Product Quality and Consumer's acceptability of cooked breast muscle of Nigerian indigenous ducks raised in different zones in Ekiti State

**Abstract** 

The rate and extent of consumer's demand for safe and quality meat products depend on the processing methods that will not alter the nutritional and eating qualities of the finished products. The study evaluated physicochemical properties and consumer's acceptability of cooked breast muscle of indigenous ducks raised in different zones in Ekiti State, Nigeria. The study was conducted at the Laboratory of Department Animal Science, Faculty Agricultural Science, of Ekiti State University, Ado-Ekiti, Nigeria. Twenty four matured indigenous ducks aged 24 months comprising 12 drakes and 12 ducks raised under extensive system were randomly selected from three different geo-graphical locations in Ekiti State, Nigeria. Animals were weighed, stunned and exsaguinated, defeathered, eviscerated, breast muscles separated, wrapped in a polythene bags and chilled at 4°C for 24hours. The breast muscles were divided into two portions, labeled according to sex and locations. The thaw loss and pH were evaluated after 24 hours. The meat samples were cooked by two methods (pan and deep fry). The cook loss, water holding capacity, shear force, lipid stability and yield of cooked breast meat were evaluated. A six- member taste panelist rated the organoleptic characteristics of cooked samples using ninepoint hedonic scale. The results showed that water holding capacity, %cook loss, % cook yield, shear force, Thiobarbituric Acid Reacting Substance (Tbars) values and sensory properties were significantly (p<0.05) influenced by cooking methods. Both sexes of location B recorded similar shear force values (8.0kg/cm<sup>2</sup>) cooked by pan fry. The overall acceptability of cooked breast meat by deep fry method was recorded from location A ducks of either sex while pan fried breast muscle were observed in location B samples of the drake meat. The study showed that drake breast muscles cooked by either method had better yield, quality and shelf stability than female counterpart

**Key words:** duck, indigenous, sex, breast meat, cooking methods

### 1.0 Introduction:

The production and demand for duck meat is very popular in most part of the world [1] but this is not so in Nigeria where only a few farmers are involved in the production. Several factors could be responsible for the short fall in the production of ducks in Nigeria; ranging from scarcity of exotic breeds that could be used to upgrade the indigenous ducks, high cost of

production and poor management system[2]. Interest of farmers could be motivated by the conversion of the duck meat to more fascinating cooked products such as fried duck meat. Methods adopted for cooking meat especially poultry could influence the meat quality, consumers' likeness and shelf stability of the products. In order to increase the demand for poultry meat more importantly duck meat in Nigeria, various methods of cooking should be used for its processing. The nature and extent of oil used for cooking muscle food is very crucial for a healthy consumption of fried meat by the consumers and keeping qualities of the finished products. In the recent times, studies have shown that consumption of highly saturated fatty acid content in muscle foods was associated with coronary heart diseases [3]. Attempt to reduce this kind of disease is a great concern to meat processors. One way to reduce the risk is by cooking meat in unsaturated fats. Frying method of cooking should be done in a healthier manner that will be very safe for the consumers. The study focused on value addition of meat from local ducks raised under extensive system using two frying methods viz-a-viz pan and deep fry and its effect on qualities, shelf stability and sensory properties.

#### 2.0 Materials and methods

# 2.1 Sample collection

Twenty four (24) indigenous ducks comprises 12 drakes and 12 ducks of 24-months of age were purchased from household keepers from three geo-political locations of Ekiti state. The birds were kept separately in the pen, fed with grains and water provided for a period of two weeks. The birds were weighed to determine live weight. The birds were immobilized mechanically, slaughtered, defeathered and eviscerated. The breast muscle was dissected from the carcass, breast muscle was filleted, weighed and wrapped in polythene bag and chilled at 4°C over night.

# 2.2 Cooking of samples

The meat samples were spiced and weighed prior cooking. Frying was done with unsaturated canola oil [4]. The breast meat of ducks was pan fried for 5 minutes per side in 100ml canola oil in a Teflon-coated pan, while deep frying was done in 500ml of canola oil for 10 minutes in a commercial stainless steel deep-fryer. Cooking oil was preheated to 180°C and samples were fried to internal temperature of 70°C and 75°C for pan fry and deep fry respectively. Cooked samples were cooled to room temperature, drained and dried with kitchen tissue paper and thereafter weighed.

# 2.3 pH determination

The pH of breast muscles sample was measured using a pH meter (MP230, Mettler, Switzerland) that was calibrated with standard pH buffers of 4.0 and 7.0. One gramme (1g) of the cooked sample was taken, blended and mixed with 10ml of distilled water. The mixture was thoroughly mixed, and pH electrode introduced into the mixture to take the pH value.

# 2.4 Water holding capacity (WHC)

The water holding capacity (WHC) was estimated according to method of Grau and Hammo [5] as described by Kwon et al.[6]. One gramme (1g) of breast meat was weighed into a Whatman filter paper (No. 1, 11cm diameter, Labsman, India). The samples were pressed between two plexiglass plates for 3 min. The areas of pressed sample and water were measured using planimeter. Water holding capacity was evaluated as 100% minus free water percent.

### 2.5 Cooking loss

Breast meat samples were cooked by two methods of pan and deep fry to an internal temperature of 75°C for 45 min respectively, surface dried, and weighed. Cooking loss was determined by expressing cooked sample (W<sub>2</sub>) weight as a percentage of uncooked samples (W<sub>1</sub>) weight by the methods of Yang et al.[7].

Cooking loss (%) =  $[(W_1-W_2)/(W_1)]\times 100$ 

# 2.6 Cooking yield (%)

The cooking yield was calculated using the method described by Kembi and Okubanjo [8]. It was expressed as the ratio of the final weight of the product to the initial weight of raw samples of meat multiplied by 100%.

# 2.7 Shear force

Cooked meat samples, 2.0cm<sup>2</sup> in diameter were sheared at three locations with Warner-Bratzler V-notch blade shearing instrument [9].

# 2.8 Thiobarbituric Acid Reacting Substance (TBARS) analysis

Tbars of the cooked samples were determined by Ali et al.'s procedure [10]. Five grammes (5g) of fried breast muscle by either method was weighed into a 50-ml test tube and homogenized with 15 ml of deionized distilled water for 10s at the highest speed. 1 ml of meat homogenate was place into a test tube, and butylated hydroxyanisole (50 μl, 10%) and thiobarbituric acid/trichloroacetic acid (TBA/TCA, ml) were added. The mixture was vortexed and incubated in a boiling water bath for 15 min until colour changed. The sample was cooled in cold water for 10 min, vortexed for the second time, and centrifuged for 15 min (2,000×g). The supernantant was decanted after cooling. The absorbance of the resulting supernatant solution was determined at 531 nm against a blank containing 1 ml of double distilled water and 2 ml of TBA/TCA solution. The amount of TBARS was expressed as milligrams of malondialdehyde per kilogram of meat.

# 2.9 Sensory Evaluation of fried breast muscle

This was evaluated according to standard procedures of AMSA [11]. A six-member semi-trained taste panel was used for rating of samples. The panelists were given unsalted biscuits and water for use in between the treatments samples. Samples were coded and presented sequentially to the panelists on a clean flat saucer and were evaluated independently of each other. The panelists rated the cooked samples on a 9-point hedonic scale on which 1=dislike extremely and 9=like extremely for aroma, flavour, tenderness, juiciness, texture and overall-acceptability, where higher values indicated higher preference for fried breast muscle from each treatment.

# 2.10 Statistical analysis

The data obtained in the study were analyzed by the analysis of variance procedure of Minitab Statistical package and Tukey pair wise was used for the separation of means at a 5% level of significance [12].

# 3.0 RESULTS AND DISCUSSION

# 3.1Physicochemical properties and cook yield

Table 1 shows the physicochemical properties and cook yield of breast muscle cooked by deep fry method in sexed ducks obtained from different geo-political zones. The water holding capacity was higher (p<0.05) in drake (70.0%) of location B (Ekiti North) than other locations.

The water holding capacity was highest (p<0.05) in female (71.3%) of location A ducks. The result of WHC obtained for deep frying was higher than those observed by Omojola et al. [4]. The high WHC content of the fried breast muscles in both sexes suggests that deep frying may not influence duck meat to losses its bound water during processing. The high WHC in the fried samples may influence better eating qualities such as juiciness and tenderness. The pH values differ significantly (p<0.05) in breast muscle of both sexes of ducks from different locations. The pH value observed was closer to the findings of Carvalho et al. [13] in chicken breast. The normal pH values observed in the study may have improved other meat qualities such as WHC, texture and tenderness of duck breast meat. The percent cook loss of drake breast muscle of location B (Ekiti North) had the least value of 9.52% while the highest of 31% was found in female duck of location A. The study showed that cook yield was highest (91%) in drake of location B while the value of 78% yield was recorded in female ducks of location C. High cook yield indicates that frying by deep method can enhance the capacity of meat processors and food vendors to earn more and this substantiate the fact that value addition plays a key role in livestock industry. The least shear force value of 4.83kg/cm<sup>2</sup> was obtained from the female of location A. Location A and B drakes had similar shear force values of 7.67 kg/cm<sup>2</sup>, but lower than those obtained in location C drake. The shear force values obtained in the study in both sexes were higher than those reported by Omojola et al.[4] and Ali et al.[10]. Breast muscle of location B had the lowest thars value for drakes while location A of female duck had the least tbars value. The tbars obtained in the study in both sexes were closer to the findings of Omojola et al.[4]. That serves an indication tool for identifying rate at which the lipid contents of the fried muscle deteriorates due to the influence of the unsaturated fatty acid present in the cooking oil.

The physicochemical properties and cook yield of pan fried breast muscles of indigenous ducks raised in different locations is as shown in Table 2. The highest WHC (74.0%) was found in the female from location C. WHC of drake breast muscle ranged from 69% (location C) and 71.0% (location B). The highest pH<sub>24-hr</sub> value of 6.1 was found in drake muscle of location A. This was similar to those observed by Kwon et al. [6] in duck, which indicates that the glucose reserved in the muscle had not been totally depleted and that the muscle was still undergoing the processing of rigour mortis. The pH values obtained for drakes from other locations (B and C) and female ducks counterparts were within the ultimate pH value for meat at 24 hours post-mortem. This may have accounted for high WHC values obtained in the cooked breast muscles by either method in both sexes. The percentage cook loss of drake muscle of location B had the least value below 10% while the female cook out values was lower than 25% but Ali et al. [10] reported higher cooking loss value in duck breast meat. This shows that pan fry method of cooking had prevented shrinkage and collapse of muscle fibre that could have resulted into release of juice in forms of water and fats due to minimal exposure of the muscle to minimal thermal heat during cooking. The study revealed that drakes cooked by pan fry had higher cook yield than the female counterparts. The highest cook yield of 94% was obtained from drake muscle of location B followed by female breast muscle of location C with 81% cook yield cooked by pan fry method.

The shear force values were lower (p<0.05) in drake of location A than locations B and C, but shear force values were similar between locations B and C samples. The shear force value obtained in the study for pan fry duck muscle was higher than the value reported by Omojola et al. [4]. Ali et al. [10] also observed lower shear force value in duck breast. The shear force value obtained in the study may have been influenced by effect of sex, age at slaughter and cooking

temperature on the muscle structure. The tbars values differ significantly (p<0.05) among ducks of different locations. This was higher than those reported by Carvalho et al. [13] in chicken breast fillet. Tbars values were extremely lower (p<0.05) in drake muscles than the female. This shows that drake's muscles had lower intramuscular fat than the female, this could support longer shelf keeping of pan fried drake meat than female meat. Low Tbars value was an indication that the rate at which lipid oxidizes in fried product was minimal especially in male as compared with female.

### 3.2 Sensory evaluation

Table 3 shows the sensory properties of breast muscles of ducks cooked by deep fry method. Significant difference (p<0.05) was observed in sensory attributes such as aroma, flavor, tenderness, juiciness, texture and overall acceptability between drakes and female ducks of raised different locations. The ratings obtained for all sensory attributes were closer to the findings of others Omojola et al.[4] and Augusty´nska-Prejsnar et al. [14]. The panelist rated the overall acceptability of deep fried duck meat from location A (Ekiti central) of both sexes higher than locations B (Ekiti North) and C (Ekiti South) samples. Overall acceptability of deep fry of drake was rated between slightly liked (6.0) of location C and liked very much (8.0) of location A, while female ducks rated from intermediately liked (5.25) of location C and liked very much (8.0) of location A.

Table 4 shows the sensory properties of breast muscles of ducks cooked by pan fry method. There were significant difference (p<0.05) between sensory attributes for aroma, flavor, tenderness, juiciness, texture and overall acceptability in drakes and female ducks of different locations. Drake breast muscle of location A had the highest rating for juiciness. High WHC, lipid and cooking procedure usually determine juiciness of meat [15,16]. The panelist rated the overall acceptability of duck meat from location A (Ekiti central) of both sexes cooked by deep fry higher (p<0.05) than locations B (Ekiti North) and C (Ekiti South) samples. The variations obtained were based on the consumers' rating using nine hedonic scales. The result of sensory properties of pan fried breast muscles of indigenous ducks rated by panelists on nine hedonic scales were not similar to those reported by Omojola et al. [4] for pan fry duck meat. The overall sensory properties of pan fry drake breast muscles rated between slightly like (6.0) of location C and liked very much (8.0) of location A. while female ducks rated from intermediately liked (5.75) of location C and liked moderately (7.0) of location A.

Table1: Physico-chemical properties and cook yield of breast muscle of indigenous ducks raised in different locations cooked by deep fry method.

Items	sex	Location A (Ekiti Central)	Location B (Ekiti North)	Location C (Ekiti South)	SEM	p-value
Raw breast weight (g)	Male	77.0°	162.0 <sup>b</sup>	137.0 <sup>a</sup>	0.01	0.00
	Female	62.0°	81.0 <sup>b</sup>	124 <sup>a</sup>	0.04	0.00

Water Holding Capacity (%)	Male	67.3°	$70.0^{a}$	69.0 <sup>b</sup>	0.13	0.09
	Female	71.3 <sup>a</sup>	$55.0^{\circ}$	$63.0^{b}$	0.34	0.00
pH@24 hrs (raw meat)	Male	$5.90^{a}$	5.77 <sup>b</sup>	5.57 <sup>c</sup>	0.03	0.30
	Female	5.95 <sup>a</sup>	5.67 <sup>b</sup>	5.53 <sup>c</sup>	0.02	0.10
Cook loss (%)	Male	$29.0^{a}$	9.52°	$18.0^{b}$	0.04	0.00
	Female	31.0 <sup>a</sup>	$29.0^{c}$	$23.0^{b}$	0.05	0.00
Cook yield (%)	Male	71.3°	91.0 <sup>a</sup>	83.0 <sup>b</sup>	0.05	0.00
	Female	69.2 <sup>c</sup>	$72.0^{b}$	$78.0^{a}$	0.05	0.00
Shear force (kg/cm <sup>2</sup> )	Male	7.67 <sup>b</sup>	7.67 <sup>b</sup>	$8.30^{a}$	0.06	0.30
	Female	4.83°	$9.00^{b}$	9.33 <sup>a</sup>	0.08	0.00
Tbars(mg malonaldehyde/kg	Male	$0.52^{b}$	$0.42^{c}$	$0.63^{a}$	0.01	0.04
meat)						
	Female	0.61 <sup>c</sup>	$0.62^{b}$	$0.72^{a}$	0.01	0.40

a, b, c- means with different superscripts on same row are significantly different (P<0.05), SEM- standard error of means, p-probability of mean effects

Table2: Physico-chemical properties and cook yield of breast muscle of indigenous ducks raised in different locations cooked by pan fry method.

Items	sex	Location A (Ekiti Central)	Location B (Ekiti North)	Location C (Ekiti South)	SEM	p-value
Raw breast weight (g)	M	88.4°	148.1 <sup>b</sup>	157.2 <sup>a</sup>	0.15	0.00
	F	$62.0^{c}$	$87.0^{b}$	130.0 <sup>a</sup>	0.05	0.00
Water holding capacity (%)	M	$70.0^{b}$	$71.0^{a}$	69.0°	0.10	0.08
	F	73.1 <sup>b</sup>	$67.0^{b}$	$74.0^{a}$	0.68	0.30
pH@24 hrs (raw muscle)	M	$6.10^{a}$	5.57 <sup>b</sup>	5.60 <sup>b</sup>	0.04	0.20
	F	5.63 <sup>b</sup>	$5.60^{c}$	5.67 <sup>a</sup>	0.02	0.90
Cook loss (%)	M	14.2 <sup>a</sup>	6.11 <sup>c</sup>	9.71 <sup>b</sup>	0.11	0.00
	F	21.4 <sup>a</sup>	20.4 <sup>b</sup>	19.3°	0.05	0.00
Cook yield (%)	M	$86.0^{c}$	$94.0^{a}$	90.3 <sup>b</sup>	0.11	0.00
•	F	79.0 <sup>b</sup>	78.3 <sup>b</sup>	81.0 <sup>a</sup>	0.06	0.01
Shear force (kg/cm <sup>2</sup> )	M	7.87 <sup>c</sup>	$8.00^{b}$	8.17 <sup>a</sup>	0.04	0.60
	F	5.60 <sup>b</sup>	$8.00^{a}$	$8.00^{a}$	0.15	0.10
Tbars(mg malonaldehyde/kg meat)	g M	0.29°	0.36 <sup>b</sup>	0.43 <sup>a</sup>	0.01	0.06
,	F	$0.56^{b}$	$0.43^{c}$	$0.60^{a}$	0.01	0.03

a, b, c- means with different superscripts on same row are significantly different (P<0.05), SEM- standard error of means, p-probability of mean effects

Table 3: Sensory properties of breast muscles of indigenous ducks raised in different locations cooked by deep fry method.

Items	sex	Location A (Ekiti Central)	Location B (Ekiti North)	Location C (Ekiti South)	SEM	p-value
Aroma	M	6.50°	$7.00^{b}$	9.00 <sup>a</sup>	0.14	0.05
	F	6.75 <sup>b</sup>	7.50 <sup>a</sup>	3.25°	0.17	0.01
Flavor	M	$6.75^{a}$	$6.50^{b}$	5.25°	0.22	0.50
	F	$7.50^{a}$	7.00 <sup>b</sup>	4.25 °	0.18	0.04

Tenderness	M	7.25 <sup>b</sup>	7.75 <sup>a</sup>	5.25°	0.22	0.20	
	F	$7.75^{a}$	5.75 <sup>b</sup>	4.25°	0.20	0.06	
Juiciness	M	$7.00^{a}$	$7.00^{a}$	$5.00^{b}$	0.18	0.20	
	F	$6.25^{a}$	5.75 <sup>b</sup>	4.25°	0.24	0.40	
Texture	M	$6.50^{b}$	$7.00^{a}$	4.75°	0.14	0.07	
	F	$7.25^{a}$	6.50 <sup>b</sup>	$4.00^{c}$	0.18	0.80	
overall	M	$8.00^{a}$	$7.25^{\rm b}$	$6.00^{c}$	0.20	0.20	
acceptability							
	F	$8.00^{a}$	7.25 <sup>b</sup>	5.25 °	0.15	0.20	

<sup>&</sup>lt;sup>a, b, c-</sup> means with different superscripts on same row are significantly different (P<0.05), SEM- standard error of means, p - probability of mean effects

Table 4: Sensory properties of breast muscles of indigenous ducks raised in different locations cooked by pan fry method.

Items	sex	Location A (Ekiti Central)	Location B (Ekiti North)	Location C (Ekiti South)	SEM	p-value
Aroma	M	5.50°	6.75 <sup>a</sup>	6.25 <sup>b</sup>	0.28	0.80
	F	5.25 <sup>b</sup>	3.75°	$6.00^{a}$	0.23	0.30
Flavor	M	$6.00^{a}$	5.75 <sup>b</sup>	$6.00^{a}$	0.15	1.00
	F	5.25 <sup>b</sup>	5.75 <sup>a</sup>	$4.00^{c}$	0.19	0.40
Tenderness	M	$6.00^{b}$	$7.00^{a}$	4.25°	0.21	0.20
	F	$7.00^{a}$	5.25°	5.75 <sup>b</sup>	0.23	0.50
Juiciness	M	$8.00^{a}$	$7.25^{\rm b}$	4.25°	0.09	0.00
	F	$6.00^{a}$	5.25 <sup>b</sup>	$5.00^{c}$	0.19	0.70
Texture	M	$6.00^{a}$	5.75 <sup>b</sup>	4.75°	0.15	0.40
	F	$6.25^{a}$	4.25°	5.25 <sup>b</sup>	0.24	0.50
overall acceptability	M	$7.00^{b}$	$8.00^{a}$	$6.00^{\circ}$	0.08	0.05
. ,	F	$7.00^{a}$	6.25 <sup>b</sup>	5.75°	0.17	0.50

<sup>&</sup>lt;sup>a, b, c</sup> means with different superscripts on same row are significantly different (P<0.05), SEM- standard error of means, p - probability of mean effects

### **CONCLUSION**

The study concluded that the locations where the indigenous ducks were raised influenced good physicochemical properties and consumer's acceptability of cooked breast muscle of ducks raised in different zones in Ekiti State, Nigeria. Shelf-life stability was enhanced by cooking methods.

# **Ethical Approval:**

Animal Ethic committee approval has been collected and preserved by the author(s)

### **REFERENCES**

1. Hird H, Chrisholm J, Brown J. The detection of commercial duck species in food using a single probe- multiple species-specific primer real-time PCR assay. Eur. Food Res.Technol.2005; 221:559-563.

- 2. Oluyemi JA. Potentialities of the indeginous species of poultry for meat and egg production in Nigeria, NAPRI. 1979:163-186.
- 3. Muchenje V, Dzama K, Chimonyo M, Strydom P, Hugo A, Raats J. Some biochemical aspects pertaining to beef eating quality and consumer health: A review. Food Chem. (2009), 112: 279–289.
- 4. Omojola AB, Hammed S, Attoh-Kotoku V, Wogar GSI, Iyanda OD, Aremo JO. Physico chemical and Organoleptic Characteristics of Muscovy drake meat as influenced by cooking methods, African Journal of Food Science. 2014; 8(4): 184-189.
- 5. Grau R, Hamm RA. A simple method for the determination of water binding in muscles, Naturwissenschaften. 1953; 40:29-30.
- 6. Kwon HJ, Choo YK, Choi YI, Kim EJ, Kim HK, Heo KN, Choi HC, Lee SK, Kim CJ, Kim BG, Kang CW, An BK. Carcass Characteristics and Meat Quality of Korean Native Ducks and Commercial Meat-type Ducks Raised under Same Feeding and Rearing Conditions, Asian Australas. J. Anim. Sci. 2014; 27(11): 1638-1643.
- 7. Yang HS, Moon SS, Jeong JY, Choi SG, Joo ST, Park GB. Effect of sodium bicarbonate injection in pre-rigor porcine *M. Longissimus lumborum* on pork quality. Asia-Aust. J Anim. Sci. 2006; 19:898-904.
- 8. Kembi SO, Okubanjo AO. Physicochemical and sensory properties of dehydrated beef patties containing soybean products. Trop.Anim. Prod. Invest.2002.
- 9. Honikel JL. Reference Methods for the assessment of physical characteristics of meat. Meat Sci.1998; 49:447-457.
- 10. Ali MS, Kang G-H, Yang H-S, Jeong J-Y, Hwang Y-H, Park G-B, Joo S-T. A Comparison of Meat Characteristics between Duck and Chicken Breast, Asian-Aust. J. Anim. Sci. 2007; 20 (6): 1002 1006.
- 11. AMSA. Research Guidelines for Cookery, Sensory evaluation and Instrumental measurement of fresh meat. National Livestock and Meat Board, Chicago, IL, USA. 1995. Retrieved from http://www.meat science.org
- 12. Minitab. Minitab version 16.1. Minitab Inc., 2007; State College, P. A., USA
- 13. Carvalho CB, Madrona GS, Corradine Sd-S, Reche PM, M. Pozza MSd-S, do Prado IN. Evaluation of quality factors of bovine and chicken meat marinated with reduced sodium content, Food Sci. Technol, Campinas.2013; 33(4): 776-783.
- 14. Augusty'nska-Prejsnar A, Ormian M, Sokołowicz Z. Physicochemical and Sensory Properties of Broiler Chicken Breast Meat Stored Frozen and Thawed Using Various Methods, Journal of Food Quality. 2018, Article ID 6754070, 9 pages https://doi.org/10.1155/2018/6754070
- 15. Cross HR, Durland PD, Seideman SC. Sensory qualities of meat. In: Muscle as food, Bechtel, P.(ed.). Academic Press, Orlando, Florida. 1986; p.286.
- 16. Aaslyng MDC, Berjerholm P, Ertbjerg HC, Andersen HJ. Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure, Food Quality and Preference. 2003; 14: 277-288.