. Flow Properties Of Encapsulated Milkfat Powders as Affected by Flow Agent. Journal of Food Science 61(6):1211-1215.
29. Papadopoulou-Mourkidou. 1993. The pyrethroid insecticides. In: Sherma J, Cairns T (eds) Comprehensive analytical profile of important pesticides.
30. . CRC Press, London, :3.
31. Parris, N., Cooke, P. H., & Hicks, K. B. 2005. Encapsulation of essential oils in zein nanospherical particles as a delivery system for antimicrobials. Journal of Agricultural and Food Chemistry 53.
32. Partanen, R., H. Yoshii, H. Kallio, B. Yang, and P. Forssell. 2002. Encapsulation of sea buckthorn kernel oil in modified starches. Journal of the American Oil Chemists' Society 79(3):219-223.
33. Pauletti, M. S. and P. Amestoy. 1999. Butter Microencapsulation as Affected by Composition of Wall Material and Fat. Journal of Food Science 64(2):279-282.
34. Qv, X.-Y., Z.-P. Zeng, and J.-G. Jiang. 2011. Preparation of lutein microencapsulation by complex coacervation method and its physicochemical properties and stability. Food Hydrocolloids 25(6):1596-1603.
35. Sanguansri, L., & Augustin, M. A. 2007. Microencapsulation and delivery of omega-3 fatty acids. . In J. Shi (Ed.), Functional food ingredients and nutraceuticals: Processing technologies 297-327.
36. Simopoulos, A. P. 2008. The Importance of the Omega-6/Omega-3 Fatty Acid Ratio in Cardiovascular Disease and Other Chronic Diseases. Experimental Biology and Medicine 233(6):674-688.
37. Sovilj, V. J. and L. B. Petrović. 2006. Influence of hydroxypropylmethyl cellulose–sodium dodecylsulfate interaction on the solution conductivity and viscosity and emulsion stability. Carbohydrate Polymers 64(1):41-49.
38. Tan, L. H., L. W. Chan, and P. W. S. Heng*. 2009. Alginate/starch composites as wall material to achieve microencapsulation with high oil loading. Journal of Microencapsulation 26(3):263-271.
39. Thimma, R. T. and S. Tammishetti. 2003. Study of complex coacervation of gelatin with sodium carboxymethyl guar gum: Microencapsulation of clove oil and sulphamethoxazole. Journal of Microencapsulation 20(2):203-210.
40. Turchiuli, C., M. Fuchs, M. Bohin, M. E. Cuvelier, C. Ordonnaud, M. N. Peyrat-Maillard, and E. Dumoulin. 2005. Oil encapsulation by spray drying and fluidised bed agglomeration. Innovative Food Science & Emerging Technologies 6(1):29-35.
41. Velasco, J., S. Marmesat, C. Dobarganes, and G. Márquez-Ruiz. 2006. Heterogeneous Aspects of Lipid Oxidation in Dried Microencapsulated Oils. Journal of Agricultural and Food Chemistry 54(5):1722-1729.
42. Wai, W. T., B. Saad, and B. P. Lim. 2009. Determination of TOTOX value in palm oleins using a FI-potentiometric analyzer. Food Chemistry 113(1):285-290.
43. Wampler, D. J., Soper, J.C., & Pearl, T. T, inventor. 1998. Method of flavoring and mechanically processing foods with polymer encapsulated flavor oils
44. Wanasundara, U. N. and F. Shahidi. 1995. STORAGE STABILITY OF MICROENCAPSULATED SEAL BLUBBER OIL. Journal of Food Lipids 2(2):73-86.
45. Weinbreck, F., M. Minor, and C. G. de Kruif. 2004. Microencapsulation of oils using whey protein/gum arabic coacervates. Journal of Microencapsulation 21(6):667-679.
46. Yin, H. and S. Sathivel. 2010. Physical Properties and Oxidation Rates of Unrefined Menhaden Oil (Brevoortia patronus). Journal of Food Science 75(3):E163-E168.
47. Young, S. L., X. Sarda, and M. Rosenberg. 1993. Microencapsulating Properties of Whey Proteins. 1. Microencapsulation of Anhydrous Milk Fat. Journal of Dairy Science 76(10):2868-2877.
48. Zhong, Q., M. Jin, P. M. Davidson, and S. Zivanovic. 2009a. Sustained release of lysozyme from zein microcapsules produced by a supercritical anti-solvent process. Food Chemistry 115(2):697-700.
49. Zhong, Q., H. Tian, and S. Zivanovic. 2009b. ENCAPSULATION OF FISH OIL IN SOLID ZEIN PARTICLES BY LIQUID-LIQUID DISPERSION. Journal of Food Processing and Preservation 33(2):255-270.
50. Zilberboim, R., I. J. Kopelman, and Y. Talmon. 1986. Microencapsulation by a Dehydrating Liquid:Retention of Paprika Oleoresin and Aromatic Esters. Journal of Food Science 51(5):1301-1306.
51. Zohuriaan, M. J. and F. Shokrolahi. 2004. Thermal studies on natural and modified gums. Polymer Testing 23(5):575-579.

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Phytochemical, physicochemical and organoleptic evaluation of apple and skim milk based functional beverage

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ABSTRACT

The growing interest in functional foods with special characteristics and health properties has led to the development of new functional beverage. Beverage based on fruits and milk products are currently receiving considerable attention. The apple contains functional nutrients such as phenolic compounds and organic acids. Milk, the best source of nutrition, provides enormous health benefits. The present study was undertaken to develop valuable functional beverage. The analysis of functional beverage was carried out for total phenolic contents, ascorbic acid, pH, acidity, total soluble solids and sensory evaluation during storage at 4°C. An increase in total phenolic contents was observed from 7.575 at zero days to 9.045 at end of storage period. The storage decreased the ascorbic acid from 3.264 to 2.203 mg/100 mL throughout the storage period. The fall in pH was observed along with an increase in acidity. The mean values were found in the range of 6.73 to 6.96 in the start and 5.44 to 5.61 at the end of storage period. The mean value for acidity was 0.019 to 0.058 % at zero days and 0.288 to 0.299 % at the end of storage period. The increase in TSS was also observed during storage time. The value ranged from 11.20 to 11.73% at initiation of experiment and 11.93 to 12.13% at the end of storage period. Sensory evaluation revealed that product is acceptable for 28 days and treatments T_2 (10% apple pulp) and T_3 (12% apple pulp) gained maximum scores during sensory evaluation. These analysis presented that dairy based apple drink can be preserved for 28 days at 4°C.

Keywords: Functional Beverage, Physicochemical, Phytochemical, storage, stability

INTRODUCTION

Functional foods like functional beverages are important for their role in health promotion and disease prevention. They provide a means to reduce the increasing burden on the health care system by a continuous preventive mechanism (Shahidi 2004). These foods and beverages are not intended only to satisfy hunger, but also provide humans with necessary nutrients to prevent nutrition-related diseases (Menrad *et al*., 2000). Besides being delicious, functional beverages are highly nutritious. Milk based beverages containing different flavors are among the most popular drinks in the world, which compete with other soft drinks due to their superior nature in organoleptic qualities (Shukla *et al*., 2003). Functional beverages containing fruit and milk not only intended to satisfy hunger, provide humans with necessary nutrients but also have a role in health promotion and disease prevention (Shahidi, 2004; Minrad *et al*., 2000). These health benefits are due to antioxidant compounds present in fruit as well as in milk and reduced the risk of many diseases. Some of which are cardiovascular diseases, cancer, diabetes mellitus, Alzheimer's disease, cataracts and age-related functional decline (Martínez-Gonzá- Lez *et al*., 2002; Slaterry *et al*., 2000; Zhang *et al*., 1995).

Apple (*Pyrus malus* L.) constitutes biologically active compounds such as certain phenolic compounds and vitamin C which are known to act as natural antioxidants besides containing the basic food components (Okuda 1993). Plant based foods contain significant levels of biologically active components (Sánchez-Moreno *et al*., 2006; Hassimoto *et al*., 2005). Phytochemicals, the plant components with discrete bio-active, have ability to provide numerous health benefits. Such phytochemicals include terpenoids, phenolics, alkaloids and fiber (Dillard and German 2000).

Milk also holds an important place among beverages because it contains a wide range of readily available nutrients. Ca, vitamin D, protein, K and Mg and other notable constituents play an important role in maintaining low body fat, decreased high blood pressure and reduced risk of hypertension, prevent cancer, cardiovascular diseases and osteoporosis (Wooten and Price 2004).Various other dairy products have also been found to be antioxidative e.g. skim milk, whey, casein and lactoferrin (Loscalzo *et al*., 2004).

During peak season apple is available in bulk, so its pulp is used with milk to produce a health promoting beverage keeping in view the above facts. The present study will reveal the development of a functional beverage

containing apple pulp and reconstituted skim milk at different concentration to evaluate the phytochemicals, physicochemical composition and sensory evaluation.

MATERIAL AND METHODS:

Apple Pulp Extraction and Preservation:

Apples of red golden variety were purchased from the super store of local market of Faisalabad. After preliminary process, Pulp was extracted after blanching for 2 minutes. The apple pulp was pasteurized at 82±2°C and preserved in sterilized bottles using sodium benzoate as a preservative as described by Hussain *et al*., 2003. The pulp was stored in refrigerator for further applications in different treatments.

Preparation of Functional beverage

Skim Milk Powder was purchased from the super store of local market of Faisalabad. After conducting several initial trials the formulation of the product was finalizes based on the sensory evaluation. The amount of water and percentage of pulp varied accordingly in all treatments (Table 2) while remaining ingredients had same amount in all treatments.The best scored levels of apple pulp selected during initial trials were added in above mixture at different concentrations as described in Table (1). The Skim milk powder was reconstituted at 80°C for 15 minutes. After that other ingredients like sucrose, pectin, sodium citrate, potassium sorbate and stabilizer were added and stirred vigorously.

Table 1: Addition of apple pulp in reconstituted skim milk for final trial

Ingredient	Amount (%)
Sugar	10
Skim milk	6
Pectin	0.2
Emulsifier	0.1
Citric acid	0.03
Sodium citrate	0.02
Ascorbic acid	0.02
Potassium sorbate	0.01
Flavour	0.09
Color	0.002
Apple pulp	As per treatment
Water	To make final volume

Table 2: Final treatment plan for dairy based apple beverage

Treatments	Apple pulp (%)
T_0	0
T_1	8
T_2	10
T_3	12
T_4	14
T_5	16

A homogenous mixture was formed after stirring for 5 mints. The beverage was then bottled in amber colored bottle and the pasteurized at 80°C for 15 minutes in water bath and finally cooled to 10°C and stored in refrigerator at 4 °C. The beverage was analyzed after 7 days of storage till 28 days. The Skim milk powder was reconstituted at 80°C for 15 minutes. After that other ingredients like sucrose, pectin, sodium citrate, potassium sorbate and stabilizer were added and stirred vigorously. A homogenous mixture was formed after stirring for 5 mints. The beverage was then bottled in amber colored bottle and the pasteurized at 80°C for 15 minutes in water bath and finally cooled to 10°C and stored in refrigerator at 4 °C. The beverage was analyzed after 7 days of storage till 28 days.

ANALYSIS

Total Phenolic Compounds of functional beverage

Total phenolic compounds were determined by using spectrophotometer following the Folin-Ciocalteu method described by Skrede *et al*., (2004) with minor modification. The spectrophotometer was set to zero absorbance with reagents at 765nm. Then the standard Gallic acid was taken in cuvette and measured the absorbance for all the concentrations.

Vitamin C (Ascorbic Acid) of functional beverage

Ascorbic acid was estimated titrimetrically as described by Ruck (1969) by using Sodium 2,6-dichlorophenol indophenol as dye.

pH of functional beverage

pH of all the samples was determined by following the method described in AOAC (1990). The samples were taken in neat and clean 50 mL beakers and pH was directly recorded by using a calibrated pH meter.

Acidity of functional beverage

The acidity was determined by following the method given in AOAC (1990). According to this method 5 mL

sample from each treatment was titrated against 0.1 N sodium hydroxide solution to a persistent pink color end point by using two or three drops of phenolphthalein indicator. The results were expressed as percent citric acid

Total Soluble Solids (TSS) of functional beverage

Total soluble solids of functional beverage were directly recorded by hand refractometer equipped with a percent scale and the results were expressed as percent soluble solids ^{o} Brix.

Sensory Analysis of functional beverage

Sensorial analysis of functional beverage was performed based on the methodology described by Harry and Hildegarde (1998). The nine point hedonic scale was employed for the evaluation of samples stored in refrigerated conditions

Statistical Analysis of functional beverage

The data thus collected was subjected to analysis by complete randomized design (2-factor factorial) and the significance of difference was determined by Duncan's multiple range test at 5 percent level of significance (Steel *et al*.,1997).

RESULTS AND DISCUSSION

Total Phenolic Contents of functional beverage

The total phenolic contents were found at the zero and 28th days of storage period with mean value of 7.575 mg/100 mL (GA equivalent) and 9.045 mg/100 mL (GA equivalent) respectively. The interaction between storage time and treatment showed significant influence on total phenolic contents higher and lower total phenolic contents in treatment T_5 and T_0 after 28 and zero days of storage period (Table 3). Treatments had significant effect on total phenolic contents. The results were in line with the findings of Kalt *et al*., (1999) who observed the increase in antioxidant capacity of strawberries and raspberries during storage at temperature > 0 ^{o}C and 20 ^{0}C. Potter *et al*., (2007) studied the characteristics of wild blueberry-soy beverages and observed that after one month of storage the total phenolic contents of the isolate juice concentrate beverage were higher.

Ascorbic Acid Contents of functional beverage

The significantly highest contents of ascorbic acid were observed at the start of experiment followed by 7th day of storage with mean values of 3.26 mg/100 mL and 3.18 mg/100 mL respectively (Table 3). The effect of treatments on ascorbic acid showed higher ascorbic acid contents in T_5 with mean values of 5.08 mg/100 mL and lower in T_0. The interaction between storage time and treatment showed significantly the higher ascorbic contents in T_5 at initiation of storage while the lower in T_0. Similar results were obtained by Maria *et al*., (2003) and Kabasakalis *et al*., (2000) who studied the ascorbic acid content of commercial fruit juices and its rate of loss upon storage.

pH of functional beverage

Highest pH value was observed at the start of the experiment. The effect of treatments on pH of the beverage showed significantly higher in T_1 followed by T_2 with mean values of 6.08 and 6.07, respectively while 5.93 was observed in T_5 the lowest among all treatments. The interaction between storage time and treatment showed the higher pH value in T_0 at the initiation of experiment while the lower pH value was estimated in T_5 after 28th days of storage (Table 4). Gradual decreasing trend in pH in stored lime juices was also found by Ziena (2000). Zaidi (1988) also found the decrease in pH of pineapple flavoured based beverage.

Acidity of functional beverage

After 28 days of storage significantly highest acidity value was observed followed by 21 days. The mean value for acidity ranges from 0.035 to 0.283% during storage period of 0 to 28 days. The effect of treatments on acidity showed significantly higher in T_5 followed by T_4 with mean values of 0.221 and 0.213 %, respectively. The interaction between storage time and treatment showed significantly the higher acidity in T_5 while lower in T_0 (Table 4). The results are in line with the findings of Caro *et al*., (2004) who observed the increasing trend in acidity during storage of Palazzelli mandarin for 12 days. Esteve *et al*., (2005) reported a significant increase in acidity of orange juice stored at 4 ^{0}C and at 10 ^{0}C after 4 and 3 weeks of storage, respectively.

Total Soluble Solids (TSS) of functional beverage

The effect of storage period on functional beverage showed significantly highest TSS value at 28 days of storage followed by 21 days of storage with mean values of 11.96 and 11.87 respectively. Total soluble solids of functional beverage were estimated significantly highest in T_0 followed by T_4 with mean values of 11.94 and 11.69, respectively. Significantly the lower TSS was observed in T_5 and T_3. The interaction between treatment and storage time showed significantly higher TSS in T_0 at 28 days of storage period while the lower in T_5 (Table 4). The increasing trend of total soluble solids in the storage of Palzzelli mandarin was reported by Caro *et al*,. (2004). Asif *et al*., (2004) also found that the storage period increased the percentage of total soluble solid from 15.40 to 17.3 % in Kalakulu, from 15.25 to 16.20 % in Golden Delicious, from 14.60 to 16.30 % in Mashhadi, from

Table 3: Phytochemical analysis of functional beverage

Property	Treatments	Storage					
		Days					
		0	7	14	21	28	Mean
Phenolic Content	T0	6.563 n	7.105 m	7.088 m	7.931 k	8.198 j	7.377 f
	T1	6.682 n	7.654 l	8.472 i	8.674 h	8.701 h	8.036 e
	T2	7.056 m	8.086 j	8.454 i	8.545 i	9.102 cd	8.248 d
	T3	8.096 j	8.190 j	8.756 gh	9.021 d	9.205 c	8.654 c
	T4	8.164 j	8.435 i	8.840 fg	9.082 cd	9.403 b	8.785 b
	T5	8.888 ef	9.491 b	8.976 de	9.477 b	9.661 a	9.299 a
	Mean	7.575 e	8.16 d	8.431 c	8.79 b	9.045 a	
Ascorbic Acid	T0	0.358 p	0.316 p	0.272 p	0.207 p	0.067 p	0.244 f
	T1	2.095 lm	2.294 k-m	1.954 mn	1.612 n	0.912 o	1.773 e
	T2	3.116 hi	2.959 hi	2.824 h-j	2.680 i-k	2.433 j-l	2.802 d
	T3	3.674 ef	3.632 e-g	3.270 f-h	3.172 h	2.791 h-j	3.308 c
	T4	4.358 cd	4.316 cd	3.772 e	3.193 gh	3.043 hi	3.736 b
	T5	5.985 a	5.617 ab	5.228 b	4.628 c	3.974 de	5.087 a
	Mean	3.264 a	3.189 a	2.887 b	2.582 c	2.203 d	

Table 5: Influence of treatments on the sensory evaluation of functional drink

Treatment	Color	Taste	Flavor	Overall acceptability
T_0	5.56^{c}	4.88^{e}	4.88^{e}	5.40^{d}
T_1	6.52^{b}	6.24^{cd}	$6.80b^{c}$	7.00^{b}
T_2	7.24^{a}	7.52^{a}	$7.12a^{b}$	7.64^{a}
T_3	7.32^{a}	7.12^{b}	7.48^{a}	7.28^{ab}
T_4	6.48^{b}	6.44^{c}	6.40^{c}	6.28^{c}
T_5	5.96^{c}	6.04^{d}	5.84^{d}	6.20^{c}

^{abcd}, Different superscripts indicates significant difference between the means at $P \leq 0.05$ probability level

Table 6: Influence of storage intervals on the sensory evaluation of functional drink

Storage interval	Color	Taste	Flavor	Overall acceptability
Day 0	7.53^{a}	7.53^{a}	7.40^{a}	7.70^{a}
Day 7	$7.1o^{b}$	6.83^{b}	7.06^{a}	7.16^{b}
Day 14	6.33^{c}	6.33^{c}	6.36^{b}	6.63^{c}
Day 21	6.00^{c}	5.70^{d}	5.83^{c}	6.10^{d}
Day 28	5.60^{d}	5.46^{d}	5.43^{c}	5.56^{e}

^{abcd}, Different superscripts indicates significant difference between the means at $P \leq 0.05$ probability level

14.46 to 16.80 % in King Amri, and from 13.15 to 15.85 % in Amri variety of apple at the end of six week storage.

Sensory Evaluation of functional beverage

The statistical analysis revealed that all sensory characteristics differ significantly with regard to the treatments as well as storage interval. The statistical analysis for color, taste, flavor and overall acceptability showed that the storage period and treatments have Significantly affect while the interaction between storage time and treatment was found to be non-significant (Table 5,6). During storage the score for color, taste, flavor and overall acceptability varied from 7.53 to 5.60, 7.53 to 5.46, 7.40 to 5.43, 7.70 to 5.56 respectively (Table 6).

The score for sensory parameter of functional beverage decreased with the storage intervals. The treatments T_2 and T_3 gained maximum sore for all above mentioned parameters while T_o attained minimum throughout the storage period of 28 days. The storage duration have a significant effect on all the sensory parameters. The product remained acceptable for 28 days of storage period under refrigerated conditions. After that, the signs of spoilage appeared in the product, fermented taste and off odor were pronounced sign of spoilage. These finding about sensory parameters are in accordance with Gonzalez & Leeson, (2000) who found that the difference in color of fruit beverage was due to the browning reaction between reducing sugars and amino acids during storage. The loss of flavour, taste and overall acceptability may be due to the degradation of ascorbic acid and furfural production during storage as described by Perez & Sanz, (2001).

CONCLUSION

The compositional in functional beverage were determined based on Phytochemical, physicochemical and organoleptic characteristics. The increase in total phenolic contents, acidity, TSS was directly whereas pH, vitamin C concentration and sensory change were inversely related with storage period.

REFERENCES:

1. AOAC. 1990. Official methods of analysis. The Association of Official Analytical Chemists Inc. 15th Ed. Arlington, U.S.A.
2. Asif, A., R. Hasnain, M.A. Khan and M. Hussain. 2004. Effect of different period of ambient storage on chemical comopsitionb of apple fruit. Int. J. Agric. Biol. 6(3): 568-571.
3. Caro, A.D., P. Antonio, V. Vincenzo and A. Mario. 2004. Changes of flavonoids, vitamin C and antioxidant capacity in minimally processed citrus segments and juices during storage. Food Chem. 84: 99-105.
4. Dillard, C.J. and J.B. German. 2000. Phytochemicals: nutraceuticals and human health. Review. J. Sci. & Agr. 80: 1744-1756.
5. Esteve, M.J., A. Frigola, C. Rodrigo and D. Rodrigo. 2005. Effect of storage period under variable conditions on the chemical and physical composition and colour of Spanish refrigerated orange juices. Food Chem. Toxicol. 43: 1413-1422.
6. Gonzalez, E.R and S. Leeson, 2000 An investigation on the preservation of kunun–zaki, an African fermented cereal based food drink. Acta Alimentaria.29: 385–92.
7. Harry T.L. and H. Hildegarde. 1998. Sensory evaluation of food. Chapman and Hall, New York.
8. Hassimoto, N.M.A., M.I. Genovese and F.M. Lajolo. 2005. Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. J. Agric. Food Chem. 53: 2928-2935.
9. Hussain, S., Saleem-ur-Rehman., M.A. Randhawa and I.Muhammad. 2003. Studies on Physico-chemical, microbiological and sensory evaluation of mango pulp with chemical preservative. J. Rec. (Sci). 14(1): 01-09.
10. Kabasakalis, V., D. Siopidou and E. Moshatou. 2000. Ascorbic acid content of commercial fruit juices and its rate of loss upon storage. Food Chem. 70: 325-328.
11. Kalt, W., F.K. Charles, M. Antonio and L.P. Ronald. 1999. Antioxidant capacity, vitamin C, phenolics and anthocycanins after fresh storage of small fruits. J. Agri. Food Chem. 47: 4638-4644.
12. Loscalzo, R., T. Iannoccari, C. Summa, R. Morelli and P. Rapisarda. 2004. Effect of thermal treatments on antioxidant and antiradical activity of blood orange juice. Food Chem. 85: 41-47.
13. Maria, C.O.C., A.M. Geraldo, W.D.F. Raimundo, S.F. Men de Sa Moreira de and M.B. Isabella. 2003. Storage stability of cashew apple juice preserved by hot fill and aseptic processes. Ceinc. Tecnol. Aliment. Campinas. 23(supl): 106-109.
14. Martínez-Gonzá-Lez, M.A., E. Fernández-Jarne, E. Martínez-Gonzá-Losa, Prado-M. Santamaría, C. Brugarolas-Brufay and M. Serrano-Martí-nez. 2002. Role of fibre and fruit in the Mediterranean diet to protect against myocardial infection: a case-control study in Spain. Euro. J. Clinical Nutr. 56: 715-722.
15. Minrad, M., B. Husing, K. Menrad, T. Reib, S. Beer-Borst and C.A. Zenger. 2000. Functional food. TA 37/2000. Bern: Schweizerischer Wissenschafts und Technologierat.
16. Nicoli, M.C., M. Anese and M. Parpinel. 1999. Influence of processing on the antioxidant properties of fruits and vegetables. Trend Food Sci. Technol. 10: 94-100.
17. Okuda, T. 1993. Natural polyphenols as antioxidants and their potential use in cancer. In: Polyphenolic phenomena (Ed. A. Scalbert), p. 221-236. INRA, Paris.
18. Perez, A.G. and C. Sanz, 2001. Effect of high oxygen and high carbon dioxide atmospheres on strawberry flavour and other quality traits. J. Agric. Food Chem., 49: 2921–30.

19. Potter, R.M., M.P. Dougherty, W.A. Halteman and M.E. Camire. 2007. Characteristics of wild blueberry-soy beverages. LWT. 40: 807-814.
20. Ruck J.A. 1969. Chemical analysis of fruits and vegetable products. Canadian deptt. Agri. Pub. No 1154
21. Sánchez-Moreno, C., L. Plaza, B. de Ancos and P. Cano. 2006. Nutritional characterization of commercial traditional pasteurized tomato juices: carotenoids, vitamin C and radical-scavenging capacity. Food Chem. 98: 749-756.
22. Shahidi, F. 2004. Functional foods: Their role in health promotion and disease prevention. J. Food Scei. 69(5): 146-149.
23. Shukla F.C., A. Sharma and B. Singh. 2004. Studies on the preparation of fruit beverages using whey and buttermilk. J. Food Sci. Technol. 41(1): 102-105.
24. Skrede, G., V.B. Larsen, K. Aaby , A.S. Jorgensen and S.E. Birkeland. 2004. Antioxidative properties of commercial fruit preparations and stability of bilberry and black current extracts in milk products. J. Food Sci. 69(9): S351-S356.
25. Slaterry, M.L., J. Benson, K. Curtin, K.N. Ma, D. Schaeffer and J.D. Potter. 2000. Carotenoids and colon cancer. American J. Clinical Nutr. 71: 575-582.
26. Steel R.G.D., J.H. Torrie and D.A. Dickey. 1997. Principles and procedures of statistics. MecGraw Hill Book Co, Inc. New York.
27. Wooten, W.J. and W. Price. 2004. Consensus report of the national medical association: The role of dairy and dairy nutrients in diet of African Americans. J. Nat. Medi. Associ. 96(12): 1-31.
28. Zaidi K.R. 1988. Manufacture of milk based fruit flavoured drink employing UHT technique. M.Sc (Hons.) Thesis, Food Technol. Uni. Agric. Faisalabad.
29. Zhang, Y.H., P.R. Taylor, T.R. Kramer and J.Y. Li. 1995. Possible immunologic involvement of antioxidants in cancer prevention. American J. Clinical Nutr. 62: 1477-1482.
30. Ziena, H.M.S. 2000. Quality attributes of bears seedless lime (citrus latifolia Tan) juice during storage. Food Chem. 71(2):167-172.

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TRANS FAT INTAKE: A SILENT THREAT TO HUMAN HEALTH - A REVIEW

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ABSTRACT

Trans fats are gaining popularity in industrial sector owing to its low cost, potential to increase shelf life of products and contribute suitable features to the food. Presently, trans fats intake is considered as a peril factor for coronary heart diseases, diabetes, cancer and other allied disparities. Vendor foods (snacks, baked products and French fries) are the largest contributor of trans-fats in human's diet. Consequently it is strongly suggested that trans-fat must be excluded from vendor foods. Vendors should use the high quality frying medium as well as avoid the repeated frying so that trans-fat could be minimized from daily consumed diet.

Keywords: Trans fats, Food, Heart diseases

INTRODUCTION

Nutritional guidelines determined the undeniable link between diet and human health. Certain health problems have resulted due to change in dietary patterns and variations in processing techniques. Incompatible dietary patterns are major causes of poor health, and increased the mortality and morbidity worldwide. These threats have forced to design safe and cost effective ways to fight certain life ominous complaints (Tarik and Ramahi, 2010). Edible fats and oils have been used by humans for thousands of years to add taste to cooked food. These play significant role in human growth and health maintenance and also provide consistency, taste and stability as a food ingredient. These are the substances which are composed of fatty acids and glycerol which may serve as a food. Fats and oils are simple lipids. Fats are solid at room temperature while oils are liquid at room temperature. This division is for ease as all fats are melted at high temperature and all oils are solidified at low temperature. Solid fat usually comprises of saturated fatty acids while liquid edible oil comprises of unsaturated fatty acids (Christy *et al*., 2003). All the fatty acids consist of hydrocarbons chain and a carboxylic group at the end of the chain. There are number of different fatty acids that occur in natural fats. Trans fatty acids (TFA) may be described as polyunsaturated and monounsaturated fatty acids holding non-coupled carbon-carbon double bonds in the trans form, disrupted by at least one methylene group. TFA did not include coupled fatty acids such as coupled linoleic acid (Kim *et al.*, 2008). Oleic acid and elaidic acid are examples of cis and trans-fats respectively. Both contain one single double bond with eighteen carbon fatty acid. But oleic acid is cis isomer and elaidic acid is trans isomer. Trans fat has adverse effect on the human health (Chavarro *et al.*, 2006). Trans fats are gaining popularity in industrial sector owing to its low cost, potential to increase shelf life of products and contribute suitable features to the food. Presently, trans-fats intake is considered as a peril factor for coronary heart diseases, diabetes, cancer and other allied disparities.

Trans fat is gaining importance in scientific research due to increasing awareness regarding the health in recent years, as it drastically affects consumer health and cause cardiovascular diseases, diabetes and cancer. Currently issued FDA pattern wants manufacturer to expose trans-fat on the food specifics label of dietary supplements and formal foods (Wilson, 2004). However food industry still uses hydrogenated vegetable fats with high trans contents for various food applications. It is a strict recommendation to all consumers from that they should carefully read the nutritional information provided on the label before making a purchase of any food commodity (Risérus *et al*., 2006). The consumption of saturated fat should be limited to as low as 7% of the energy and also the intake of trans-fats should not reach 1% of the energy. Also, keep the cholesterol intake to less than 300 mg per day (Hunter, 2005).

SOURCES OF TRANS FATS

Primarily trans-fats come from two origins. The first one is the partial hydrogenation procedure which is used for the transfer of vegetable oils into semi solid or solid fats to give it desirable melting attributes which is desirable for certain food items such as margarines and shortenings. This practice increases the keeping quality of the oil and also provides oxidation stability. Partial hydrogenation is done for the production of vanaspati, margarine and shortenings, which result in unsaturation reduction. Hydrogenation alters the fat viscosity and saturates the double bond of fatty acid. Product formed as a result of hydrogenation behaves like saturated fats and

inhibit the enzymatic destruction of essential fatty acids and increase the shelf life of product (Dimitrios *et al*., 2003). Vegetable oil and shortening are mostly used in frying and baking. For baking purpose margarine has ample plasticity and considered as a substitute for butter in dietary fats (Anwar *et al.,* 2006). The second one is the natural occurrence of TFA in the fats of ruminants which is produced in their stomach through hydrogenation of cis-unsaturated fatty acids by microbial activity (Richter *et al.*, 2009). Animal fats hold about 3% of TFA while partial hydrogenated vegetable oils comprise 30% TFA (Daniel *et al.,* 2005). Some other origins of trans fatty acids are commercially produced snacks, baked products, margarines and fried foods (Aftab *et al*., 2008). Collectively, it can be stated that consumption of hydrogenated fats, deep- fried foods and baked goods are major sources of trans-fats in developed and developing countries.

Perilous effects of trans fat

Tran's fat produced from the natural and industrial sources have different effect on human health. Industrially produced trans-fats are more dangerous and perilous than those of naturally present in food (Chardigny *et al*., 2007). Tran's fat behaves like saturated fats and is a leading cause of cardiovascular complications, cancer, diabetes, obesity, pro-inflammatory response and endothelial dysfunction.

Cardiovascular diseases

Two Dutch researches, Mensink and Katan in 1990 gave the evidence that trans-fatty acids have an unwanted effect that may cause heart diseases. They carried out experiment on 35 women and 25 men in the Netherlands, examining solid diets with different contents of elaidic, oleic, and saturated fatty acids. They examined that trans-fatty acids decrease HDL (high-density lipoprotein) cholesterol and increase LDL (low density lipoprotein) cholesterol. The ratio of LDL and HDL cholesterol was more with the trans-fatty acid diet than with the saturated fatty acid diet. Correspondingly other scientists revealed that the intake of trans fatty acids increases the level of low-density lipoprotein (LDL), lowers the degrees of high-density lipoprotein (HDL) and raises the ratio of total cholesterol to HDL cholesterol (Motard-Belanger et al., 2008). Omega-3 fatty acid is an important indicator of lower prevalence of cardiovascular diseases; associated with heart attack preventing proteins. According to Larque *et al*. (2000) partial deficiency of Omega-3 fatty acids is caused by partially hydrogenated vegetable oil (PHOV) thus increasing the danger of heart attack.

One more approach for evaluating the above data as provided by Hunter (2005) is to compare the degrees of linoleic acid and trans-fatty acids used in the diet. Consumption of linoleic acid for men 30 to 51 years old is 18g/day. Proper consumption of linoleic acid would supply 5.5 % of energy which is more than the relatively low level provided by trans-fatty acid that is about 2 to 4 % of energy. On the whole, when consumption of trans-fat is increased as compare to linoleic acid then level of linoleic acid is decreased due to conversion of linoleic acid into trans-fat. High level of trans-fat in the membrane of human red blood cells may be involved in unpredictable heartbeat and abrupt cardiac death (Katz, 2002). Studies have shown that increased consumption of trans-fat in diet can increase the inflammation in the arteries which is a dangerous factor for coronary heart disease. Inflammation and coronary heart disease in women is linked with elevated contents of trans-fat in the membrane of their red blood cells. Trans-fats encouraged the low grade inflammation and cause cardiovascular diseases (Sun *et al*., 2007). Hu *et al.*, (1997) reported that for each 3% % increase in trans-fat calories when they are consumed the risk of coronary heart disease doubled. He also reported that reducing trans-fat energy consumption about 2 % with unsaturated fats that are not trans the risk of coronary heart disease is reduced to about more than halves percent. Correspondingly, Mozaffarian *et al.* (2006) conclude that the extensive intake of partially hydrogenated oils increase the danger of coronary heart diseases.

Diabetes

Mozaffarian *et al.* (2006) revealed that trans-fatty acids are not only associated with the risk of coronary heart disease diseases but also interfere with the insulin sensitivity and results in the diabetes. According to Bray *et al.,* (2002); meal containing high level of trans fat specially elaidic acid compare with other fatty acids indicate that first one produced more resistance against insulin and raise level of insulin at the same level of blood sugar. Riserus (2006) estimated that the trans-fat consumption on a long period damage the insulin activity and add to diabetes risk. They significantly change insulin binding capacity and influence insulin receptors. Studies have shown that there is a positive affiliation of trans-fat consumption and insulin sensitivity. Studies have indicated that danger of type 2 diabetes in women can be lowered by lowering the consumption of trans-fat in their diet and by increasing the intake of polyunsaturated fatty acids (Salmeron *et al.*, 2001).

Cancer

In control study it was also investigated that an association also exist between cancer of large intestine and trans-fat intake (Bakker *et al.*, 1997). It also provides verification that a relationship exists between trans-fatty

acid and colon cancer risk. Chavarro *et al.* (2006) also reported that these are also linked with an increase in prostate cancer danger. Another study revealed that a serum level of trans-fat is also one factor adding to increase the chance of cancer in women (Chajes *et al.*, 2008).

Other health implications

For several years dietary fats and their relationship to obesity has been a burning issue. Inquiry on monkeys shows that trans-fat may increase abdominal fat and weight gain, even with the similar caloric consumption. Study on asthma and allergies in children aged 13 to 14 years indicated a positive affiliation between trans-fatty acid consumption and these diseases. It was discovered that higher consumption of trans-fatty acid increase the chance of asthma and allergic cold in children (Weiland *et al.*, 1999). Some studies revealed that consumption of trans-fat can adversely affect the pregnant women because trans-fat are shifted from mother to fetus (Decsi, 2001). As a result new born baby has high concentration of trans-fat and low concentration of polyunsaturated fatty acids, which are crucial for the proper growth and development. High concentration of trans-fat can adversely affect the development of central nervous system of new born baby (Wandall, 2008).

ELIMINATION OF TRANS FAT FROM DIET

Getting rid of trans-fat is not an easy task but due to its adverse health effects it is the need of the time to eliminate the trans-fat from our diet. So, it is necessary to take the following actions.

Nutritional labeling of food products

FDA has issued a guideline in 2003 that made it obligatory to disclose trans-fat content on the label (FDA, 2005). Studies have indicated that new information, which may be supplied either through media or nutrition label can influence the food products choice of consumer. Moreover, previous studies also propose that food industries will also bring product reformulation, which will improve diet for all consumers ultimately (Kim *et al.*, 2000). Similarly such ingredients should be banned which are high in trans-fat contents (Eckel *et al.*, 2007).

Processes/ plant genotype modification

Now a day, the food industry is trying to cut down TFAs in their food items and at the same time maintain the morphological and toothsome features of the food product. They are currently developing technology to reduce or eliminate TFA content first by alteration of the hydrogenation process to develop partially hydrogenated fats with low TFA content such as precious catalyst hydrogenation, electro catalytic hydrogenation and supercritical fluid state hydrogenation. Secondly, alteration of the fatty acid constitution of oil seed through genetic engineering techniques, plant breeding and interesterification of mixed fats (Jang *et al.*, 2005).

CONCLUSION

Edible fats and oils are important for development and maintenance of good health. They provide taste, consistency, and stability to the food when added as an ingredient. Due to the increasing nutritional awareness regarding the health, Trans fat in dietary fats is of great importance because it is the cause of cardiovascular diseases, diabetes and cancer. Trans fat is geometric isomer of cis fat and it is formed during partial hydrogenation of oil. Trans fat behaves like saturated fats even they are unsaturated. Consequently it is strongly suggested that trans-fat must be excluded from foods. Vendors should use the high quality frying medium as well as avoid the repeated frying. So that trans-fat could be minimized from daily consumed diet so as to improve the quality and nutritional value of foods.

REFERENCES:

1. Aftab K, Sherazi STH, Mahesar SA, Bhanger MI, Talpur M, Younis M, Rauf A (2010). GC–MS quantification of fatty acid profile including trans fatty acids in the locally manufactured margarines of Pakistan. Food Chem. 109:207-211.
2. Anwar F, Bhanger MI, Latif S, Manzoor M (2006). Lipid content and fatty acid composition of some deep fried and fast foods from Sindh Pakistan. J. Chem. Soc. Pak. 28(4):374-379.
3. Bakker N, Veer PV, Zock PL (1997). Adipose fatty acid and cancers of the breast, prostate and colon: an ecological study. Int. J. Cancer. 72:587-597.
4. Bray GA, Lovejoy JC, Smith SR, Delany JP, Lefever M, Hwang D, Ryan DH, York DA (2002). The influence of different fats and fatty acids on obesity, insulin resistance and inflammation. J. Nutr. 132:2488-2491.
5. Chajes V, Thiebaut ACM, Rotival M, Gauthier E, Maillarad PV, Boutron-Ruault MCB, Joulin V, Lenoir GM, Clavel-Chapelon F (2008). association between serum trans-monounsaturated fatty acids and breast cancer risk in the E3N-EPIC study. Am. J. Epidemiol. 167(11):1312-1320.
6. Chardigny JM, Malpuech-Burger C (2007). Impact of trans fatty acids on cardiovascular diseases. J. Food Chem. 19(4):198-202.
7. Chavarro J, Stampfer M, Campos H, Kurth T, Willtt W (2006). A prospective study of blood trans fatty

acids and risk of prostate cancer. J. Cancer Epidimol. Biomark. Prev. 17(1):95-101.
8. Christy AA, Egeberg PK, Ostensen ET (2003). Simultaneous quantitative determination of isolated trans fatty acids and conjugated linoleic acids in oils and fats by chemometric analysis of the infrared profiles. Vibrational Spectrosco. 33: 37–48.
9. Daniel DR, Thompson LD, Shriver BJ, Chich-Kang W, Hoover LC (2005). Non hydrogenated cottonseed oil can be used as a deep fat frying medium to reduce trans-fatty acid content in french fries. J. Am. Diet Assoc. 105:1927-1932.
10. Decsi T, Burus I, Molnar S, Minda H, Veitl V (2001). Inverse association between trans isomeric and long chain polyunsaturated fatty acids in cord blood lipids of full-term infants. Am. J. Clin. Nutr. 74: 364-368.
11. Dimitrios T, Vasilios A, Zografos S, Haralambos K (2003). Fatty acid content of margarines in the Greek market (including trans-fatty acids): A contribution to improving consumers' information. Int. J. Food Sci. Nutr. 54:135-141.
12. Eckel RH, Borra S, Lichtenstein AH, Yin-Piazza SY (2007). Understanding the complexity of trans fatty acid reduction in the American diet: American Heart Association *trans* Fat Conference 2006: Report of the trans Fat Conference Planning Group. Circulation. 115: 2231- 2246.
13. FDA (Food and Drug Administration). FDA acts to provide better information to consumers on trans fats. 2005. Available at http://www.fda.gov/oc/initiatives/transfat/. Accessed on 17 March 2008.
14. Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Rosner BA, Hennekens CH, Willett WC (1997). Dietary fat intake and the risk of coronary heart disease in women. N. Engl. J. Med. 337(21):1491–1499.
15. Hunter JE (2005). Dietary level of trans fatty acids: basis for health concerns and industry effort to limit use. J. Nutr. Res. 25:499-513.
16. Jang ES, Jung MY, Min DM (2005). Hydrogenation for low trans and high conjugated fatty acids. CRFSFS. 1:22-30.
17. Katz AM (2002). Trans-fatty acids and sudden cardiac death. J. Circ. 105:699-671.
18. Kim BH, Lumor SE, Akoh CC (2008). Trans-Free margarines prepared with canola oil/Palm stearin/Palm kernel oil-based structured lipids. J. Agric. Food Chem. 56:8195-8205.
19. Kim SY, Nayga RM, Capps OR (2000). The effect of food label use on nutrient intakes: an endogenous switching regression analysis. J. Agric. Econ. 25:215-231.
20. Larque E, PerezLlamas F, Puerta V, Giron MV, Suzarez MD, Zamora S, Gil A (2000). Dietary trans fatty acids effect decosahexaenoic acid concentration in plasma and liver but not brain of pregnant and fatal rats. Pediatr. Res. 47(2):278-283.
21. Mensink RP, Katan MB (1990). Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. New England J. Med. 323:439-445.
22. Motard-Belanger A, Charest A, Grenier G, Paquin P, Chouinard Y, Lemieux S, Couture P, Lamarche B (2008). Study of the effect of trans fatty acids from ruminants on blood lipids and other risk factors for cardiovascular disease. Am. J. Clin. Nutr. 87: 593-599.
23. Mozaffarian D (2006). Trans fatty acids effect on systematic inflammation nad endothelial function. Ather. Suppl. 7(2):29-32.
24. Richter EK, Shawish KA, Scheeder MR, Colombani PC (2009). Trans fatty acid content of selected Swiss foods: The Trans Swiss Pilot study. J. Food Compos. Anal. 22:479-484.
25. Riserus U (2006). Trans fatty acids and insulin resistance. Athero. Suppl. 7(2):37-39.
26. Salmeron J, Hu FB, Manson JE (2001). Dietary fat intake and risk of type 2 diabetes in women. Am. J. Clin. Nutr. 73: 1019–1026.
27. Sun Q, Ma J, Campos H, Hu FB (2007). Plasma and erythrocyte biomarkers of dairy fat intake and risk of ischemic heart disease. Am. J. Clin. Nutr. 86: 929–37.
28. Tarik M, Ramahi MD (2010). Cardiovascular Disease in the Asia Middle East Region: Global Trends and Local Implications. Asia Pac. J. Public Health. 22:83-89.
29. Wandall B (2008). The controversy over trans fatty acids: effects early in life. Food Chem. Toxicol. 46: 3571-3579.
30. Weiland SK, Mutuis EV, Husing A, Asher MI (1999). Intake of trans fatty acid and prevalence of childhood asthma and allergies in Europe. Lancet, 353:2040-2041.
31. Wilson E (2004). Trans fat update. Manuf. Confect. 84:73-78.

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Microbiological Safety Concern of Filled Bakery Products in Lahore

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ABSTRACT

The primary intention of present study was to determine the incidence of microbial load & some pathogens in filled bakery products available in bakeries & supermarkets. A total number of 122 samples (65 chicken sandwiched burger and 57 butter cream pastries) were microbiologically analyzed. By using the standard microbiological techniques like spread plate method, pour plate method & streak plate method to examined the microbial count in these products. Further isolation & identification of microbes was performed by using selective microbiological media & bacterial biochemical identification kits. Among the chicken sandwiched burgers 75.38% of the samples had reasonable and satisfactory microbiological quality with appropriate aerobic plate count and absence of tested pathogenic microorganisms. But 4 samples were found to be potentially hazardous due to the presence of *Salmonella* and *Staphylococcus aureus* in three & one samples respectively. Whereas 64.91% sample of the buttered cream pastries were of good microbiological quality. 12.28% butter cream pastry samples were classed as unsatisfactory due to high aerobic plate count, high coliform count and presence of the fungal contaminants. Overall 62.29% of the sampled filled baked products were of satisfactory quality while only 3.27% samples had poor microbiological quality including those which were potentially hazardous to the health of the consumer. To save consumer health & spoilage of the products good hygienic practices & HACCP system should be implemented during production of filled bakery products.

Keywords: Chicken sandwich burger, Butter cream pastries, Salmonella, Staphylococcus aureus, Spread plate method, Pour plate method, Streak plate method

INTRODUCTION

Today bakery products make a major component of our daily diet now. In the past people used to prepare and consume fresh food at home. Now the trends are changing as the people are trying to keep up with the hectic routines of busy life. The food sector is evolving accordingly to keep up with the need of the time and many ready-to-eat and semi ready-to-eat products are offered at bakery and the fast food outlets. Among these ready-to-eat food product the bakery products offer convenience and balanced diet at the same time for the busy and health conscious customers (Musaiger *et al.*, 2007).

These backed products as compared to the perishable food items are not only limited to bakeries only, rather these are distributed over wide area from manufacturing point. Baked products have become important part of service industry, hospitals, supermarkets and fast food chains. Microorganisms play important role in the manufacturing of baked products and also in the flavoring of the baked product, but microbes also play major role in the spoilage of the baked products due to inadequate preparatory steps (cooling, slicing and transport) (Ponte, 1987).

In addition to bacteria other microbes like molds also play major role in the spoilage of the baked products. The fresh bakery products are sterile but soon become contaminated when it comes in contact with the air and other material. Improper handling also introduces contamination as bakery workers are major source of contaminants. Frazier *et al.* (1988) found 96.3% positive bacterial culture from workers of a bakery industry. Foods are served after passing through a long chain of steps involved in production, processing, distribution and marketing. Man stands as the ultimate consumer at the head of many food chains and because of bio-magnification; they may get exposed to diverse

harmful agents in increasing concentrations (Rahman, 1997).

The major contaminant present in the filled backed products are Coliform, *Escherichia coli*, *Staphylococcus*, *Salmonella*, Molds, Yeast and overall microbial load in the filled bakery products. These contaminants are added due to a number of problems including contaminated raw materials of objectionable quality, unhygienic conditions and un-satisfactory storage. *Salmonella* is a well-known food borne zoonotic infectious agent that account for major share as the causative agent of food borne illnesses (Thorns, 2000). The processing of product near the raw eggs and poultry meats may also lead to contamination of *Salmonella spp*. and these poultry products may also be potential source of contamination as raw ingredient in the filled baked products (Antunes *et al.*, 2003). The lack of hygienic measures and GMPs in the bakeries lead to introduction of *Staphylococcus*, coliform and *Escherichia coli* (M'hir *et al.*, 2007). The re-use of single use filling piping bag might be a source of contamination in the filling that later on may cause food poisoning as the filling after being filled with the contaminated piping bags will stay unchecked and consumed without any necessary treatment to kill these contaminants. The handlers themselves are source of contamination as presence of *Staphylococcus* on the food handlers is well known (Acco *et al.*, 2003) and if the products are handled without observing food safety principles it contaminates the product with *Staphylococcus*. *Staphylococcus aureus* is considered the third most important cause of disease in the world amongst the reported food-borne illnesses (Zhang *et al.*, 1998).

Among coliform, *Escherichia coli* is considered to be the most important pathogen that causes food borne illnesses. The organism is known to cause large outbreaks originating with the consumption of contaminated food. Illnesses caused by E. coli infection can range from self-limited, watery diarrhea to life-threatening manifestations such as hemolytic uremic syndrome or thrombotic thrombocytopenic purpura (Padhye and Doyle, 1992). In the present study, microbiological quality of the filled bakery products like butter cream cake and chicken sandwiches was analyzed. The filled backed products are potential vehicle for food poisoning causing organisms as the filled products are not heat treated prior to consumption leaving any contaminant introduced during handling and storage. The filling provides favorable growth conditions like moisture level (water activity), physical state and nutrients for the contaminants already present in the ingredients of the filling or added during processing, handling and storage. Thus these contaminants become the cause of the food borne illnesses (Abrahamson *et al.*, 1952).

The present study focuses on evaluating the incidence of Coliform, *Escherichia coli*, *staphylococcus*, *Salmonella*, Molds, Yeast and observing the overall microbial load in the filled bakery products available in our surrounding bakeries & supermarkets. This will indicate us that our daily consumable bakery products are microbiologically safe or not. These products may pose a serious threat to consumer in form of food poisoning outbreak.

Materials and Methods

A total number of 122 samples (65 chicken sandwiched burger and 57 butter cream pastries) were aseptically collected from different localities of Lahore. The samples were collected from supermarkets, fast food outlets and conventional bakeries. The samples were collected in sterile plastic bags immediately after purchase and put in sampling box with ice packs (4-8 °C). Samples were usually analyzed within 4 hours of collection in Food Microbiology Laboratory, Bureau Veritas, Lahore.

To determine the microbial count of the samples Aerobic Plate Count was performed on Plate Count Agar (Oxoid CM0325, UK).The samples were diluted in the phosphate buffered saline (PBS) by adding one gram sample in 9mL of phosphate buffered saline. The sample were serially diluted in the phosphate buffered saline. One mL from the dilutions was plated on Aerobic Plate Count Agar by spread plate technique. The plates were incubated at 30-32 °C for 24-72 hours. The colony count was performed with conventional plate count method (Larry and James, 1998). For performing count of Coliform and screening *Escherichia coli* the 1mL of sample diluted in phosphate buffered saline (1:10) were spread on Violet Red Bile Agar (Merck) with spread plate method and incubated at 37°C for 24-48 hours. For detection of presence of *Staphylococcus aureus* in the samples, the sample was mixed in phosphate buffered saline (1:10) were cultivated on Mannitol Salt Agar (Oxoid CM085, UK). 1mL sample was spreaded and incubated at 37°C for 24-48 hours. Similarly yeast and molds were also checked on the Potato Dextrose Agar (Oxoid CM009, UK) and the plates were incubated at 25°C.

Table 1: Media used for microbiological analysis and the incubation conditions

Microorganisms	Medium	Temperature	Time (hours)	Conditions Aerobic/Anaerobic
Aerobic Plate Count	Plate Count Agar (CM0325)	30°C	24-72	Aerobic
Coliform	Violet Red Bile Agar (Merck)	37°C	24-48	Aerobic
Escherichia coli	MRVP Broth (CM0043)	37°C	24	Aerobic
	Tryptone Water (CM0087)	37°C	24	Aerobic
	Simmons Citrate Agar (CM0155)	37°C	24	Aerobic
Staphylococcus aureus	Mannitol Salt Agar (CM085)	37°C	24-48	Aerobic
Yeast & Molds	Potato Dextrose Agar (CM009)	25°C	2-5 days	Aerobic
Salmonella	Buffered Peptone Water (CM009)	37°C	24	Aerobic
	Tetrathionate Broth (CM0137)	37°C	24	Aerobic
	Bismuth Sulphit Agar (CM201)	37°C	24-48	Aerobic
	Hekton Enteric Agar (CM0419)	37°C	24-48	Aerobic
	Tripple Sugar Iron Agar (CM0277)	37°C	24-48	Aerobic

Table 2: Guidelines for the microbiological quality of various ready-to-eat foods

Microorganism	Satisfactory	Acceptable	Unsatisfactory	Potentially Hazardous
Aerobic Plate Count	$<10^5$	10^5-10^6	$>10^6$	N/A
Coliform Count	<100	100-10^4	$>10^4$	N/A
Salmonella	not detected in 25g sample			detected in 25g sample
E. coli (total)	<20	20-100	>100	N/A
S. aureus	<20	20-<100	100-$<10^4$	$>10^4$

Table 3: Results of microbiological examination of the food samples

Product	Microbiologically Acceptable		Microbiologically Unacceptable	Potentially Hazardous	
	Satisfactory	Acceptable	Unsatisfactory	Potentially Hazardous	Total
Chicken Sandwiched Burger	49(75.38%)	6(9.23%)	6(9.23%)	4(6.15%)	65(100%)
Butter Cream pastries	37(64.91%)	13(22.80%)	7(12.28%)	0(0.0%)	57(100%)
Total	86(62.29%)	19(15.57%)	13(10.65%)	4(3.27%)	122(100%)

For the isolation of *Salmonella* 25 gram sample was suspended in 225mL Buffered Peptone Water (Oxoid CM009, UK) and incubated at 37°C for 24 hours. Then for selective enrichment 0.1mL from Buffered Peptone Water was added into 10mL Tetrathionate Broth (Oxoid CM0137, UK) made according to the manufacturer's directions by adding supplements. After enrichment the culture was directly streaked onto agar plates of Bismuth Sulphit Agar (Oxoid CM201, UK) and incubated for 24-48 hours at 37°C. The suspected colonies were checked by gram staining and growing on Hekton Enteric Agar (Oxoid CM0419, UK) and Tripple Sugar Iron Agar Slants (Oxoid CM0277, UK). The further identification & confirmation of *Escherichia coli* & *Salmonella* was made by plating on MacConkey agar using the RapID ONE System (Remel) followed the instruction of the manufacturer.

Results and Discussion

A total of 122 bakery products including 65 chicken sandwiched burgers and 57 butter cream pastries were analyzed for the microbiological quality. The results were compared with Public Health Laboratory Services (PHLS) guidelines for the microbiological quality of some ready-to-eat foods (Gilbert *et al.*, 2000) as described in the Table 2. The results of the analysis after comparison with the standard are arranged in the Table 3.

Among the chicken sandwiched burgers 75.38% of the samples had reasonable and satisfactory microbiological quality with appropriate Aerobic Plate Count and absence of tested pathogenic microorganisms. But 4 samples were found to be potentially hazardous due to the presence of *Salmonella* & *Staphylococcus aureus* in three & one samples respectively. Whereas 64.91% sample of the buttered cream pastries were of good microbiological quality. 12.28% butter cream pastry samples were classed as unsatisfactory due to high Aerobic Plate Count, high Coliform Count and presence of the fungal contaminants. Overall 62.29% of the sampled filled baked products were of satisfactory quality while only 3.27% samples had poor microbiological quality including those which were potentially hazardous to the health of the consumer. Previous researchers were also observed similar findings (Kumar *et al.*, 2011). The results of the study are displayed as a bar chart (Figure-1) where the quality of both the sample is compared. It is evident that the chicken sandwiched burgers have better satisfactory product rate than the butter cream pastries but at the same time the former shows some potentially hazardous products. The variation is due to the different nature of the products. The *Salmonella* contamination is suggested to be from the poultry related ingredients like the poultry egg and chicken meat. The Salmonella is lead contaminant of the products of poultry origin (Antunes *et al.*, 2003). *Salmonella* causes serious disease condition in the humans and in the past several outbreaks of salmonella associated infection due to the consumption of contaminated poultry product have been reported by Evans *et al.*, (1999).

Staphylococcus aureus was identified from one of the sample of chicken sandwiched burger. Its known that the Staphylococcus are normal flora of the human skin and nasal mucosa. The transmission of the organism from food handler to the product is well documented (Acco *et al.*, 2003; Wei and Chiou, 2002). In this regard the hygiene and proper training of the food handler is of prime importance as it effects the quality of the product and also plays role in the community health.

The percentage of sample with greater than normal Aerobic Plate Count and Coliform Count was more in the butter cream pastries. This is due to the nature of the product as the cream topping of the pastries provide favorable environment for the contaminants to grow and also the post manufacturing handling of the product magnifies the microbial load of the product. The cream have perfect moisture and nutritious contents to support the growth of the contaminants. The incidence of fungal contamination was again higher in the butter cream pastries.

The presence of yeast & mold indicate the after baking contamination. The fungal spores are killed by baking process (Knight and Menlove, 1961) which leaves post backing contamination to be the only source for fungal spoilage. The fungal spores are present in the air of bakeries and high level of contamination of the air is often attributed to the high contamination of the bakery floor. Air conditioning further facilitates the movement of the spores in every nook and corner, thus further exacerbating the problem of contamination of fungal spores. The results is contamination and spoilage of the bakery products due to molds (Sadozai *et al.*, 2009). High sugar contents in the filled products of bakery origin tent to support the growth of the mold better than other microbes, but some other yeast and bacteria can also attack the products.

Figure 1: Bar Chart Diagram of Results of microbiological examination of the Food samples.

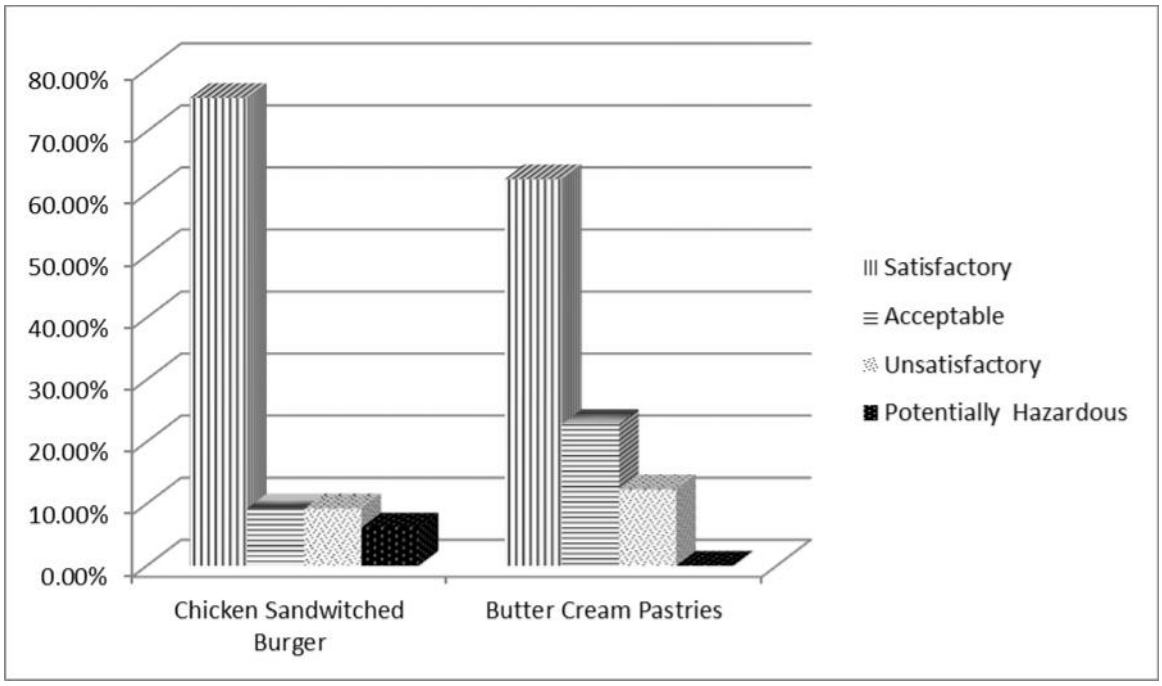

Figure 2: *E.coli* on MacConkey Agar

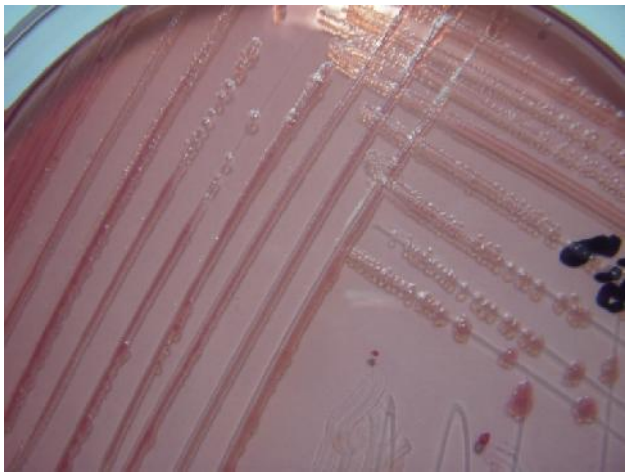

Figure 3: Salmonella on MacConkey Agar

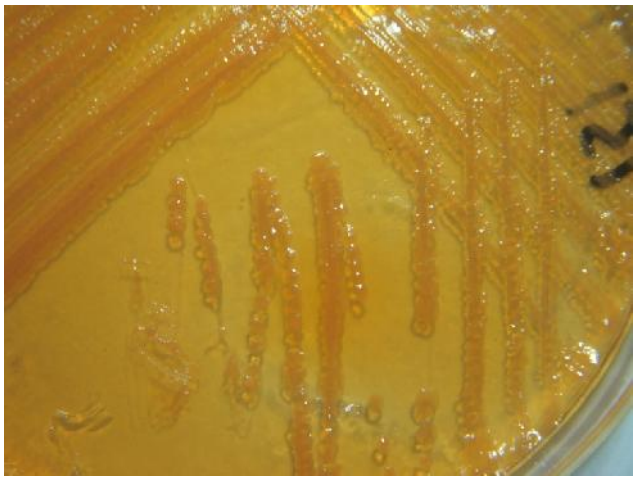

Figure 4: RapID ONE Panel kit

CONCLUSION

The present study revealed the microbiological & hygienic status of filled bakery products. Overall, the microbiological status of filled bakery products was slightly good with 77.86% samples yielding acceptable results. While 10.65% samples were found unsatisfactory & only 3.27% samples was potentially hazardous. Presence of *Salmonella & Staphylococcus* in chicken sandwich burger indicated an alarming situation for final consumer. So the chicken sandwich burger samples may be a potential risk for staphylococcus intoxication for consumer. *Salmonella* may also cause severe enteric problems like diarrhea, dysentery, abdominal pain & vomiting in its consumer. So we have to improve the hygienic status of production process starting from raw material to final product manufacturing. Meanwhile we have to design a project to evaluate the point of microbial contamination in filled bakery products. It will involve the microbiological analysis of raw ingredients, baked stuff, filling material & ultimately the final product. Further communication and education of businesses community may assist in improving food hygiene and handling practices in bakeries.

References

1. Abrahamson, A. E., R. Field, L. Buchbinder and A.V. Catelli. 1952. A study of the control of the sanitary quality of custard-filled bakery products in a large city. J. Food Sci. 17: 268-277.
2. Acco, M., F.S. Ferreira, J.A. Henriques, and E.C. Tondo. 2003. Identification of multiple strains of Staphylococcus aureus colonizing nasal mucosa of food handlers. Food Microbiol. 20: 489-493.
3. Antunes, P., Réu, C., J. C. Sousa, L. Peixe and N. Pestana. 2003. Incidence of Salmonella from poultry products and their susceptibility to antimicrobial agents. Int. J. Food Microbiol. 82: 97-103.
4. Evans, M. R., R.L. Salmon, L. Nehaul, S. Mably, L. Wafford, M.Z. Nolan-Farrell and C.D. Ribeiro. 1999. An outbreak of Salmonella typhimurium DT 170 associated with kebab meat and yoghurt relish. Epidemiol. Infection. 122: 377-383.
5. Frasier, W.C. and D.C. Westhoff. 1988. Food Microbiology. 4th ed. Tata McGraw-Hill Publishing Company Limited. p. 66.
6. Gilbert, R. J., J. De Louvois, T. Donovan, C. Little, K. Nye, C.D. Ribeiro and F.J. Bolton. 2000. Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. PHLS Advisory Committee for Food and Dairy Products. Communicable disease and public health/PHLS. 3: 163.
7. Knight, R. A., and E.M. Menlove. 1961. Effect of the bread-baking process on destruction of certain mould spores. J. Sci. Food and Agri. 12: 653-656.
8. Kumar, H., R. Palaha, D. Sharma, V. Sharma, D. Singh and A. Kaur. 2011. Microbiological quality analysis of the pastry sold in the Jalandhar city and public perception about the pastry. Int. J. Food Safety. 13: 361-366.
9. Larry, M. & T.P. James. 1998. Aerobic Plate Count. In: Bacteriological Analytical Manual, 8th ed, Revision A. FDA. USA.
10. M'hir, S., M. Mejri and M. Hamdi. 2007. Microflora distribution and species ratio of Tunisian fermented doughs for bakery industry. Afr. J. Biotechnol. 6: 2122-2129.
11. Musaiger, A. O., J. H. AL-Jedah and R. D'Souza. 2007. Nutritional Profile of Bakery Products Consumed in Bahrain. Pak. J. Nutr. 6: 228-233.
12. Padhye, N. & M. Doyle. 1992. Escherichia coli Q157:H7: epidemiology, pathogenesis, and methods for detection in food. J. Food Protection. 55: 555-565.
13. Ponte, J.G. 1987. Bakery Products. 2nd ed. AVI Book, Van. Nostrand Reinhold, New York.
14. Rahman, M. A. 1997. Microbes in Health and Hygiene. Key note paper presented at the 15th Annual Conference of Bangladesh Society of Microbiologists., BAU, Mymensingh. 4-5 Sept. 1997.
15. Saddozai, A. A., and S. Khalil. 2009. Microbiological status of bakery products available in Islamabad. Pak. J. Agri. Res. 22: 93-96.
16. Thorns, CJ. 2000. Bacterial food-borne zoonoses. Rev Sci Tech. 19: 39-226.
17. Wei, H. L., and C.S. Chiou. 2002. Molecular subtyping of Staphylococcus aureus from an outbreak associated with a food handler. Epidemiol. infection. 128: 15-20.
18. Zhang, S., J. Iandolo and C. Stewart. 1998. The enterotoxin D plasmid of Staphylococcus aureus encodes a second enterotoxin determinant (sej). FEMS Microbiol. Lett. 168: 227–233.

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Effect of pretreatment of plantain *(Musa Parasidiaca)* flour on the pasting and sensory characteristics of Biscuit Product

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ABSTRACT

The effect of pre-treatment on the pasting and sensory characteristics of plantain biscuit was investigated. The plantain flour was produced using different treatments; the use of sodium metabisulphite ($Na_2S_2O_5$), blanching at 80°C for 10min and unblanched plantain flour. The protein, fat and carbohydrate content of the flour samples are significantly difference ($p<0.05$) and the unblanched sample had the highest value (6.04%) in protein. Result showed that there were significant difference ($P< 0.05$) in the bulk densities and water absorption capacity. The plantain treated with sodium metabisulphite had the highest value (1.05mg/100g) in sodium content. Pasting time and the final viscosity are significantly difference. The sensory evaluation results showed a significance difference in the appearance, colour, taste, flavour, aroma, crispiness, and the overall acceptability, but plantain treated with sodium metabisulphite was rated the highest by the panelist.

Keywords: Pre-treatment, Pasting, Sensory, Plantain biscuit

INTRODUCTION

Plantain (*Musa paradisiacal*) is an important staple food in Central and West Africa, which along with bananas provides 60 million people with 25% of their calories. According to FAO (2004), over 2.11 million metric tons of plantains are produced in Nigeria annually. However, about 35-60% post harvest losses had been reported and attributed to lack of storage facilities and inappropriate technologies for food processing (Olorunda and Adelusola, 1997). When processed into flour it is used traditionally for preparation of gruel which is made by mixing the flour with appropriate quantities of boiling water to form a thick paste (Mepba *et al*., 2007). The use of plantain flour for production of baked goods if feasible would help to lessen our total dependence on imported wheat.

The chemical composition of plantain varies with the variety, maturity, degree of ripeness and where it is grown (soil type). The water content in the green plant is about 61% and increases on ripening to about 68%. The increase in water is presumably due to the breakdown of carbohydrates during respiration. Green plantain contains starch which is in the range of 21 to 26%. The starch in the unripe plantain is mainly amylose and amylopectin and this is replaced by sucrose, fructose, and glucose during the ripening stage due to the hydrolysis of the starch (Marriott *et al*., 1981).

The carbohydrate content reduces to between 5 to 10% when ripe. The sugar content is between 0.9 to 2.0% in the green fruit but becomes more prominent in the ripe state. The titrable acidity of plantain is about twice that of sweet potato (Aurand *et al*., 1987). Plantains therefore have a high carbohydrate content (31 g/100 g) and low fat content (0.4 g/100g). They are good sources of vitamins and minerals (Adeniji, *et al*., 2006), particularly iron (24 mg/kg), potassium (9.5 mg/ kg), calcium (715 mg/kg), vitamin A, ascorbic acid, thiamin, riboflavin and niacin. The sodium content (351 mg/kg) is low in dietary terms hence recommended for low sodium diets (Izonfuo and Omuaru, 1988; Stover and Simmonds, 1987; Welford *et al*., 1988). The amino acid components include; alanine, aminobutyric acid, glutamine, asparagine, histidine, serine, arginine and leucine. The ascorbic acid is high compared to that of banana. As a starchy staple food, plantain supply about 1 g protein/100 g edible portion (USDA, 2009). As a healthy adult requires about 0.75 g protein kg-1 day-1 (Burton and Willis, 1976), plantain alone cannot meet adult protein needs. The fat content of plantains and bananas is very low, less than 0.5%, and so fats do not contribute much to the energy content. Although the total lipid content remains essentially unchanged during ripening, the composition of fatty acids, especially within the phospholipids fraction has been observed to change, with a decrease in their saturation (Ogazi, 1996). The energy value of a food derives from the sum of its carbohydrates, fat and protein content. In the case of plantain, the carbohydrate fraction is by far the most important. The sugars and starches that make up this fraction are present in varying concentrations according to the state of the ripeness of the fruit. The two main components of this starch are amylose and amylopectin, present in a ratio of about 1:5.

Sugars comprise only about 1.3% of total dry matter in unripe plantains, but rises to around 17% in the ripe fruit.
Plantain for local consumption undoubtfully, plays a role in food and income security and has the potential to contribute to national food security and reduce rural poverty.
This crucial role is still largely ignored by policy makers and therefore special public awareness effort is required to sensitize policy makers in both producing and donor countries. Despite the importance of plantain, major constraints threatening its cultivation in terms of pest and disease infestations, soil fertility, planting materials, postharvest losses, marketing constraints, particularly poor road system and lack of infrastructure and storage facilities and much of the fruits harvested go waste. Plantain processed into flour can store for up to a maximum of two years.
This investigation is aimed at processing a local cultivar of plantain into stable flour as a way of extending the shelf life of ripe plantain fruits, to add value to plantain for both the local markets and for export, thereby ensuring food security. Low cost processing methods, such as solar drying etc., were employed to obtain a product that was then subjected to various analyses to determine the quality and acceptability of the resulting product.

MATERIALS AND METHODS

Matured green plantain fruit (*Musa parasidiaca*) was obtained from the International Institute of Tropical Agriculture in Ibadan, Oyo state, Nigeria.

Preparation of plantain flour

The matured green plantain fruits bunch was cut into individual fruits and was defingered and weighed. The plantain was washed, peeled and cut to approximately (2 mm thick) using the stainless steel knife. Sodium metabisulfite ($Na_2S_2O_5$) (2.0%) was prepared by dissolving 2 g of the salt in approximately 100 ml of distilled water, the plantain slices were poured inside the 25ml of prepared 2.0% sodium metabisulfite. The sulphited pulp was then dried in the oven dryer at 60°C for 24 hours to obtain dry chips, the dried chips were milled using the milling machine, and other treatments were done as shown in fig. 1

Production Method

Sugar (75g) was added to margarine (125g) in a Kenwood mixer and mixed at medium speed until fluffy (for about 12 minutes). Egg (1) and milk were added while mixing and then mixed for a total of approximately 30 minutes. Sifted flour (250g), 1 teaspoon of baking powder, ½ teaspoon of banana flavour, was slowly added into the mixture. The mixture was kneaded until dough formation. It was then rolled on a flat rolling board sprinkled with flour to a uniform thickness of about 0.4cm using wooden rolling pin and guiding stick. Circular cookies of 5.8cm to 6cm diameter were cut, placed on oiled baking trays and baked at 160 °C for about 15 min.

Proximate Analyses

The proximate analyses were determined using the procedure of (AOAC 1990).

Mineral content determination

The dry ashing procedure was used for mineral content determination was. Five (5) grams of each of the samples were accurately weighed into porcelain crucibles and pre-ashed until the sample was completely charred on a hot plate. The pre-ashed samples were thereafter ashed in the muffle furnace at 500 degrees Celsius till the ash was white for about 2 hours. After ashing, the crucibles were transferred into the desiccator to cool and the reweighed. Each sample was quantitatively transferred into volumetric flasks by carefully washing the crucibles with 1ml nitric acid, then with portions of dilute nitric acid. All washings were transferred to individual volumetric flasks, repeating the washing procedure twice. The solutions were diluted to volume with deionized water and were used for individual mineral determination using the appropriate standards and blank. The content of the minerals; Calcium, Iron, sodium, copper, were determined with the Atomic Absorption Spectrophotometer (Buck Scientific, Model 210).

Phosphorus Content

Phosphorus was determined using the Spectrophotometric method.

Physical and functional properties

Bulk Density determination of Plantain Flour

This was determined using the method described by (Wang and Kinssela 1976).

Swelling Power and Solubility: This was determined by the method described of Leach et al., (1957).

Water absorption capacity (WAC): This was determined using the method described by Sosulski (1962).

Pasting properties

It was determined using the rapid viscous Analyzer (Newport scientific, 1998). Sample (3.5g) was weighed to the nearest 0.01g into a weighing vessel prior to transfer into the text canister. 2.5 of distilled water were dispensed into test canister. The sample was transferred onto the water surface in the canister. A paddle was placed into the canister and its blade was rigorously jogged through the sample up and down 10 times. Jogging was repeated to ensure that the samples

remaining on the water surface or on the paddle were dissolved. The paddle and canister assembly were inserted firmly into the paddle coupling so that the paddle is properly centered. The measurement cycle was initiated by depressing after initiation and terminated automatically (IITA, 2001).

Sensory evaluation of biscuit

The Multiple Comparism test method was used, 3 samples of plantain biscuit and the control were served to a 15 semi-trained panelists who are familiar with the sensory attributes. A 9-point hedonic scale was designed to measure the degree of preference of the samples. The samples were presented in identical containers, coded with 3-digit random numbers served simultaneously to ease the possibility of the panelists to re-evaluate a sample. The categories were converted to numerical scores ranging from 1 to 9, with 1 as the lowest and 9 as the highest level of preference (Iwe, 2002). Necessary precautions were ensured to prevent carry-over flavour during the tasting by ensuring that panelists pass a piece of cracker biscuit in their mouths or rinse with water after each stage of sensory evaluation. Data obtained were subjected to analysis of variance (ANOVA). Means were separated using Duncan multiple range test (SPSS 16.0).

RESULTS AND DISCUSSION

Effect of pretreatment on the proximate analysis of wheat flour and plantain flour

The proximate analysis of the control and the plantain flour sample are shown on table 1. It was observed that the protein content of wheat flour (12.82%) was significantly difference at $p<0.05$ from the plantain flour samples. However the blanched plantain flour has the lowest value 5.16%. The moisture content goes a long way in suggesting the shelf life of the product. The fat content of the plantain flour was significantly different from the wheat flour. The plantain flour treated with sodium metabisulphite ($Na_2S_2O_5$) was low (2.27%). The lower level of fat in the samples gave a higher probability of a longer shelf-life in terms of the onset of rancidity (Ihekoronye and Ngoddy, 1985). The ash also the sodium metabisulphite treatment applied to the plantain. There was significant difference ($p < 0.05$) in the moisture content of wheat flour (11.51%), and the treated plantain flour samples content of wheat flour was lower than the plantain flour. The unblanched plantain flour has the highest implication of this could be that some of the protein is either leached or denatured by the blanching process (Oluwalana and Oluwamukomi, 2011), and flour has protein content of (6.04%) as compared to the plantain flour treated with the sodium metabisulphite ($Na_2S_2O_5$) which has (4.27%). The crude fibre and carbohydrate of the wheat flour was significantly difference ($p < 0.05$) from the plantain flour, as well as the treated plantain flour sample.

Effect of pretreatment on the functional properties of wheat flour and plantain flour

The effect of pretreatment on functional properties of plantain flour is represented on table 2.The bulk density of the plantain flour was significantly difference ($p < 0.05$) from the wheat flour, the blanched plantain flour sample had the lowest value of 0.25 g/ml, this is contrary to a report made by Tagogoe (1994) and Fagbemi (1999) which showed that bulk density increased as a result of blanching/heat treatment prior to drying. The reduction in the bulk density in blanched samples will be an advantage in the bulk storage and transportation of the flour. This is usually affected by the particle size and density of the flour and it is very important in determining the packaging requirement, materials handling and application in wet processing in the food industry The water absorption capacity was significantly different ($p < 0.05$) in all the flour samples, but the wheat flour had the highest value of 171.40 % of water absorption capacity and the unblanched plantain sample had the lowest value 125.17%. There was no significant difference ($p > 0.05$) between the wheat flour 40.54% and both the blanched has 39.49% and sodium metabisulphite ($Na_2S_2O_5$) plantain flour has 38.18% water absorption capacity. The unblanched plantain flour was significantly difference ($p < 0.05$). Swelling power is an indication of the absorption index of the granules during heating (Loos *et al*., 1981). The solubility of the wheat flour was significantly difference ($p < 0.05$) from the plantain flour, but the plantain flour treated with the sodium metabisulphite ($Na_2S_2O_5$) and the blanched sample are not significantly difference ($p > 0.05$) from each other. There are significance differences ($p < 0.05$) in the pH of the samples.

Effect of pretreatment on the proximate composition of plantain biscuit

The proximate composition of the plantain biscuit is shown on table 3. The results shows that there were no significant differences ($p > 0.05$) in protein between the plantain biscuit treated with sodium metabisulphite ($Na_2S_2O_5$) 5.12% and blanched plantain biscuit 5.27%. The wheat biscuit has the highest value of protein (11.83%) and this is due to the high gluten in wheat flour.

Table 1: Effect of pretreatment on the proximate composition of plantain flour and wheat flour

SAMPLES	ASH	MOISTURE (%)	FAT (%)	PROTEIN (%)	CRUDE FIBRE (%)	CARBOHYDRATE (%)
NPF	2.26±0.01	6.68±0.02	2.27±0.01	4.27±0.08	3.52±0.12	80.99±0.05
BPF	2.76±0.02	5.16±0.03	2.55±0.02	4.31±0.09	2.28±0.07	82.94±0.20
UPF	3.08±0.02	6.27±0.01	2.75±0.02	6.04±0.19	4.44±0.03	77.43±0.23
WF	0.49±0.02	11.51±0.02	1.40±0.01	12.82±0.02	0.81±0.01	72.98±0.06

Mean ± standard error

Table 2: Effect of pretreatment on the pH and functional properties of plantain and wheat flour

SAMPLES	BULK DENSITY (g/ml)	SWELLING POWER (%)	SOLUBILITY (%)	WATER ABSORPTION CAPACITY (%)	pH
NPF	0.76±0.00	38.18±0.04	6.80±0.01	139.19±0.01	6.29±0.00
BPF	0.25±0.00	39.49±0.01	6.47±0.00	130.26±0.01	6.25±0.00
UPF	0.49±0.00	48.89±0.01	5.57±0.01	125.17±0.01	6.12±0.01
WF	0.70±0.00	40.54±2.62	4.67±0.26	171.40±0.01	6.01±0.00

Mean ± standard error

The moisture content in the plantain biscuit samples which ranges between (5.90 to 7.10 %) was significantly difference ($p < 0.05$) from the wheat biscuit (12.83%). The ash content of the wheat biscuit (2.99%) was significantly difference ($p < 0.05$) from the plantain biscuit samples, but the unblanched biscuit has the highest value (3.50%) for ash content. The fat content of wheat biscuit (7.52%) was significantly difference ($p < 0.05$) from the plantain biscuit samples which ranges between (3.49 to 4.73%). There was a significant difference ($p < 0.05$) in crude fibre between the wheat biscuit and the plantain biscuit samples, the crude fibre in wheat is lower (1.01%) than the plantain biscuit samples. Carbohydrates are significantly difference ($p < 0.05$) from each other in all the samples.

Effect of pretreatment on the mineral composition of plantain flour

The mineral composition of the plantain flour is shown on table 4. The results shows that the sodium content in the plantain flour are significantly difference ($p < 0.05$), but the plantain flour treated with sodium metabisulphite ($Na_2S_2O_5$) has the higher value 1.05mg/100g. This may be due to the chemical treatment given to the plantain using the sodium metabisulphite which increased the sodium content in the plantain. There are significant differences ($p < 0.05$) in the iron content among the treatments of the plantain flour. In phosphorus there was significant difference ($p < 0.05$) among the plantain flour, but there was no significant difference ($p > 0.05$) between the plantain flour treated with sodium metabisulphite ($Na_2S_2O_5$) 0.28mg/100g and the blanched samples 0.34mg/100g. The calcium content in the plantain flour decreased significantly ($p < 0.05$) with the treatments; the plantain treated with sodium metabisulphite ($Na_2S_2O_5$) has the lowest value 1.27mg/100g. There was significant difference ($p < 0.05$) in copper of the plantain samples, but the plantain flour sample treated with sodium metabisulphite has the lowest value (0.53mg/100g).

Effect of pretreatment on the pasting properties of plantain flour and wheat flour

The pasting properties of plantain flour and wheat flour are shown on table 5.The pasting properties of starch are used in assessing the suitability of its application as functional ingredient in food and other industrial products (Oluwalana, *et al*., 2011). The most important pasting characteristic of granular starch dispersion is its amylographic viscosity (Aviara *et al.,* 2010). The pasting temperature of the plantain samples ranges between 64.55°C to 86.65°C and the control (wheat flour) is 83.45°C.but the blanched plantain flour sample had the lowest pasting temperature. The pasting temperature is a measure of the minimum temperature required to cook a given food sample (Sudha *et al.,* 2007), it can have implications for the stability of other components in a formula and also indicate energy costs (Newport Scientific, 1998). The peak time is a measure of the cooking time (Adebowale *et al.,* 2005). The plantain flour sample treated with sodium metabisulphite ($Na_2S_2O_5$) had the lowest peak time 4.50 min and the control sample (wheat flour) had the highest peak time 5.80 min. Peak viscosity, which is the maximum viscosity, developed during or soon after the heating portion of the pasting test, is lower in the control sample (wheat flour) 215.33 RVU and highest in the blanched plantain flour sample 293.92 RVU. Peak viscosity is often correlated with the final product quality. It also provides an indication of the viscous load likely to be encountered during mixing (Maziya-Dixon *et al*., 2004). Higher swelling index is indicative of higher peak viscosity while higher solubility as a result of starch degradation or dextrinization results in reduced paste viscosity (Shittu *et al.,* 2001). The hold period sometimes called shear thinning, holding strength, hot paste viscosity or trough due to the accompanied breakdown in viscosity is a period when the sample was subjected to a period of constant temperature (usually 95°C) and mechanical shear stress. It is the minimum viscosity value in the constant temperature phase of the RVA profile and measures the ability of paste to withstand breakdown during cooling (Newport Scientific, 1998). The blanched plantain flour had the highest value of the hold period 259.25 RVU while the plantain flour treated with sodium metabisulphite ($Na_2S_2O_5$) had the lower value 186.75 RVU, the control sample wheat flour and the unblanched plantain flour ranges between (200.67 and 200.83 RVU) respectively. This period is often associated with a breakdown in viscosity (Ragaee *et al.,* 2006). It is an indication of breakdown or stability of the starch gel during cooking. The lower the value the more stable is the starch gel. The breakdown is regarded as a measure of the degree of disintegration of granules or paste stability (Newport Scientific, 1998). The breakdown viscosities of the plantain flour samples ranges between 34.67 to 46.92 RVU and the control sample (wheat flour) had the lowest breakdown viscosity value 14.67 RVU. The viscosity after cooling to 50°C represents the setback or viscosity of cooked paste. It is a stage where retrogradation or reordering of starch molecules occurs. It is a tendency to become firmer with increasing resistance to enzymic attack. It also has effect on digestibility. Higher setback values are synonymous to reduced dough digestibility (Shittu *et al.,* 2001) while lower setback during the cooling of the paste indicates

Table 3: Effect of pretreatment on the proximate composition of plantain biscuit and wheat biscuit

SAMPLES	ASH (%)	MOISTURE (%)	FAT (%)	PROTEIN (%)	CRUDE FIBRE (%)	CARBOHYDRATE (%)
NPB	2.54±0.03	7.10±0.03	3.49±0.02	5.12±0.02	2.64±0.02	79.13±0.07
BPB	2.69±0.01	5.90±0.02	3.89±0.01	5.27±0.02	2.15±0.01	80.11±0.02
UPB	3.50±0.02	6.94±0.03	4.73±0.02	7.07±0.08	3.79±0.02	73.97±0.12
WB	2.99±0.02	12.83±0.02	7.52±0.02	11.83±0.03	1.01±0.01	63.83±0.01

Mean ± standard error

Table 4: Effect of pretreatment on the mineral composition of plantain flour

SAMPLE	COPPER (mg/100g)	CALCIUM (mg/100g)	SODIUM (mg/100g)	PHOSPHORUS (mg/100g)	IRON (mg/100g)
NPF	0.53±0.01	1.27±0.01	1.05±0.01	0.28±0.01	1.67±0.02
BPF	0.59±0.01	1.36±0.01	0.52±0.01	0.34±0.01	1.33±0.01
UPF	0.65±0.01	1.52±0.01	0.47±0.01	0.74±0.05	1.25±0.01

Mean ± standard error

Table 5: Effect of pretreatment on pasting properties of plantain flour and wheat flour

SAMPLE	PEAK VISCOSITY (RVU)	TROUGH (RVU)	BREAKDOWN (RVU)	FINAL VISCOSITY (RVU)	SET BACK (RVU)	PEAK TIME (min)	PASTING TEMPERATURE (°C)
NPF	241.00	186.75	54.25	222.58	35.83	4.50	65.10
BPF	293.92	259.25	34.67	346.83	87.58	5.21	64.55
UPF	247.75	200.83	46.92	297.50	96.67	5.33	86.65
WF	215.33	200.67	14.67	271.92	71.25	5.80	83.45

Mean ± standard error

Table 6: Effect of pretreatment on the sensory characteristics of plantain biscuit and wheat biscuit

SAMPLE	APPEARANCE	CRUST COLOUR	CRUMBS COLOUR	TASTE	CRISPINESS	FLAVOUR	AROMA	OVERALL ACCEPT ABILITY
WB	8.20±0.26	8.07±0.18	7.93±0.21	8.13±0.35	7.40±0.42	7.86±0.31	7.40±0.42	8.20±0.28
BPB	5.67±0.39	5.20±0.37	6.07±0.33	5.67±0.54	5.93±0.53	5.40±0.58	5.46±0.54	5.80±0.47
UPB	5.67±0.37	6.00±0.47	5.93±0.45	5.67±0.54	5.93±0.49	5.13±0.49	5.73±0.48	6.27±0.42
NPB	5.27±0.47	5.73±0.44	5.67±0.48	6.33±0.36	6.20±0.39	6.00±0.34	6.20±0.41	6.47±0.31

Mean ± standard error

lower tendency while lower setback during the cooling of the paste indicates lower tendency for retrogradation (Sandhu *et al.,* 2007). The final viscosity of the blanched plantain flour had the highest value 346.83 RVU and the plantain flour sample treated with sodium metabisulphite ($Na_2S_2O_5$) had the lower value 222.58 RVU. The final viscosity for the control sample (wheat flour) is 271.92 RVU. The setback value for the control sample (wheat flour) is 71.25 RVU. The plantain flour treated with sodium metabisulphite had the lowest setback value 35.83 RVU. The setback viscosity indicates the tendency of the dough to undergo retrogradation, a phenomenon that causes the dough to become firmer and increasingly resistant to enzyme attack (Ihekoronye and Ngoddy, 1985), and has a serious implication on the digestibility of the dough when consumed. Higher setback values are synonymous to reduced dough digestibility (Shittu *et al.,* 2001) while lower setback during the cooling of the paste indicates lower tendency for retrogradation (Sandhu *et al.,* 2007).

Effect of pretreatment on the sensory characteristics of the plantain flour biscuit

The result for sensory evaluation is represented on table 6. This result was evaluated in terms of appearance, colour (crust and crumbs), taste, crispiness, flavor, aroma, and overall acceptability. The appearance of the control (wheat biscuit) was significantly difference ($p < 0.05$) from the plantain flour. However, there was no significant difference ($p > 0.05$) between the plantain samples, but the plantain biscuit treated with sodium metabisulphite was shown to be the least liked by the panelist. The control (wheat biscuit) was significantly difference ($p < 0.05$) in crust colour, the unblanched plantain biscuit sample was rated the highest among the plantain biscuit by the panelist. There was significant difference ($p < 0.05$) in the crumbs colour between the wheat biscuit and the plantain biscuits, but the least liked by the panelist was the biscuit treated with sodium metabisulphite. Crispiness is perceived when food is chewed between molars, and is usually expressed in terms of hardness and fracturability (Noor, *et al*., 2009). There was no significant difference ($p > 0.05$) in crispiness between the wheat biscuit and the plantain flour treated with sodium metabisulphite ($Na_2S_2O_5$). The study shows that there were no significant difference ($p > 0.05$) in taste between the blanched plantain biscuit and the unblanched plantain biscuit, but there was significant difference ($p < 0.05$) between the control (wheat biscuit) and the plantain biscuits. The plantain biscuit treated with sodium metabisulphite was rated highest among the plantain biscuit by the panelist. The flavor of the control (wheat biscuit) was significantly difference ($p < 0.05$) from the plantain biscuits, the unblanched plantain biscuit was shown to be the least liked by the panelist. The aroma was significantly difference ($p < 0.05$) between the control sample and the plantain biscuit sample. There was significant difference ($p < 0.05$) in terms of overall acceptability between the control (wheat biscuit) and the plantain biscuit. Among the plantain biscuit there is no significant difference ($p > 0.05$) between them, the plantain biscuit treated with sodium metabisulphite was rated highest among the plantain biscuit by the panelist.

CONCLUSION

The flour produce with different treatments has significant difference ($p<0.05$) in its proximate analysis except that the protein for blanched plantain flour and the sodium metabisulphite plantain flour has no significant differences ($p>0.05$). The pretreatment had effect on the functional properties of the plantain flour samples. Flour treated with sodium metabisulphite had the highest water absorption capacity and this gave it is higher affinity to absorb water during production. The absorbent nature of the said flour has quantitative advantage and can be regarded as been economical. The final viscosity was low in the plantain flour treated with sodium metabisulphite ($Na_2S_2O_5$) and this might be due to low protein content present in the flour sample. The sensory characteristics of the plantain biscuit were significantly difference ($p < 0.05$). However the plantain biscuit treated with sodium metabisulphite had the highest overall acceptability. The use of plantain in biscuit making, and other food products, would greatly enhance the utilization of this crop in developing countries like Nigeria and West Africa in whole, where the crop has not been optimally utilized. Further work should be done on the microbiological examination and shelf stability of the plantain flour products to improve the quality of the product.

REFERENCE

1. Adebowale, A.A, S.A. Sanni and F.O. Oladapo. 2008. Chemical, functional and sensory properties of instant yam breadfruit flour. Niger. Food J. 26(1): 2-12.
2. Adebowale, Y.A, I.A Adeyemi and A.A. Oshodi 2005. Functional and physic chemical properties of flour of six Mucuna Species. Afr. J of Biotech. 4 (12): 1461-1468
3. Adeniji T.A, L.O. Sanni, I.S. Barimalaa and A.D. Hart 2006. Determination of micronutrients and colour variability among new plantain and banana hybrids flours. World J. Chem. 1(1): 23-27.
4. AOAC, 1990. Official Methods of Analysis of the Association of Official Analytical Chemists, 15th ed., AOAC, Arlington, Virginia, USA.
5. Aurand, W.L. 1987. Food composition and analysis of food, Von Nostrand Reinhold, New York: 135-138.
6. Aurand, W.L, A.E. Wood and R.M. Wells.1987. Food composition and analysis, 4th edition, Van Nostrand Reinhold, 115 Fifth Avenue, New York: 19-34.
7. Burton, T.B, R.F. Willis 1976. Human nutrition, 4th edition, McGraw Hill Book Company, New York: 566-588.

8. Aviara N.A., O.A. Onuh and S.A. Ehiabhi. 2010. Physical properties of *Mucuna flagellipes* nuts. Seed Science and Biotechnology, 4: 59–68.
9. Fagbemi, T.N. 1999. Effect of Blanching and Ripening on Functional Properties of Plantain (Musa aab) Flour. Foods Hum. Nutr., 54: 261- 269.
10. FAO (Food and Agricultural Organization) 2004. Food and agricultural indicators. ESSA Oct. 2005. FAO Rome. http://www.fao.org/es/ess/top/country.html.
11. Ihekoronye A.I and P.O. Ngoddy 1985. Integrated food science and technology for the tropics. 1st ed. McMillan publishers: 261- 291.
12. IITA (2001).Operation manual for the series 3 rapid visco analyzer using thermocline for windows Newport scientific pty.Lmt.
13. Iwe, M.O. 2002. Handbook of Sensory Methods and Analysis. Rojoint Communications Services Ltd, Uwani – Enugu Nigeria.
14. Izonfuo W.A.L, V.O.T Omuaru. 1988. Effect of ripening on the chemical composition of plantain peels and pulps (*Musa paradisiaca*). J. Sci. Food Agric. 45: 333-336.
15. Leach H.W, L.D. McCowan and T.J. Schoch 1957. Structure of the Starch Granule: Swelling Power and Solubility Patterns of Different Starches. Cereal Chem., 36: 534-544.
16. Loos, P.J., L.F. Hood and A.J. Graham. 1981. Isolation and characterization of starch from breadfruit. Cereal Chem., 58: 282-286.
17. Marriott, J, M. Robinson and S. K. Karikari. 1983. Starch and sugar transformations during ripening of plantains and bananas. J. Sci. Food Agric. 32: 1021-1026.
18. Maziya-Dixon B, A.G.O. Dixon, and A.A. Adebowale 2004. Targeting different end uses of cassava: genotypic variations for cyanogenic potentials and pasting properties. A paper presented at ISTRC-AB Symposium, 31 October – 5 November 2004, Whitesands Hotel, Mombassa, Kenya
19. McDonald P, R.A. Edwards and J. F. D. Greenhalgh. 1973: Protein concentrates. In: Animal Nutrition. Second Edition: 398-418.
20. Mepba, H.D, L. Eboh and S.U. Nwaojigwa, 2007. Chemical Composition, Functional and Baking Properties of Wheat – Plantain Composite Flours. Afr. J. Food Agric, Nutr. Dev., 7(1): 4-5.
21. Newport Scientific (1998). Applications manual for the Rapid Visco Analyzer using thermocline for windows. Newport Scientific Pty Ltd., 1/2 Apollo Street, Warriewood NSW 2102, Australia: 2-26.
22. Noor S, G.M. Ali, U. Rashid, M. Arshad, S. Ali and Y. Zafar. 2009. Optimization of callus induction and regeneration system for Pakistani wheat cultivars Kohsar and Khyber-87. Afr. J. Biotechnol. 8(20): 5565-5569.
23. Ogazi, P.O. 1985. Quality Assessment of Plantain Fruits for Dehydration. Nig. Food J., 4(1): 125-130.
24. Olorunda, A.O and M.A. Adelusola. 1997. Screening of Plantain / Banana Cultivars for Import, Storage and Processing Characteristics. Paper Presented at the International Symposium on Genetic Improvement of Bananas for Resistance to Disease and Pests 7 – 9th September, (IRAD, Montpellier, France).
25. Oluwalana, I.B and M.O. Oluwamukomi. 2011. Proximate composition, rheological and sensory qualities of plantain (*Musa parasidiaca)* flour blanched under three temperature regimes. Afr. J. of Food Sci. Vol. 5(14): 769 – 774.
26. Oyewole, O.B., L.O. Sanni and M.A. Ogunjobi. 1996. Production of biscuits using cassava flour. Nig. Food J., 14: 24-28.
27. Ragaee, S., E.M. Abdel-Aal and M. Noaman. 2006. Antioxidant activity and nutrient composition of selected cereals for food use. Food Chem**.** 98: 32-38.
28. Sandhu, K. S, N. Singh and N.S. Malhi 2007. Some properties of corn grains and their flours I: Physicochemical, functional and chapati-making properties of flours. Food Chem., 101: 938-946.
29. Shittu T.A, O.O. Lasekan, L.O. Sanni and M.O. Oladosu. 2001. The effect of drying methods on the functional and sensory characteristics of pukuru-a fermented cassava product. ASSET-An Intern. J., 1(2): 9-16.
30. Sosulski, F. W. 1962. The centrifuge methods for determining flour absorption in hard red spring wheat. Cereal chem. 39: 344-349.
31. Stover, R.H and N.W. Simmonds. 1987. *Bananas*. 3rd ed. Wiley. New York, USA: 97-103.
32. Sudha, M.L., R. Vetrimani and K. Leelavathi. 2005. Influence of fibre from different cereals on the rheological characteristics of wheat flour dough and on biscuit quality. Food Chem. 100: 1365-1370.
33. Tagodoe, A. 1994. Functional Properties of Raw and Precooked Taro Colocasia Esculenta Flour. Int. J. Food Sci. Tech., 29: 457-482.
34. USDA, 2009. National Nutrient Database for Standard Reference, http://www.nal.usda.gov/fnic/foodcomp/plantain/.
35. Wang, J.C and J.E. Kinsella. 1976. Functional properties of novel proteins: Alfalfa leaf protein. J. Food Sci., 41: 286-289.
36. Welford A, L. Izonfuo and F. Victor 1988. Effect of ripening on the chemical composition of plantain peels and pulps (*Musa paradisiaca*). J. Sci. Food Agric. 45: 333-336.

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Ochratoxin A in Cereal Products, Potential Hazards and Prevention strategies: A Review

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ABSTRACT

Mycotoxins are microbial agents which cause food or feed-borne intoxications. Mycotoxins are undesireable compounds often found in cereal grains and forages. Production of mycotoxins is dependent on environmental conditions and agricultural practices. Moisture level ranges between 13% to 18% and temperatures between 20 C and 30 C can increase fungal growth rate during growing seasons as well as during transport and storage. Extensively, mycotoxins are aflatoxins (Afs), ochratoxin A (OTA), Fusraium and Patulin. Ochratoxin is naturally occurring mycotoxin produced by fungi Aspergillus and Penicillium. Family of ochratoxin consist of three member ochratoxin A, B and ochratoxin C. Ochratoxin A is most abundant and most toxic produced mainly by Aspergillus ochraceus, A. carbonarius and Penicillium verrucosum. Penicillium verrucosum mainly produced the ochratoxin A in cereals. A. ochraceus is responsible for ochratoxin A production in coffee, grapes and spices. Conclusively, findings of some researchers related to the mycotoxins, their production and concentration especially ochratoxin A in different cereal products and effect of processing on the ochratoxin A contents and its health implication on human health have been reviewed.

Key Words: Mycotoxins, Ochratoxins A, Toxicity, Health implications, Preventive measures

INTRODUCTION

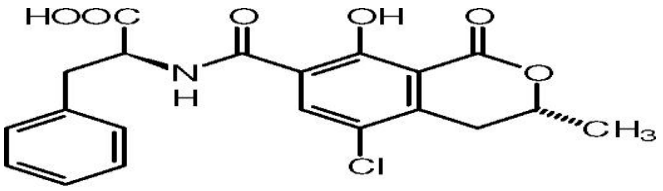
Mycotoxins are natural food and feed contaminants, mainly produced by moulds of genera *Aspergillus*, *Penicillum* and *Fusarium*. The number of mycotoxins known to exert toxic effect on human and animal health is constantly increasing as well as the legislative provisions taken to control their presence in food and feed. Extensively considered mycotoxins are aflatoxins (AFs), ochratoxin A (OTA), *Fusarium* toxins and patulin (Miraglia and Brera, 2000). A valuable review on the most prominent aspects of mycotoxins is given by the CAST Report (CAST, 2003).They can cause acute and chronic toxic effects in animals and can be transferred into products, or may affect human health directly. They are relatively small molecules with highly diverse chemical structures and biological activity. The production of mycotoxins is not essential for the fungal growth or reproduction, but could be a "virulence factor" for some plant diseases and act against other microorganisms and higher organisms (Puschner, 2002). Plant stressors such as draught or over-irritation, insect damage and pesticide exposure result in a higher susceptibility to fungal infection, whereas the production of mycotoxins may be due to stress or altered conditions for the fungus. Mycotoxin production does not only depend on the genotype of a certain strain alone, but also on a range of environmental factors including humidity, temperature, water activity, processing-errors and insect damage, which have an influence on fungal growth and metabolism (CAST, 2003). Most mycotoxins are stable chemical compounds and cannot be destroyed by processing and heat treatment of feed and foodstuffs. When the fungal metabolites present in foods in sufficiently high levels, they can have toxic effects, either acute (for example, liver or kidney deterioration), chronic (for example, liver cancer), mutagenic, or teratogenic.

The resulting symptoms range from skin irritation to immunosuppression, birth defects, neurotoxicity, and death (Murphy et al., 2006). Problems associated with mycotoxin contamination and the economic losses resulting will continue to be seen in food and agriculture industries. Therefore, it can be predicted that food and feed are always contaminated with toxins to a greater or less extent, and with increasing accuracy of analysis (lower detection limits) toxins can be detected in more and more cases.

Ochratoxins

Ochratoxins are mycotoxins produced by several species of *Aspergillus* and *Penicillium*. Among different types of ochratoxins, ochratoxin A is most important, other being the methyl and ethyl ester of OTA which is also known as ochratoxin C (OTC), ochratoxin B (OTB), its methyl and ethyl ester and ochratoxin α. Structurally all the ochratoxins comprise of polyketide-derived dihydroisocoumarin moiety which is linked via the 7-carboxy group to L-β-phenylalanine by an amide bond. Most economically important form of ochratoxin, while OTB and OTC are less important and less common. The empirical formula of OTA is C20H18O6NCl and the molecular weight is 403.82. It is crystalline solid, white in color, slightly soluble in water but highly soluble in the polar organic solvents. OTA is also soluble in the aqueous sodium bicarbonate solution. Ochratoxin A is mainly produced by *Penicillium verrucosum* and several species of *Aspergillus*. Ochratoxin A (OA) has nephrotoxic and immunosuppressive properties (Murphy et al., 2006).

Ochratoxins are widely spread metabolites mainly produced by some toxigenic species of fungi as *Aspergillus* and *Penicillium* (Sedmikova *et al*., 2001; Bayman *et al*., 2002). Major OTA-producing fungus in northern Europe is *P. verrucosum* , more important in warmer climatic zones is *A. ochraceus* (Cairns-Fuller *et al.*, 2005).



ochratoxin A

Major OTA-producing fungus in northern Europe is *P. verrucosum* , more important in warmer climatic zones is *A. ochraceus* (Cairns-Fuller *et al.*, 2005). More *Aspergillus* species have been found to produce OTA, for example *A. sulphureus*, *A. melleus*, *A. sclerotiorum, A. alliaceus*, (Bayman and Baker, 2006), *A. albertensis*, *A. lanosus* (Palumbo *et al*., 2007). OTA is important because of the contamination of ochratoxin A of agricultural products including cereals and grains and influence chronic effect on human exposure (Dehelean, 2011). Natural occurrence in maize and maize-based products is a worldwide problem of OTA contamination (Duarte *et al*., 2010). Maize kernels are a good substrate for mould infection and production of mycotoxins harmful to both humans and animals. *A. niger* is commonly isolated from maize (Shah *et al*., 2010) and a high incidence of *A. carbonarius* has been also reported (Alborch *et al*., 2011). Both species are the main source ofochratoxin in corn and other food products in both subtropical zones and tropical of the world (Palencia *et al*., 2010). There is highest contamination of OAT in cereal grains, and to a lesser extent in grapes, wine, dried vine fruits and grape juice (Clark and Snedeker, 2006).

Production of ochratoxin A

Production of ochratoxins is influenced by various factors including medium composition, temperature and water activity. The fungi responsible for the production of OTA in the cool and temperate regions are mainly *Penicillium verrucosum* or *P. nordicum* .*P. nordicum* is found in the cheese and meat products while *P. verrucosum* contaminates the cereal crops. For the production of OTA in the tropical and semitropical regions *Aspergillus*

ochraceus also referred to as *A. allutaceus* var. *allutaceus*, is mainly responsible and other species of *Aspergillus* i.e. *A. niger* var. *niger* (Biffi *et al*., 2004) .

OTA occurrence in cereal grains

There are multifactorial differences in OTA contamination between cereals. Therefore, it is very difficult to establish relationship between OTA content and individual factor, and therefore it is very hard to expect with all confidence that each type of cereals have the OTA content Gonzalez-Osnaya *et al*. (2007). Garcia et al. (2009), showed association with the early development of predictive mycotoxicology, which have very high importance of food spoilage prevention. Eskola (2002) studied that occurrence and production of ochratoxin A in grains of cereal is largely depends upon the condition of the grain at time of harvest, drying conditions and the storage facilities. Scudamore *et al*. (2003) demonstrated that in the particular circumstances of Western and Northern Europe, Canada and other moderate zones, cereals have high possibility for the production of ochratoxin A because of cereal have high moisture content, sometimes more than 20%. The production of ochratoxin A in grains is due to inadequate aeration or long term storage without adequate drying (Uysal *et al*., 2009). Shah *et al*. (2010) Studied aflatoxin B1 and ochratoxin A and Proimate analysis of maize from Swat Valley, KPK of Pakistan and reported that Upper and Lower Regions, showed AFB1 concentration from zero to 30.92 ppm with average values of 14.94 and 16.22 ppm. Ochratoxin A contamination level was in the range of < 0.001 to 7.32 ppm. Juan *et al*. (2008a) demonstrated that whole-grain cereal samples have high concentration OTA as compared to samples of non-whole-grain cereals (33% versus 14%). Organic whole-grain rye sample showed maximum value which is (27 ng/g). Only seeds or whole grain cereals is important because surface of the grain is site where most of fungi are present, so there are high chances of contamination on the surface of grain is expected.

Jaun *et al*. (2007) collected 61 bread samples of bread from Portugal which were analyzed for ochratoxin A determination. For the determination Liquid Chromatography with Fluorescence Detection was used. Detection limits and quantification were 0.03 and 0.09 ng/g by LC-MS/MS and 0.015 and 0.03 ng/g, using LC–FD. Presence of ochratoxin A in wheat bread is 12.9% and in maize bread 70% . Maize bread showed the highest ochratoxin A level and only one sample surpassed the maximum limits of European legislation which is established for ochratoxin A in cereal products. A. carbonarius grows at rather lower temperatures than A. niger and produces the large number of spores with temperature at 30 centigrade. They also have ability to grow at reduced moisture contentwith maximum germination occurs at 0.85 aw at 25 and at the temperature 30 degree centigrade. A. niger is commonly present in warm climates, because it grows optimally at the relatively high temperatures of 35-37 centigrade. (Magnoli et al., 2007). It is important to mention within few days A. niger isolates are able to produce OTA in commercially grown crops (JECFA, 2008).

OTA levels in contaminated food

Apart from measuring OTA in human fluids and tissues, exposure can also be estimated by measuring OTA levels in contaminated food that may have been consumed. Studies on some foods show that there are differences between the contamination level of different batches of food, and even within the batches, the mycotoxin might not be homogeneously distributed but be restricted to a small part of the batches (Speijers, 2001). Furthermore, the occurrence of mycotoxins can fluctuate considerably in time. Sometimes the mycotoxin concentration can be high for a certain episode, whereas for another it might be negligible low. It is difficult to compare OTA levels

between countries or between types of food, as data on the occurrence of OTA in food and beverages are not available for many commodities in many countries, and the data that are available are often out of date and/or incomplete. The consumption data used were mainly based on intake in Europe . The European Commission (2000) calculated and summarised intake figures for OTA. The total mean intake of OTA for Europe was estimated to be 3.7 ng/kg body weights per day, assuming a body weight of 60 kg.

Table 1: Relative contribution of different food categories to human OTA exposure

Food category	OTA level (μg/kg)	Intake (g)	Daily intake of OTA (ng/kg body weight/ day)	% of total intake
Cereals	0.94	230	3.58	57.8
Wine	0.32	240	1.23	20.8
Grape juice	0.39	69	0.44	7.3
Coffee	0.76	24	0.30	5.1
Pork meat	0.17	76	0.21	3.5
Beer	0.023	260	0.09	1.6
Dry fruits	2.2	2.3	0.08	1.1
Pulses	0.19	25	0.08	1.1
Cocoa	0.55	6.3	0.06	0.8
Poultry	0.041	53	0.06	0.8

Human exposure

Mycotoxins can affect human and animal health, as mentioned before. In general, animals are directly exposed to mycotoxins through the consumption of mouldy feedstuff. Human exposure can be via one of two routes; direct exposure due to the consumption of mouldy plant products, or indirect exposure through the consumption of contaminated animal products, containing residual amounts of the mycotoxin ingested by the food producing animals (Boutrif and Bessy, 2001). However, animal derived food products contribute to a lesser extent to human OTA exposure, with the exception of babies and infants, due to their high consumption of milk and milk products, and their specific metabolism (Kuiper-Goodman, 1998).

Toxicity and health implications

Metabolism

OAT is absorbed through gastrointestinal tract. Ochratoxin A is absorbed from the stomach due to its acidic properties in most species. Ochratoxin A is circulated through blood, mainly to the kidneys, and at lower concentrations to the muscle, fat and liver, with a proportion metabolised into the non-toxic metabolite and other less toxic metabolites. Ochratoxin A has a long serum half-life in non-ruminant animals and in humans (72-120 h in pigs, 840 h in a human subject). Cows and sheep (ruminant animals) are normally resistant to the toxic effect of OTA because before the absorption into blood it is hydrolyzed into the metabolites of non-toxic substances in the stomachs by protozoa. (Kiessling *et al.*,1984).

Acute toxicity

The acute toxicity of OAT is generally low, although large species differences and sensitivity are seen with oral LD50 values ranging widely in different species. Oral LD50 values has been demonstrated to range from 1 mg/kg bw in pigs, 0.2 mg/kg bw in dogs 3.3, 46-58 mg/kg bw in mouse and mg/kg bw in chicken, and. Effects of acute poisoning such as multifocal haemorrhages in various organs and fibrin. At present, there are no documented cases of acute toxicity reported in human.

Chronic toxicity

The chronic effects of ochratoxin A are of great importance. It has been shown to be hepatotoxic, nephrotoxi, teratogenic and immunotoxic to species of animals and carcinogenic to different species of animals.

Neurotoxicity

It has been shown that the administration of OTA during the gestation period in rats encouraged malformations in different portion of the central nervous system . In the same way, Soleas *et al.* reported that OTA can cause certain lesions as well as damage at the in cerebral system. Thus, OAT seems to be highly toxic for the nervous cells and able to reach at any time the neural tissue.

Teratogenicity

OTA is a potent teratogen to laboratory animals. It cross the placenta during the gestation period and store in fetal tissue causing morphological anomalies. The mechanism has not been clearly defined and may involve an indirect effect through maternal action and/or a direct effect on the developing conceptus. Thus, intensity of malformations depends on the gestative period and route of administration.

Immunotoxicity

OTA presents a powerful immunosuppressor effect under the certain conditions, Necroses of lymphoid tissues which shows there high sensitivity to the OTA. OTA seems to play a role in the inhibition of the peripherals T and B lymphocytes proliferation and stops the production of interleukin 2 and its receptors. Moreover, it stops the production of killer cells as well as the production of interferon. The administration of OTA to many animal species causes variable effects on the osseous marrow and immunity response. Thus, this molecule is considered to be the origin of:

- Regression of the thymus
- Lymphopenia,
- Suppression of the immunity response.

Following these results, OTA is clearly taken as an important immune-suppressor agent.

Carcinogenesis

OTA is reasonably expected to be a human carcinogen based evidence of carcinogenicity in experimental animals. When this molecule was administered in the diet, hepatocellular tumors, renal cell tumors, hepatomas, and hyperplastic hepatic nodules were observed in male mice.

Prevention or management of Mycotoxins Contamination

For preventing fungous growth on grains, you should dry them fast and wholly and maintain them on dry place. For preventing OTA forming by *A. ochraceus*, activity of the grain by moisture would reduce to less than 0.8. The most influential method of grains storing includes; steaming, airing. Sealed storing and controlled atmosphere especially in tropical and mid tropical region in which insect damage is critical problem. Controlled atmosphere storing is based on applying atmosphere by low oxygen or high density of dioxide carbon. Using improved atmosphere to control insect helps to the control of fungus

Improved Farm management

Research on pre-harvest OAT contamination has revealed that on favorable conditions fungus can attack on plants in the field and therefore, result OAT contamination. Certain commodities such as peanuts, corn and cottonseed growing under stress condition are vulnerable for fungus attack. To prevent pre-harvest mycotoxin contamination, good management practices, such as using sound, fungus free seeds for planting, controlling insects and plant disease and controlling irrigation practices,

should be implemented. Furthermore, mycotoxin contamination can also be minimized by using proper adjustment and operation of harvesting equipment during crop harvesting (CAST, 2003).

Temperature

The use of proper temperature management during storage could prevent mycotoxins contamination in food or food products. To avoid mycotoxin contamination during transportation containers or sacks must be free from any contamination before taking mature products. The food overripe, damaged, fermented or fallen onto the soil must be discarded and eliminated even off the field, as they are likely to have high mycotoxin levels or to harbor mycotoxin-producing molds which could be rapidly propagate (Perez de Obanos, *et al*., 2005). Blend with poor quality products or with other commodities is also unadvisable (Lopez-Garcia, *et al*., 2008). The food and food products have to be homogeneously and fastly dried under clean and environmentally controlled conditions. In the case of tropical crops, it is very important not to store fresh products (FAO, 2006). Piles have to be turned over in order to promote their aeration and to prevent mycotoxigenic mold development (Kouadio *et al*., 2006). The FAO/WHO/UNEP, 1999c has recommended that carrying out a fast drying and, in general, under a moisture level of 10%. In coffee, it has been demonstrated that sun drying, less expensive than mechanical dryers, entails greater risks of mycotoxin contamination (Suarez-Quiroz *et al*., 2005). Moreover, Lopez-Garcia *et al*., 2008 have pointed out the importance of using small driers to avoid long periods of wet products storage.

Adsorbing Gages

For many years modified atmospheres or alternative gases have been examined for the medium and long term storage of cereal grain destined for food/feed. While fungi involved In biodeterioration of grain are considered to be obligate aerobes, many are actuallymicroaerophilic, being able to survive and grow in niches where other species cannot grow and thus dominate specialized grain ecosystems. In many cases decreasing O2 by 0.14% is required before growth can be substantially reduced. Increasing CO2 to N2 50% is required for inhibition of mycelial growth. Some fungi, e.g. *P. roqueforti*, are able to grow and infect grain at 80% CO2 provided at least 4% O2 is present. The use of integrated post-harvest systems for prevention of deterioration entails modifying O2 and CO2 simultaneously and the use of (O2 free) N2. The tolerance to low O2 and high CO2 is also influenced by interactions with grain type and water availability. The drier the grain, the more effective the treatment. Modified atmosphere storage is used for control of both moulds and insects in moist stored grain. Regimes sufficient for moulds may not however be effective against some storage insects, which can survive and grow over a wider equilibrium relative humidity range. Modified atmosphere storage has been examined for the storage of moist grain especially for animal feed. Studies with *P. verrucosum* and *A. ochraceus* with up to 50% CO2 suggest that spore germination is not markedly affected, although germ tube extension and hence colonization is significantly inhibited by 50 to 75% CO2, especially at 0.90 to 0.995 aw for both *P. verrucosum* and *A. ochraceus* (Cairns-Fuller *et al*., 2005). Growth and OTA production were highest in air, followed by 25 and 50% CO2 regardless of the aw level tested on wheat grain. Generally, CO2 and aw together cause an enhanced inhibitory effect, although this was not synergistic.

Removing Ochratoxin

There is much strategy for reducing OTA level. These methods are used for eliminating or reducing OTA level. These different technology are ranked according physical, chemical and biologically, microbiologically methods. Ideal toxin method is easy and expensive and don't

produce toxin compound or don't change quality parameters of material (Hundhausen *et al*., 2005).

Physical Methods

Physical methods include division, sorting, purification; peeling, peeling procedure's aim is removing the most contaminated one. They include using materials as additive food in which absorbed through OTA, hence reduce biologic frequency (Riley and Norred, 1999).

Chemical Methods

These methods require compounds for removing . We use ammonium, alkali hydrolyze, bisulfites and ozone in some procedures they has been reported as an effective compound for eliminating OTA and other mycotoxins. Although some chemical residue may remain, we don't study there is reducing on taste and quality of cared foodstuff (Riley and Norred, 1999).

Microbiologic Methods

By the aids of microorganism for decomposing, absorb or changing OTA, to remove toxin from contaminated products or when eating mycotoxin .Carboxypeptidase A could damage OTA. Using a toxigenic *A. niger* strain has been suggested as carboxypeptidase source (Varga et al., 2000). Other enzymes in which get from *A. niger* and damage OTA includes: lipase (Stander et al, 2000), enzymatic crude and metalloenzyme. We have one carboxypeptidase from phaffia rhodozyma in which damage more than 90% of OTA (Abrunhosa *et al*., 2007). In addition to it special bacteria belongs to *Streptococcus*, *Bifidobacterium*, *Lactobacillus*, *Butyribrio*, *phenylobacterium, pleurotus*, *Saccharomyces*, *Bacillus* and *Acinetobacter* and special fungus belongs to *Aspergillus*, (*A. fumigatus*, *A.* niger, *A. carbonarius*, *A. japonicus*, *A. versicolor*, *A. wentii*, *A. ochraceus)* and *Botrytis*, *Cladosporium*, *Phaffia*, *Penicillium*, *Rhizopus* (*R. stolonifer*, *R. oryzae*) could damage more than 95% of OTA on in vivo (Peteri *et al*., 2007).

Conclusion

Ochratoxin contamination cause serious health disparities. Therefore, keeping toxins in low levels in foodstuff is of great importance. To minimize contamination, foodstuff should be kept below 14% moisture level. As, these are moderately stable compounds therefore cooking has no effect on the reduction of mycotoxin level. To control this emerging problem it is compulsarily needed to pass some regulations to decrease mycotoxigenic moulds in food. To control mycotoxins growth at stores by controlling the moisture contents and preventing the cereals from damage. Its imperative to reduce mould contamination by opting the international standards for safe storage of food and feed; because, the contaminated products endanger human health seriously.

REFERENCE

1. Abunrosa, L., L. Santo, A. Venancio. 2006. Degradation of ochratoxin-A by proteases and crude enzyme extract of Aspergillus niger. Food Biotechnol. 20: 231-236.
2. Alborch, L., M.R. Bragulat, M.L. Abarca, F.J. Cabanes. 2011. Effect of water sactivity, temperature and incubation time on growth and ochratoxin A production by Aspergillus niger and Aspergillus carbonarius on maize kernels. Int. J. Food Microbiol. 147: 53-57.
3. and brew preparation on the ochratoxin A content in coffee infusion. Food Addit. Contam. 22:463–471.
4. Bao, L., R. Krska, T. Goto, M. Arcinas, A. Morales-Diaz, J. Baldi and R.E. Poms. 2006. Impacts of mycotoxin regulations on world trade. Retrieved May 7, 2009 from: http://en.engormix.com/MA mycotoxins/articles/impacts-mycotoxin-regulations- world_139.htm.

5. Bayman, P., J. Baker, L. Doster, M.A. Michailides, T. J. Mahoney. 2002. Ochratoxin production by the Aspergillus ochraceus group and Aspergillus alliaceus. Appl. Envir. Microbio. 68: 2326-2329.
6. Bayman, P., J. Baker, L. Doster, M.A. Michailides, T. J. Mahoney. 2002. Ochratoxin production by the Aspergillus ochraceus group and Aspergillus alliaceus. Appl. Envir. Microbio. 68: 2326-2329.
7. Bhattacharya, K., S. Raha. 2002. Deteriorative changes of maize, groundnut and soybean seeds by fungi in storage. Mycopathol. 155: 135-141.
8. Biffi, R., M. Munari, L. Dioguardi, C. Ballabio, A. Cattaneo, C.L. Galli and P. Restani. 2004. Ochratoxin A in conventional and organic cereal derivatives: a survey of the Italian market. Food Addit. Contam. 21: 586-591.
9. Blesa, J., H. Berrada, J.M. Soriano, J.C. Molto and J. Manes. 2004. Rapid determination of ochratoxin A in cereals and cereal products by liquid chromatography. J. Chromatogr. A. 1046: 127-131.
10. Boutrif, E. and C. Bessy. 2001. Global significance of mycotoxins and phycotoxins. In: Mycotoxins and phycotoxins in perspective at the turn of the millennium. Koe, W.J., Samson, R.A., van mond, H.P., Gilbert, J. and Sabino, M. (eds.). Ponsen and Looyen, Wageningen, The Netherlands, 3-16.
11. Bullerman, L.B., A. Bianchini. 2007. Stability of mycotoxins during food processing. Int. J. Food Microbiol. 119: 140-146.
12. Cabanas, R., M.R. Bragulat, M.L. Abarca, G. Castella, F.L. Cabañes. 2008. Occurrence of Penicillium verrucosum in retail wheat flours from the Spanish market. Food Microbiol. 25: 642-647.
13. Cairns-Fuller, V., D. Aldred, N. Magan. 2005. Water, temperature and gas composition interactions affect growth and ochratoxin A production by isolates of Penicillium verrucosum on wheat grain. J. Applied Microbiol.vol. 99: 1215-1221.
14. Clark, H.A. and S.M. Snedeker. 2006. Ochratoxin A: Its cancer risk and potential for exposure. J. Toxicol. Envir. Health. 9: 265–296.
15. Council for Agricultural Science and Technology (CAST). 2003. Mycotoxins. In: Risks in plant, Animal and Human system. Task Force Report, No. 139. Cast, Ames, USA, 199 S.
16. date: 2008/03/10).
17. Dehelean, C.A., E. Alexa, S. Feflea, P. Georgeta and P. Camelia. 2011. Ochratoxin A: a toxicologic evaluation using in vitro and in vivo bioassays. In Analele Universităţii din Oradea - Fascicula Biologie Tom. 2: 99-103.
18. Domijan, A.M., M. Peraica, Z. Jurjevic, D. Ivic, B. Cvjetkovic. 2005. Fumonisin B1, fumonisin B2, zearalenone and ochratoxin A contamination of maize in Croatia. Food Addit. Contam. 22: 677-680.
19. Duarte, S.C., A. Pena, C.M. Lino. 2010. A review on ochratoxin A occurrence and effects of processing of cereal and cereal derived food products. Food Microbiol.27: 187-198.
20. Elmholt, S., P.H. Rasmussen. 2005. Penicillium verrucosum occurrence and ochratoxin A contents in organically cultivated grain with special reference to ancient wheat types and drying practice. Mycopathologia. 159 :421-432.
21. Eskola, M., P. Parikka and A. Rizzo. 2001. Trichothecenes, ochratoxin A and zearalenone contamination and Fusarium infection in Finnish cereal samples in 1998. Food Addit. Contam. 8: 707-718.

22. European Commission. 2000. Exposure assessment to certain contaminants. Reports of the Scientific Committee on Food (SCF), Health and Consumer Protection Directorate-General, Brussels, Belgium.

23. FAO. 2006. Reducing OTA in coffee. Available at: http://www.coffee-ota.org/ (accession
24. Feed Sci. Technol. 133: 149–166.
25. Food Addit. Contam. 25: 231–240.
26. Garcia, D., A.J. Ramos, V. Sanchisa and S. Marín. 2009. Predicting mycotoxins in foods: a review. Food Microbiol0.. doi:10.1016/j.fm.2009.5.014.
27. Gonzalez, L., C. Juan, J.M. Soriano, J.C. Molto and J. Manes. 2006. Occurrence and daily intake of ochratoxin A of organic and non-organic rice and rice products. Int. J. Food Microbiol. 107: 223-227.
28. González-Osnaya, L., J.M. Soriano, J.C. Molto and J. Mañes. 2007. Dietary intake of ochratoxin A from conventional and organic bread. Int. J. Food Microbiol. 118: 87-91.
29. Hundhausen, C., C. Bösch-Saadatmandi K. Augustin, R. Blank, S. Wolffram and G. Rimbach, 2005. Effect of vitamin E and polyphenols on ochratoxin Ainduced cytotoxicity in liver (HepG2) cells. J. Plant Physiol. 162: 818-822.
30. JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2008. Safety Evaluation of Certain Mycotoxins in Food. WHO Food Additives Series 59. Retrieved 18, 2009 from. http://whqlibdoc.who.int/publications/2008/9789 24 1660594_eng.pdf. Jørgensen, K., Rasmusse.
31. JECFA, Joint FAO/WHO Expert Committee on Food Additives. 2001. Safety evaluation of certain mycotoxins in food. FAO, Food and nutrition paper 74, food additive series 47, Rome, Italy, 281-415.
32. Juan, C., J.C. Molto, C.M. Lino and J. Mañes. 2008a. Determination of ochratoxin A in organic and non-organic cereals and cereal products from Spain and Portugal. Food Chem. 107: 525-530.
33. Kouadio, A.I., N.B. Agbo, A. Lebrihi, F. Mathieu and M. Dosso. 2006. Effect of the frequency of the mixing of coffee cherries put out for drying on the kinetics of drying and the relationship to ochratoxin A production. Food Addit. Contam. 23: 295–304.
34. Kuiper-Goodman, T. 1998. Food safety: mycotoxins and phycotoxins in perspective. In: Mycotoxins and phycotoxins- developments in chemistry, toxicology and food safety. Proceedings of the IX IUPAC, International Symposium. Miraglia, M., van Egmond, H., Brera, C. and Gilbert, J. (eds.), Fort Collins, Alaken Inc, Colorado, USA, 25-48.
35. Lee, H.B., N. Magan. 2000. Impact of environment and interspecific interactions between spoilage fungi and Aspergillus ochraceus on growth and ochratoxin production in maize grain. Int. J. Food Microbiol. 61: 11-16.
36. Lopez-Garcia, R., C. Augusto Mallmann and M. Pineiro. 2008. Design and implementation of an integrated management system for ochratoxin A in the coffee production chain.
37. Magnoli, C.E., A.L. Astoreca, S.M. Chiacchiera and A.M. Dalcero. 2007. Occurrence of ochratoxin A and ochratoxigenic mycoflora in corn and corn based foods and feeds in some South American countries. Mycopathol. 163: 249-260.

38. Miraglia, M. and C. Brera. 2000. Mycotoxins in grains and related products. Food analysis by HPLC. ed 2., pp: 452-493.
39. Murphy, P. A., S. Hendrich, C. Landgren, and C. M. Bryant. 2006. Food mycotoxins: An update. J. Food Science. 71: 51-65.
40. Palencia, E. R., D.M. Hinton, and C.W. Bacon. 2010. The black Aspergillus species of maize and peanuts and their potential for mycotoxin production. . In Toxins. 2: 399-416.
41. Palumbo, J. D., T.L. O'Keeffe, N.E Mahoney. 2007. Inhibition of ochratoxin A production and growth of Aspergillus species by phenolic antioxidant compounds. Mycopathologia. 164: 241-248.
42. Perez de Obanos, A., E. Gonzalez-Penas and A. Lopez de Cerain. 2005. Influence of roasting
43. Peteri, Z., J. Teren, C. Vagvolgyi and J. Varga. 2007. Ochratoxin degradation and adsorption caused by astaxanthin-producing yeasts. Food Microbiol. 24: 205-210.
44. Puschner, B. 2002. Mycotoxins. Veterinary Clinics of North America Small Animal Practice, 32: 409-419.
45. Riley, R.T. and W.P. Norred, 1999. Mycotoxin prevention and decontamination—A case study on maize. Food Nutr. Agric. 23: 25-32.
46. Scudamore, K.A., 2005. Prevention of ochratoxin A in commodities and likely effects of processing fractionation and animal feeds. Food Addit. Contam. Part A 22, 17-25.
47. Sedmikova, M. H. Z. Reisnerora, I. Dufkova, F. Burta. 2001. Potential hazard of simultaneous occurrence of aflatoxin B1 and ochratoxin A. Vet. Med. vol. 46: 69-174.
48. Shah, H. U., T.J. Simpson, S. Alam, K.F. Khattak and S. Perveen. 2010. Mould incidence and mycotoxin contamination in maize kernels from Swat Valley, North West Frontier Province of Pakistan. Food Chem. Toxicol. 48: 1111-1116.
49. Speijers G.J.A. abd H.P. Van Egmond. 1993. Worldwide ochratoxin A levels in food and feeds. Human Ochratoxicosis and its Pathologies. 231: 85-100.
50. Stander, M.A., U.T. Bornscheuer, E. Henke and P.S. Steyn, 2000. Screening of commercial hydrolases for the degradation of ochratoxin A. J. Agr. Food Chem., 48: 5736-5739.
51. Suarez-Quiroz, M., O. Gonzalez-Rios, M. Barel, B. Guyot, S. Schorr-Galindo and J.P. Guiraud. 2005. Effect of the post-harvest processing procedure on OTA occurrence in artificially contaminated coffee. Int. J. Food Microbiol. 103: 339–345.
52. Valle-Algarra, F.M., E.M. Mateo, A. Medina, F. Mateo, J.V. Gimeno-Adelantado and M. Jiménez. 2009. Changes in ochratoxin A and type B trichothecenes contained in wheat flour during dough fermentation and breadbaking. Food Addit. Contam. - Part A 26, 896-906.
53. Varga, J., K. Rigó and J. Téren. 2000. Degradation of ochratoxin A by Aspergillus species. Int. J. Food Microbiol., 59: 1-7.
54. Weidenbörner, M., C. Wieczorek, S. Appel and B. Kunz. 2000. Whole wheat and white wheat flourdthe mycobiota and potential mycotoxins. Food Microbiol. 17: 103-107.