tion on the expression of Na-dependent amino acid accumulation by mammalian small intestine. Valine uptake displayed a high Na-dependency in intestine from both fed and 72 h fasted rats. Uptake under Na-free conditions was, however, consistently higher in the jejunum but not the ileum following starvation. At present, we have no explanation for this regional difference.

The membrane events responsible for these dietary-induced changes in valine accumulation are unresolved. One possible explanation comes from studies of the potential difference across the brush border membrane  $(V_m)$  which is an important component of the total driving force for Na-linked amino acid uptake into enterocytes  $^{10}$ .

The process of cell movement along the villus is associated with marked changes in  $V_m$ , the value being highest at the villus tip  $^{11}$ . This hyperpolarisation may be related to the structural and functional differentiation which occurs during enterocyte migration since both an elongation of individual microvilli and a raised intracellular K concentration have been observed during the initial phase of enterocyte development. Recently we reported that starvation results in a hyperpolarisation of the microvillus membrane of both the jejunum and ileum The enhanced valine accumulation shown in figures 1 and 2 powered by the increased membrane potential difference may, in turn, be due to a slower cell turnover time and reduced transit along the villus  $^{15}$ . Lengthening the residence time would result presumably in a population of more mature cells at the villus tip.

The systemic and/or luminal factors responsible for the changes in active valine accumulation are unknown. It is of interest, however, that several studies have implicated pancreatic glucagon in the adaptation to transport following starvation<sup>16-18</sup>. In addition, recent work has shown that the chronic administration of this hormone to rats induces a hyperpolarisation of the microvillus membrane<sup>19</sup>. Preliminary observations on the effect of glucagon on the localisation of [<sup>3</sup>H] valine uptake during enterocyte migration reveal striking similarities to the changes reported in this present study, i.e. valine uptake occurs earlier during enterocyte development resulting in a significantly higher maximum grain density at the villus tip<sup>19</sup>. It is therefore possible that the increased secretion rate of the hormone during fasting<sup>20</sup> is an important factor in the adaptation of valine transport.

In conclusion, it appears that starvation results in an enlarged functional absorptive surface area and an increased ability of individual enterocytes to accumulate valine. This adaptation may compensate, at least in part, for the reduced anatomical surface area which has been observed during fasting<sup>4</sup>.

- 1 Acknowledgments. We wish to thank MW Smith, PS James and IS King (AFRC Institute of Animal Physiology, Babraham, Cambridge) for facilities and advice regarding autoradiography. Financial support from the British Digestive Foundation and Eli Lilly and Company is appreciated.
- 2 Reprint requests to E.S.D., Department of Physiology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW32PF, England.
- 3 Debnam, E.S., and Levin, R.J., J. Physiol., Lond. 252 (1975) 681.
- 4 Ross, G. A., and Mayhew, T. M., Experientia 40 (1984) 856.
- 5 King, I.S., Sepulveda, F.V., and Smith, M.W., J. Physiol., Lond. 319 (1981) 355.
- 6 Krebs, H. A., and Henseleit, K., Hoppe-Seyler's, Z. physiol. Chem. 218 (1932) 33.
- 7 Murgatroyd, L.B., Med. Lab. Sci. 33 (1976) 67.
- 8 Menge, H., Sepulveda, F. V., and Smith, M. W., J. Physiol., Lond. 334 (1984) 213.
- 9 Smith, M. W., Experientia 37 (1981) 868.
- 10 Schultz, S.G., Am. J. Physiol. 233 (1977) E249.
- 11 Cremaschi, D., James, P.S., Meyer, G., and Smith, M.W., Comp. Biochem. Physiol. 78A (1984) 661.
- 12 Smith, M. W., Paterson, J. Y. F., and Peacock, M. A., Comp. Biochem. Physiol. 77A (1984) 655.
- 13 Cremaschi, D., James, P.S., Meyer, G., Rossetti, G., and Smith, M.W., J. Physiol. Lond. 354 (1984) 363.
- 14 Debnam, E.S., and Thompson, C.S., J. Physiol., Lond. 355 (1984) 449.
- 15 Stevens-Hooper, C., and Blair, M., Expl Cell Res. 14 (1958) 175.
- 16 Debnam, E. S., Q. J. expl Physiol. 67 (1982) 587.
- 17 Rudo, N.D., Lawrence, A.M., and Rosenberg, I.H., Gastroenterology 69 (1975) 1265.
- 18 Rudo, N.D., Rosenberg, I.H., and Wissler, R.W., Proc. Soc. expl Biol. Med. 152 (1976) 277.
- 19 Debnam, E.S., and Thompson, C.S., J. Physiol., Lond. 364 (1985) 81P.
- 20 Potter, D. E., and Morris, J. W., Experientia 36 (1980) 1003.

0014-4754/86/080945-04\$1.50 + 0.20/0 © Birkhäuser Verlag Basel, 1986

## Effects of storage and processing on the nutritive value of certain Nigerian foods

M.E. Ukhun

Chemistry Department, University of Benin, Benin City (Nigeria), 18 March 1985

Summary. The effects of storage and processing on the nutritive value of two Nigerian foods – raw cowpea (Vigna unguiculata) flour and palm oil, have been investigated. Increased retention of both thiamine and riboflavin as a result of increased water activity  $(A_w)$  were recorded for cowpea flour stored for 6 months. A storage temperature of 5 °C led to minimal losses of both vitamins in the stored flour. Differences in physico-chemical characteristics were observed between palm oil produced by the traditional method and that produced by a modern commercial method. Increasing  $A_w$  led to decreasing loss of unsaturation in the traditionally-produced palm oil during 4-week storage at ambient temperature (25 °C).

Key words. Palm oil; raw cowpea flour; storage; nutritive value.

The problem of inadequate food supply, especially in the developing countries, is related not only to the total output of raw agricultural production but also to post-harvest factors such as processing and storage. These two post-harvest factors must be taken into consideration in evaluating the adequacy (in terms of total quantity and nutritional wholesomeness) of food supplies in developing countries like Nigeria.

Although cowpea (Vigna unguiculata) is consumed, primarily, for its high protein content<sup>9</sup>, it could also act as a good source of some vitamins. Accordingly, the influence of water activity<sup>6</sup> and

of temperature on the thiamine and riboflavin contents of the stored raw flour, prepared from the seeds, was investigated.

The traditionally-produced palm oil marketed in the Nigerian traditional open markets is used by a large segment of the population, probably for reasons of cost. However, it would appear that no comparative studies have yet been undertaken to compare the physico-chemical quality attributes of the traditionally-produced palm oil with those of palm oil produced by modern commercial methods. The present studies address this problem. The differences in atmospheric humidity under which palm oil is

marketed in the open traditional markets justifies an examination of the effects of  $A_{\rm w}$  on the oil.

Materials and methods. Cowpea seeds (Vigna unguiculata) were obtained from the National Seeds Co., New Orleans, USA. These seeds were then milled into a flour using a Hobart Manufacturing Co. Mill (Model No. 3430). The flour was such that it was able to pass through a No. 1 Standard Sieve having a Tyler Equivalent of 16 mesh.

Four hundred grams of the flour were weighed, in duplicate, into 500-ml glass beakers. These beakers, in duplicate, were then kept in each of three desiccators and water activities of 0.11, 0.33 and 0.75 respectively, established in the samples by the method of Rockland 11. The desiccators were stored at an ambient temperature of 25 °C, on a laboratory bench. Similar duplicate samples were stored at three different temperatures of 5, 25 and 40 °C in temperature-controlled storage rooms at a uniform  $A_{\rm w}$  of 0.75. All the samples were stored for 6 months.

The thiamine and riboflavin contents of the cowpea flour were determined immediately after milling and, thereafter, on a monthly basis, over the 6-month storage period, by the fluorimetric method of the Association of Vitamin Chemists<sup>1</sup>. The fluorimeter was a G.K. Turner Associates type (Model No. B 111). Duplicate analyses of each sample were carried out under subdued light. Results were analyzed by Analysis of Variance (ANOVA)<sup>2</sup>.

Traditionally-produced palm oil was obtained from traditional open markets in Benin City, Nigeria, while palm oil produced by modern commercial methods was obtained from the Nigerian Institute for Oil Palm Research, (NIFOR), Benin City, Nigeria. Smoke point (SP), iodine value (IV), peroxide value (PV) and free fatty acid (FFA) content were determined by the method described by Devine and Williams<sup>3</sup>. Refractive index (RI) was determined using an Abbé Refractometer while specific gravity (SG) determinations were in accordance with methods described by Joslyn<sup>4</sup>.

Fatty acid profiles of the oils were determined by gas-liquid chromatography of the methyl esters of the component fatty acids prepared by the method of Metcalfe et al.<sup>8</sup>. The Pye series 104 gas chromatograph was equipped with a flame ionization detector. The steel column (1.7 mm × 4 m) was packed with DEGA adsorbed on 80/120 mesh celite. The instrument was operated at an isothermal temperature of 190°C and the chart speed was 160 mm/h. Carrier gas was nitrogen at a flow rate of 35 ml/min.

Peak identification was by retention times in comparison with those of standard fatty acid methyl esters, while fatty acid quantification was by triangulation. For 4-week storage studies, 50 g samples of each of the palm oil were stored in 500-ml glass beakers kept in 3 desiccators where water activites ( $A_{\rm w}$ ) of 0.11, 0.33 and 0.75, respectively, were established by the method of Rockland<sup>11</sup>. All the desiccators were then stored at 25 °C on a laboratory bench.

Results and discussion. There is an indication in table 1 that increases in water activity from 0.11 to 0.33 and then to 0.75 led to increased retention (reduced losses) of both riboflavin and

Table 2. Physico-chemical characteristics of palm oil produced by two different methods

	POT	PON
Iodine value (IV)	*61, 42, 54, 56	64
Peroxide value (PV) (mEq/kg oil)	34	6
Free fatty acid (FFA) as palmitic	3.19	0.42
Specific gravity (SG), (25°C)	0.9038	0.9179
Smoke point (SP) (°C)	40	52
Refractive index (RI) at 25°C	1.4595	1.4604
<sup>C</sup> 14:0 (%)	1.7	1.8
C16:0 (%)	40.3	36.2
<sup>C</sup> 18:0 (%)	6.5	7.4
C <sub>18:1</sub> (%)	42.1	49.4
<sup>C</sup> 18:2 (%)	6.5	10.0

POT = Palm oil produced by Nigerian traditional method; PON = Palm oil produced by modern commercial method (NIFOR);  $^*$  = Values from left to right are those for prestorage samples, and samples stored at  $A_w$  0.11, 0.33 and 0.75 respectively, for 4 weeks at 25 °C.

thiamine in the raw cowpea flour. At all the water activities, there were increasing vitamin losses with increasing length of storage. The changes in vitamin content with changes in water activity were statistically significant (p < 0.01). The trend was observed despite the fact that both vitamins, being water soluble, would be predicted to decrease in amount (decreased retention) with increasing water activity as was observed by Kamman, Labuza, and Warthesen<sup>5</sup> in their studies with pasta. It could be that in the raw cowpea flour used in the present studies, oxidation-linked destruction of both vitamins may have been the prevailing mechanism in riboflavin losses at water activities between 0.11 and 0.33. Ukhun<sup>13</sup> has reported reduced oxidation, which should lead to increased vitamin retention, at the water activity of 0.33 in raw cowpea flour. The further decrease in vitamin losses (increased retention) when the water activity was increased from 0.33 to 0.75, would be difficult to explain along the same lines, since at this water activity, oxidative reactions would, in fact, be expected to increase<sup>13</sup> and the losses of both vitamins therefore accelerated. It would appear that at the water activity of 0.75, the mechanisms operating in the losses of the vitamins were beginning to be mitigated by the dilution effect of a high water activity.

It could be speculated that a combination of autoxidation-linked losses, photoconversion to lumiflavin by the ambient laboratory light in the case of riboflavin, and enzymic transformations, among other factors, may have been important in the losses of the two vitamins in the flour. This may have been possible because the cowpea flour, prepared without any thermal process and with the histological disintegration accompanying milling, would have been subjected to quite a wide array of chemical reactions.

The reduced losses of both riboflavin and thiamine from the raw cowpea flour with decreases in storage temperature are consistent with reduced reaction rates, both enzymic and non-enzymic, at the lower storage temperatures.

Table 1. Riboflavin and thiamine contents (mg/100g flour, wet basis) of raw milled cowpea flour stored at different water activities and temperatures for 6 months

Month	A <sub>W</sub> * Riboflavin			Thiamine		Temperature (°C)** Riboflavin			Thiamine			
	0.11	0.33	0.75	0.11	0.33	0.75	5	25	40	5	25	40
0 +	0.163	0.163	0.163	0.863	0.863	0.863	0.163	0.163	0.163	0.863	0.863	0.863
1	0.092	0.114	0.134	0.722	0.782	0.843	0.162	0.113	0.105	0.861	0.841	0.544
2	0.043	0.085	0.115	0.611	0.726	0.813	0.163	0.117	0.098	0.862	0.814	0.353
3	0.006	0.054	0.096	0.530	0.664	0.772	0.161	0.095	0.064	0.865	0.771	0.204
4	0.003	0.023	0.075	0.461	0.612	0.743	0.162	0.076	0.032	0.863	0.741	0.065
5	_d	0.021	0.074	0.392	0.567	0.710	0.160	0.076	_d	0.861	0.712	_d
6	d	0.018	0.072	0.322	0.511	0.686	0.159	0.071	_d	0.858	0.688	_d

<sup>+ =</sup> Corresponding readings obtained immediately after milling and before storage; d = No measurable amount of vitamin by the fluorimetric method used; \* = At a uniform temperature of 25 °C; \*\* = At a uniform water activity of 0.75.

The fact that the IV of the traditionally produced palm oil is lower than that for the NIFOR palm oil (table 2) means that there is a lower amount of the unsaturated fatty acids in the traditionally produced palm oil. This is probably due to differences in the production methods. Essentially, the traditional "soft oil process" for producing palm oil in Nigeria involves the boiling of palm oil fruits in wooden or metal vats, manual kneading of the boiled fruits, separation of extracted oil from the fibrous residue and packaging. In all of these steps, the level of hygiene can, at best, be regarded as low. Air is not excluded as a matter of deliberate policy and the types of containers and vats used are not carefully selected to minimize contamination by pro-oxidant metals such as iron and copper. The oil is usually sold in glass bottles or open vat containers which are exposed to the warm humid environment and not shielded from light. All of these factors would tend to promote some oxidation of the palm oil. The NIFOR palm oil, on the other hand, is produced by modern scientific methods which take into account the need to retard lipid oxidation during processing and wholesale storage. The PV recorded in table 2 also indicate that the traditionallyproduced palm oil had undergone greater oxidation than the NIFOR oil. The PV of the traditionally-produced palm oil is also indicative of rancidity<sup>10</sup>. The higher free fatty acid content of the traditionally-produced palm oil compared with that for the NIFOR palm oil indicates the lack of a refining process and probable contamination by lipase-secreting microbes. Since the SG of edible oils is related to the degree of unsaturation of the component fatty acids<sup>4</sup>, there is consistency in the fact that the NIFOR palm oil which had the higher IV (higher unsaturation) also had the higher SG (table 2). The same consistency is observed with regard to the RI values which also tend to increase with increasing unsaturation, while the higher smoke point, shown in table 2, for the NIFOR palm oil, is consonant with its lower free fatty acid content.

The fatty acid profiles of both types of palm oil show high levels of the saturated fatty acids. The profiles are consistent with the low IV for the oils. While a low level of unsaturated fatty acids will enhance stability to oxidation and attendant spoilage, the relationship of low levels of lipid unsaturation to the development of certain disease conditions makes it nutritionally undesirable. The NIFOR palm oil had a higher content of the essential fatty acid linoleic acid and of linolenic acid, probably because it had been subjected to a lower degree of oxidative abuse during processing and subsequent storage.

Results in table 2 also show that increase in  $A_w$  led to higher IV for the oil (higher unsaturation); this could be due to a decrease in lipid oxidation. This observation is in line with the reported retarding effects of high  $A_w$  on lipid oxidation<sup>7</sup>.

- Association of Vitamin Chemists, (Method of Vitamin Assay), 3rd edn. N.Y. Intersc. Publ. 1966.
- 2 Bhattacharyya, G.K., and Johnson, R.A., in: Statistical Concepts and Methods, John Wiley and Sons, New York 1977.
- 3 Devine, J., and Williams, P. N., in: The Chemistry and Technology of Edible Oils and Fats, p. 161. Pergamon Press, N. Y./Oxford/London/Paris 1961.
- 4 Joslyn, M.A., in: Methods in Food Analysis, 2nd edn, p. 402. Academic Press, New York 1970.
- 5 Kamman, J. F., Labuza, T. P., and Warthesen J. J., J. Food Sci. 46 (1981) 1457.
- 6 Labuza, T.P., Proc. 3rd Intl. Cong., Food Sci. Technol., p. 618. Washington, D.C. 1971.
- 7 Loncin, M., Bimbenet, J. J., and Lenges, J., J. Food Technol. 3 (1969)
- Metcalfe, L.D., Schmitz, A.A., and Petka, J.R., Analyt. Chem. 38 (1966) 514.
- 9 Oyenuga, V. A., in: Nigeria's food and feeding stuffs, p. 79. Ibadan University Press, Ibadan, Nigeria 1968.
- 10 Pearson, D., in: The Chemical Analysis of Food, 7th edn, p. 495. Churchill Livingstone, London 1976.
- 11 Rockland, L. B., Analyt. Chem. 32 (1960) 1375.
- 12 Scheig, R., Am. J. clin. Nutr. 21 (1968) 300.
- 13 Ukhun, M.E., Food Chem. 14 (1984) 35.

0014-4754/86/080948-03\$1.50 + 0.20/0 © Birkhäuser Verlag Basel, 1986

## Effect of epidermal growth factor on growth and maturation of fetal and neonatal rat small intestine in organ culture<sup>1</sup>

C.N. Conteas, J.M. DeMorrow and A.P.N. Majumdar

Department of Medicine, Veterans Administration Medical Center, Martinez (California 94553, USA), and Departments of Medicine and Biological Chemistry, University of California, Davis (California 95616, USA), 30 September 1985

Summary. Small intestinal explants from pre- and post-natal rats were incubated in an organ culture system in the absence and presence of epidermal growth factor (EGF). The rate of synthesis of small intestinal DNA and protein as well as the activity of lactase and alkaline phosphatase increased rapidly between 17 and 20-day gestational age, whereafter they declined. The maximal incorporation of <sup>3</sup>H-thymidine and <sup>14</sup>C-alanine into DNA and protein, respectively, was significantly stimulated by EGF (100 ng/ml). EGF had no effect on the activity of either lactase or alkaline phosphatase in the small intestinal explants.

Key words. Small intestinal growth; DNA synthesis; protein synthesis; disaccharidases; EGF; fetal development; organ culture.

Epidermal growth factor (EGF), which is structurally and functionally similar to urogastrone<sup>2</sup> has long been known to stimulate proliferation and differentiation (keratinization) of the epidermis<sup>3,4</sup> and to enhance growth and maturation of the fetal pulmonary epithelium<sup>5</sup>. More recently, it has come to light that EGF also promotes growth of the digestive tract during the early stages of development, as evidenced by increased DNA synthesis, ornithine decarboxylase activity and protein and nucleic acid content of the stomach and small intestine following administration of EGF to growing rats and mice<sup>6-8</sup>. This, together with the fact that milk possesses EGF-immunoreactivity<sup>9</sup> and that both EGF and milk stimulate DNA synthesis in cultured fibroblast<sup>10</sup> suggests further that EGF may play an important role in

regulating growth during the early stages of life. Recently we have demonstrated that prolonged administration of EGF to non-weaned suckling rats (undernourished) not only stimulates growth of the stomach and small intestine but also increases body weight<sup>8</sup> indicating that EGF induces overall growth of the animals. On the other hand, Calvert et al.<sup>11</sup> have observed that in mice administration of EGF during the later stages of pregnancy produces no change in either fetal body weight or small intestinal DNA or protein content but significantly increases intestinal alkaline phosphatase, trehalase and glucose-6-phosphatase activities suggesting that prenatal functional maturation, but not growth of the small intestine, is stimulated by EGF. To further determine the role of EGF in the regulation of growth and