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Statistical modelling of a process for the extraction of Maillard reactive product (MRP) and Maillard reactive product precursors (MRPP) of *Chrysophyllum albidum* pulp origin

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Abstract

Crude Maillard reactive product (MRP) and Maillard reactive product precursors (MRPP) of C. albidum pulp origin were extracted by varying temperature 0 C (30-70), time (min) (10-30) and addition of sodium carbonate solution (%) (2-10 mL) using the central composite rotatable designs. Equations for predicting objective indices namely; crude melanoidin, free amino acids, reducing sugar and relative reducing power were developed and adequacy confirmed using residual assessment and analysis of variance. Addition of sodium carbonate solution and other process variables could be manipulated for the extraction of high quality MRP and MRPP of *C. albidum* origin. Such extracts offer product with pleasant olfactory note, attractive optical and therapeutic functionality that would find application in both food and non-food products.

Introduction

Melanoidins are the dark brown polymeric compounds formed from the reaction between reducing sugars and amino acids, peptides, and proteins. The phenomenon is known as Maillard reaction or non-enzymatic browning. It is a major reactions taking place during thermal processing, cooking and storage of foods (Phisut and Jirapon, 2013). Melanoidins occur extensively in food and biological materials (Dolphen and Thiravetyan, 2011). Melanoidins are of importance in Food Science and Technology with respect to colour and flavour. Also, food and drink with melanoidins may offer substantial health promoting effects (Ibarz *et al*., 2013).

Shedid (2010) reported that Maillard reaction products notably, melanoidins exhibit strong antioxidant activity through scavenging oxygen radicals or chelating metals. In another study, Ibarz *et al.* (2013) reported that antioxidant capacity of MRPs and other melanoidins substantially contribute to the shelf life of heat-treated foods. Substantial amount of melanoidins are found in beverages such as coffee, cocoa, bread, malt and honey and cooked meat.

African star apple botanically named *Chrysophyllum albidum* (Linn) belongs to the plant family Sapotaceae. It is a wild plant found in Nigeria, Uganda, Niger republic, Cameroon and Cote d'ivore (Adewusi and Bada, 1997). *Chrysophyllum albidum* is nutritionally high in ascorbic acid, vitamin A, protein (5.89 %), calcium (14.4 %) phosphorus (8.58 %) and sugars (Ureigho, 2010). The fleshy pulp of the fruit is eaten as snack and relish by both young and old (Cenrad, 1999). African star apple is a climacteric fruit and found only in season therefore, it is highly perishable. In a series of study conducted to extend shelf life of African star apple (flesh) pulp. It was observed that the pulp is a candidate for preparation of natural Maillard reactive product or melanoidin. Preference for whole-natural product suggests that MRP and MRP precursors from *C. albidum* would be favoured in comparison to MRP of synthetic origin. In this paper, reports on development of statistical model for the extraction of crude MRP and MRPP of *C. albidum* pulp origin is accomplished.

Materials and Methods

Materials

Chrysophyllum albidum pulp (dehydrated and stored), sodium carbonate, ascorbic acid, glucose, DNS, glycine, and other chemicals used were of analytical grade.

Extraction procedure

Dark with pleasant smell dehydrated *C. albidum* pulp was grated manually and the flakes were used in the subsequent crude MRP and MRPP extraction process. Extraction process independent variables

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were time, temperature and sodium carbonate solution. Further details on extraction variables on the experimental design are presented in Table 1. The lowest and highest levels of independent variables were based on the results of preliminary investigation

Table 1. Process variables used in the central composite rotatable design (k = 3)

Independent variables	Code	Levels				
Sodium carbonate (g/mL)/5%	X1	2 mL	4 mL	6 mL	8 mL	10 mL
Temp (^{0}C)	X2	30	40	50	60	70
Time (min)	X3	10	15	20	25	30

Note: 10 g of sodium carbonate in 200 mL of water
0.2 g of sample in 2.2 mls of solution
Dried African star apple pulp was flaked using domestic grater prior to use

Experimental design

A central composite rotatable design for K = 3 was used (Cochran and Cox, 1957; Lapin, 1997). The 3-factor, 5-level design generated 20 sample combinations comprising eight points peculiar to 23 factional, six star points and six central points for replication. The effects of independent variables namely: time, temperature and amount of sodium carbonate were noted for extraction of the crude MRP extract of *C. albidum*. The responses were crude melanoidins, free amino acids (FAA), total reducing sugar (TRS), relative reducing power (RRP) of the extracts solution were evaluated. Step-wise regression analysis were performed on the obtained data to yield equations for predicting extraction of the crude constituents of dehydrated dark *C. albidum* pulp for appropriate application.

Quantification of crude melanoidins

MRP (crude melanoidins) were quantified spectrophotometrically as described by Meza *et al.* (2012). Although the molecular structure of melanoidins has not been determined specific extraction coefficient value was assumed for the crude melanoidin as reported here under in this study. Crude melanoidin was quantified using the Lambert-Beer modified formula:

$$C = A/ba$$

Where C = melanoidin concentration
A = the absorbance of extract solution
b = length of the spectrophotomer's cell (cm)
a = the specific extraction coefficient (L $g^{-1}cm^{-1}$)

The value of 'a' used was 1.1289 L $g^{-1}cm^{-1}$ the standard calibration curve was constructed by plotting absorbance values as a function of the melanoidins concentrations and melanoidins were spectrophotometrically determined at 420 nm.

Determination of free amino acids

Free amino acids in the extract of the grated dark brown dehydrated *C. albidum* pulp samples were further extracted with 80 % ethanol (v/v) in accordance with the method of Odibo *et al*. (1990) with little modification. The free amino acids in the ethanolic extract were estimated using the ninhydrin colorimetric method (Rosen, 1957) using glycine as standard.

Determination of reducing power

Reducing power in the extracts of the grated dark brown dehydrated *C. albidum* pulp samples were determined in accordance with the method of Oyaizu (1986). Simply, each sample (1 mg/mL) in ethanol (2.5 mL) was mixed with sodium phosphate buffer (pH 6.6). The buffered sample was mixed with conditioning reagents (1% K_3-Fe-CN_6, 10% TCA, 0.1% $FeCl_3$) centrifuged, diluted using distilled water and absorbance was measured at 700 nm. Higher absorbance indicates a higher reducing power.

Measurement of reducing sugar

The concentration of soluble reducing sugars in the extracts of the grated dark brown dehydrated *C. albidum* pulp samples were measured using a 3,5 dinitrosalicylic acid (DNS) method (Chaplin, 1986). Immediately after heating, the solution was diluted with water 10 times and 0.2 mL of the diluted sample was transferred to a test tube where it was mixed with 2 mL of the DNS reagent. After mixing it with a vortex, the samples were heated in the water bath at 100^{0}C for 10 min followed by rapid cooling in iced water. The optical densities of the samples were measured at 570 nm using a (Bausch & Lomb Rochester NY, USA) spectrophotometer. Glucose was used for the preparation of a standard curve.

Statistical analysis

The central composite orthogonal design was analysed as reported by Cochran and Cox (1957) and modified by Lapin (1997). Each of the x-matrix was multiplied by the y-column (response) to obtain corresponding sums of products that is 0y to 23y for X_0 to X_2X_3. Consequently, the coefficients b_o to b_{23} were calculated as:

$$b_o = 0.166338(0y) - 0.056791\Sigma(iiy) \quad (1)$$

$$bi = 0.073224(iy) \quad (2)$$

$$bii = 0.062500(iiy) + 0.006889\Sigma(iiy) - 0.056791(0y)) \quad (3)$$

$$bij = 0.125000(ijy) \quad (4)$$

Table 2. regression coefficients for the quadratic model equation for the extraction of melanoidin like constituent/product of *C. albidum* pulp

Sum of products	Melanoidin g L-1	Free amino acids mg/mL	Reducing sugar	RRP	Regression coefficient	Melanoidin	Free amino acids	Reducing acids	RRP
0y	*2.1074*	*20.68*	*50.26*	*1.400*	*Bo*	*0.1427992*	*1.99675374*	*3.99291998*	*0.108972275*
Y	*0.1612*	*3.122*	*10.67*	*0.3216*	*b1*	*0.01180371*	*0.22860533*	*0.78130008*	*0.023548838*
2y	*0.3639*	*3.128*	*6.08*	*0.2808*	*b2*	*0.02664621*	*0.22904467*	*0.445201920.*	*0203548838*
3y	*0.3112*	*3.88*	*7.181*	*0.3068*	*b3*	*0.022787308*	*0.284160377*	*0.525821544*	*-0.022465123*
11y	*1.1016*	*7.916*	*24.38*	*0.6499*	*b11*	*-0.2563139*	*-0.50463839*	*-0.8008*	*-0.023858918*
22y	*1.1861*	*8.533*	*25.14*	*0.7659*	*b22*	*-0.02035014*	*-0.4660759*	*-0.7533*	*-0.016608919*
33y	*1.3703*	*8.962*	*27.38*	*0.7659*	*b33*	*-0.00883764*	*-0.4392634*	*-0.61330156*	*-0.016608919*
12y	*-0.0076*	*0.33*	*1.23*	*0.7659*	*b12*	*-0.00095*	*0.04125*	*0.15375*	*0.008375*
13y	*0.0524*	*-0.17*	*1.41*	*0.067*	*b13*	*0.00655*	*-0.02125*	*0.17625*	*0.001625*
23y	*0.0364*	*0.33*	*-4.97*	*0.013*	*b23*	*0.00455*	*0.04125*	*-0.62125*	*-0.000875*
Σiiy	*3.6580*	*25.411*	*76.90*	*-0.007*					

RRP = g ascorbic acid reducing activity equivalent/mL of extract. Free amino acid = mg glycine equivalent/mL of extract
Total reducing sugar = mg glucose equivalent/mL of extract

The quadratic model was fitted using the regression coefficient and the predicted response calculated for each of the observed values. The model was observed for adequacy by subjection to residual analysis and analysis of various.

Results and Discussion

In a bid to dehydrate *Chrysophyllum albidum* pulp and store same, a dark brown product was accidentally obtained. The product was characterised with pleasant smell. In addition, no visual spoilage was observed. When tested it was strictly acidic with pleasant taste. Intuition suggests formation of melanoidin from the reaction of sugars and protein, amino acid and peptides inherent to *C. albidum* pulp (Ureigho, 2010). In this study, three independent variables namely temperature, time and sodium carbonate as constraints were employed for the extraction of melanoidin and melanoidin precursors from the dried dark brown *C. albidum* pulp. Temperature, time are well known extraction process variables, however, the use of sodium carbonate for extraction was used on the result of screening experiment conducted on range of additive-chemicals that could facilitate dissolution of constituents of the dark brown *C. albidum* pulp. Consequently, the constraints (Table 1) were explored in this study. Extraction effects of constraints was evaluated using four technological parameters namely, melanoidin, free amino acids, total reducing sugar and relative reducing power (an index of antioxidative activity) as responses (Table 2). Therefore, MRP, MRPP and RRP were modelled for prediction of the extracted crude constituents.

The central composite orthogonal design to fit the polynomial model for the extractions of the crude melanoidin constituents of *C. albidum* was accomplished as elicited by Cochran and Cox (1957) and modified by Lapin (1997); Tomaino *et al.* (2010). The computed sums of products and regression coefficients to fit the model are shown in Table (2).

Crude melanoidin content (CMC)

The basis of this study hinged on the importance of melanoidin as colourant, flavourant and its associated therapeutic value. Therefore, the extracted melanoidin contents of the naturally formed crude melanoidin of *C. albidum* origin. Thus the quadratic model takes the form:

$$CMC = 0.1427992 + 0.01180371X_1 + 0.02664621X_2 + 0.022787308X_3 - 0.02563139X_1^2 - 0.0203501414X_2^2 - 0.00883764X_3^2 - 0.00095X_1X_2 + 0.00655X_1X_3 + 0.00455X_2X_3$$

The predicted crude melanoidin content for each of the experimental runs and their respective residual are shown in Table 3. Examination of the residuals suggests that the fitted model was reasonably adequate. The assertion was confirmed on model testing. In addition, the analysis of variance to test the fitness of the model is presented in Table 4. The first and second order terms were significant as shown by the higher calculated F-ratio in comparison with the tabulated values. However, since the calculated F-ratio for the lack of fit was lower than the tabulated value, adequacy of the model is affirmed.

Free amino acids content (FAA)

FAA is one of the precursors of melanoidin product (Bedinghaus and Ockerman, 1995) and it is inherent to *C. albidum* pulp hence there is need to assess its presence in the *C. albidum* crude extract. FAA is one of the two precursors that react to form melanoidin (Xiao-Ming *et al.*, 2010). FAA content was evaluated with view to determine FAA associated with the crude melanoidin of natural origin from *C. albidum*. FAA is an important nutrient when the extract is planned to be used as a base for food drink

Table 3. Residual Analysis of assessed parameters

Exp	Crude melanoidin			Free amino acids			Total reducing sugar			Relative reducing power		
Run	Observed	Predicted	Residual	Observed	Predicted	Residual	Observed	Predicted	Residual	Observed	Predicted	Residual
1	0.0264	0.037	-0.0106	0.13	-0.09365	0.22365	0.22	-0.2137	0.4337	0.01	-0.00565	0.015645
2	0.04	0.0493	-0.0093	0.50	0.32359	0.1764	1.88	0.690	1.19	0.05	0.021335	0.028665
3	0.065	0.083	-0.018	0.30	0.199	0.101	2.19	1.61	0.58	0.02	0.020545	-0.00055
4	0.089	0.0915	-0.0025	0.75	0.78159	-0.03159	2.50	3.1281	-0.6281	0.067	0.081045	-0.01405
5	0.035	0.0603	0.0253	0.55	0.43471	0.11529	2.50	1.7267	0.7733	0.04	0.037745	0.002255
6	0.089	0.099	-0.01	0.75	0.76691	-0.0169	2.9	3.3337	-0.4337	0.06	0.071245	-0.01125
7	0.106	0.1245	-0.0185	0.8	0.89271	-0.0927	0.02	1.0695	-1.0495	0.02	0.060435	-0.0404
8	0.142	0.1594	-0.0174	1.25	1.38991	-0.13991	3.00	3.2917	-0.2917	0.1	0.127455	-0.02746
9	0.08	0.05	0.03	0.02	0.18579	-0.16579	0.04	0.41555	-0.37555	0.01	0.001547	0.00845
10	0.10	0.0902	0.0098	1.00	0.95278	0.04722	3.20	3.0438	0.1562	0.09	0.080344	0.00966
11	0.06	0.0403	0.0197	0.04	0.2933	-0.2533	0.01	1.12235	-1.1124	0.001	0.02736	-0.02636
12	0.15	0.12983	0.02017	1.20	1.06366	0.13634	3.50	2.61995	0.88	0.14	0.096659	0.0433
13	0.09	0.0795	0.0105	0.04	0.27606	-0.23606	0.5	1.3735	-0.8735	0.001	0.02425	-0.02325
14	0.185	0.156	0.029	1.35	1.2319	0.1181	3.8	3.14229	0.6577	0.14	0.09977	0.04023
15	0.14	0.1428	-0.0028	2	1.9968	0.0032	3.0	3.9929	-0.9929	0.14	0.10897	0.03103
16	0.14	0.1428	-0.0028	2	1.9968	0.0032	5.0	3.9929	1.0071	0.10	0.10897	-0.00897
17	0.14	0.1428	-0.0028	2	1.9968	0.0032	3.5	3.9929	-0.4929	0.095	0.10897	-0.01397
18	0.11	0.1428	-0.0328	2	1.9968	0.0032	4.0	3.9929	0.0071	0.095	0.10897	-0.01397
19	0.18	0.1428	-0.0372	1.5	1.9968	-0.4968	4.5	3.9929	0.5071	0.095	0.10897	-0.01397
20	0.14	0.1428	-0.0028	2.5	1.9968	0.5032	4.0	3.9929	0.0071	0.126	0.10897	0.01703

Residual = observed –predicted

or fermentation purposes. Therefore, the predicted model for FAA in the crude melanoidin of natural origin was determined. The quadratic model takes the form:

$$FAA = 1.99675374 + 0.22860533X_1 + 0.22904467X_2 + 0.284160377X_3 - 0.50463839X_1^2 - 0.46607589X_2^2 - 0.4392634X_2^3 + 0.04125X_1X_2 - 0.02125X_1X_3 + 0.04125X_2X_3$$

The predicted FAA for each of the experimental runs and their residuals are presented in Table 3. Their residuals suggest that the fitted model is adequate. The claim on adequacy of the model fitness was verified by conduction of analysis of variance test (Table 4). The first and second order terms were significant as revealed by higher calculated F-ratio in comparison with the tabulated values and the calculated F-ratio for the lack of fit was lower than the tabulated value, consequently attesting adequacy of the model. Prediction of FAA during extraction of crude constituents of melanoidin of *C.albidum* pulp is important for giving insight to FAA in extracts with view to make necessary correction for product of interest notably food drinking or fermented product. Since amino acid is a nitrogen source, a requisite for microbial fermentation.

Total reducing sugar (TRS)

Similarly, total reducing sugar is a complementary constituent inherent to *C. albidum* pulp that takes part in melanoidin formation: Therefore, during extraction of crude melanoidin of natural origin from *C. albidum* pulp, reducing sugars were also extracted. Therefore, predictive model for total reducing sugar was determined. The quadratic model takes the form:

$$TRS = 3.99291998 + 0.7813000bX_1 + 0.44520192X_2 + 0.525821544X_3 - 0.8008X_1^2 - 0.7533X_2^2 - 0.6133X_2^3 + 0.15375X_1X_2 + 0.17625X_1X_3 - 0.62125X_2X_3$$

Using same assessment protocol herein reported for previous model parameters, the fitness of TRS model was evaluated using residual analysis (Table 3) and analysis of variance (Table 4) and were adequate.

Relative reducing power (RRP)

The extracts showed reducing activity. Reducing activity, an antioxidant index is one of the property of melanoidin products from coffee and cocoa earlier assessed by some authors notably Sanchez-Gonzalez *et al.* (2005). As stated in the beginning of this report melanoidin has therapeutic value which include antioxidative, antibacterial etc (Xiao-Ming *et al.*,2010). This is the reason for evaluating the RRP, an index of antioxidative activity of the crude extract. The extracts in all the experimental runs exhibited reducing activity which implies that the extracts can function as primary antioxidant (Meza *et al.*,2012). The quadratic model takes the form:

$$RRP = 0.1089723 + 0.02354884X_1 + 0.020561299X_2 + 0.02246512X_3 - 0.02385892X_1^2 - 0.016608929X_2^2 - 0.01660892X_2^3 + 0.008375X_1X^2 + 0.001625X_1X^3 - 0.000875X2X3$$

Both the residual analysis (Table 3) and analysis of variance (Table 4) showed that the developed model was found adequate. Articulation of adequacy was same as for previous models.

Table 4. Analysis of variance (ANOVA) for the predictive model

Dependent variable	Statistical term	DF	SS	MS	F-Ratio Calculated	F-Ratio Tabulated
CM	*First order*	*3*	*0.01869072549*	*0.0062302*	*12.5447*	*5.41*
	Second order	*6*	*0.01491134*	*0.0024852*	*5.004*	*4.95*
	Lack of fit	*5*	*0.0045719*	*0.00091438*	*1.8411*	*5.05*
	Error	*5*	*0.0024832*	*0.00049664*		
	Total	*19*	*0.0406572*			
Free amino acids	*First order*	*3*	*2.53269983*	*0.8442*	*8.442*	*5.41*
	Second order	*6*	*8.03216*	*1.3387*	*13.387*	*4.95*
	Lack of fit	*5*	*0.305*	*0.061*	*0.61*	*5.05*
	Error	*5*	*0.5*	*0.1*		
	Total	*19*	*11.36988*			
TRS	*First order*	*3*	*14.819*	*4.94*	*9.88*	*5.41*
	Second order	*6*	*22.652*	*3.775*	*7.55*	*4.95*
	Lack of fit	*5*	*7.12272*	*1.424*	*2.849*	*5.05*
	Error	*5*	*2.5*	*0.5*		
	Total	*19*	*47.09372*			
RRP	*First order*	*3*	*0.020239*	*0.0067463*	*17.59*	*5.41*
	Second order	*6*	*0.014196*	*0.00236228*	*6.1538*	*4.95*
	Lack of fit	*5*	*0.0086985*	*0.001740*	*4.53716*	*5.05*
	Error	*5*	*0.0019175*	*0.0003835*		
	Total	*19*	*0.045051*			

DF = Degree of Freedom SS = Sum of Square MS = Mean Square, CM= Crude melanoidin, TRS= Total reducing sugar

Conclusion

Extraction of dark brown crude constituents of dehydrated-stored *Chrysophyllum albidum* origin using three process variables yielded constituents that could essentially be dietary colourant with antioxidant activity was accomplished in this study. Using residual assessment and analysis of varaiance (ANOVA), the models developed were found adequate to extract the crude constituents that are essentially MRP and melanoidins precursors. Extracts could find application as dietary colourant and bases for preparation fermented and non-fermented food drink.

References

Adewusi, H.G. and Bada, S.O. 1997. Preliminary information on the ecology of *Chrysophyllum albidum* in West and Central Africa: In proceedings of a national workshop on the potentials of the star apple in Nigeria, pp 16-25.

Bedinghaus, A.J. and Ockerman, H.M. 1995. Antioxidant Maillard reaction products from reducing sugars and free amino acids in cooked ground pork. Journal of Food Science 60: 992-995.

Cenrad, Q. 1999. Centre for Environmental Renewable Natural Resources Management, Research and Development Jericho, Ibadan, Publication. No CEN 011/1999.85.

Chaplin, M.F. 1986. Monosaccharide. In Chaplin MF, Kennedy JF, editors.Carbohydrates analysis. Oxford IRL Press pp 3.

Cochran, W.G. and Cox, G.M. 1957. Experimental Design 2nd Edition, John Wileyand Sons, New York USA.

Dolphen, R. and Thiravetyan, P. 2011. Adsoption of melanoidins by chitinnanofibers. Chemical Engineering Journal 166: 890-895.

Ibarz, A., Echavarria, A.P. and Pagan, J. 2013. Antioxidant activity of the melanoidin fractions formed from D-glucose and D-fructose with L-asparagine in the Maillard reaction. Scientia Agropecuaria 4: 45-54.

Lapin, L.L. 1997. Modern Engineering Statistics Wards worth Publishing Company,Belmont, CA, USA

Meza, N.G., Perez-Hernandez, L.M., Chavez-Quiroz, K. and Medina-Juarez, L. 2012. Phenolic characterization, melanoidins and antioxidant activity of some commercial coffees from *Coffea arabica* and *Coffea canephora*. Journal of Mexico Chemical Society 56 (4): 430-435.

Odibo, F.C., Nwabunnia, F. and Osuigwe, D.I. 1990. Biochemical changes during fermentation of Telfaria seeds for ogiri production. World Journal of Microbiology and Biotechnology 6: 425-427.

Oyaizu, M. 1986. Studies on products of browning reaction: antioxidative activitiesof products browning reaction prepared from glucosamine. Japan Journal of Nutrition, 44: 307-315.

Phisut, N. and Jiraporn, B. 2013. Characteristics and antioxidant activity of Maillard reaction products derived from chitosan-sugar solution. International Food Research Journal 20 (3): 1077-1085.

Rosen, H. 1957. A modified ninhydrin colorimetric analysis for amino acids.
Archives of Biochemistry and Biophysics 67: 10-15.

Sanchez-Gonzalez, I., Jimenez-Escrig, A, Saura.-Calixto, F. 2005. *In vitro* antioxidant activity of coffee brewed using different procedures (Italian, espresso and filter). Food Chemistry 90: 133-139.

Shedid, S.A. 2010. Chemical composition and antioxidant activity of Maillard reaction products generated from Glutathione or cysteine/glucose. World Applied

Science Journal 9(10): 1148-1154.

Tomaino, A., Martorana, M., Arcoraci, T., Monteleone, D., Giovinazzo, C. and Sanja, A. 2010. Antioxidant activity and phenolic profile of Pistachio (*Pistacia vera* L., variety Bronte) seeds and skins. Biochimica 92: 1115-1122.

Ureigho, U.N. 2010. Nutrient values of *Chrysophyllum albidum* Linn African star apple as a domestic income plantation species. African Research Review 4(2): 50-56.

Xiao-Ming, Z., Feng-Lin, G., Jin-Moon, K. Shabbar, A., Shu-Qin, X. and Zheng-Xing, C. 2010. Structure and antioxidant activity of high molecular weight Maillard reaction products from Casein-glucose. Food Chemistry 120: 505-511.