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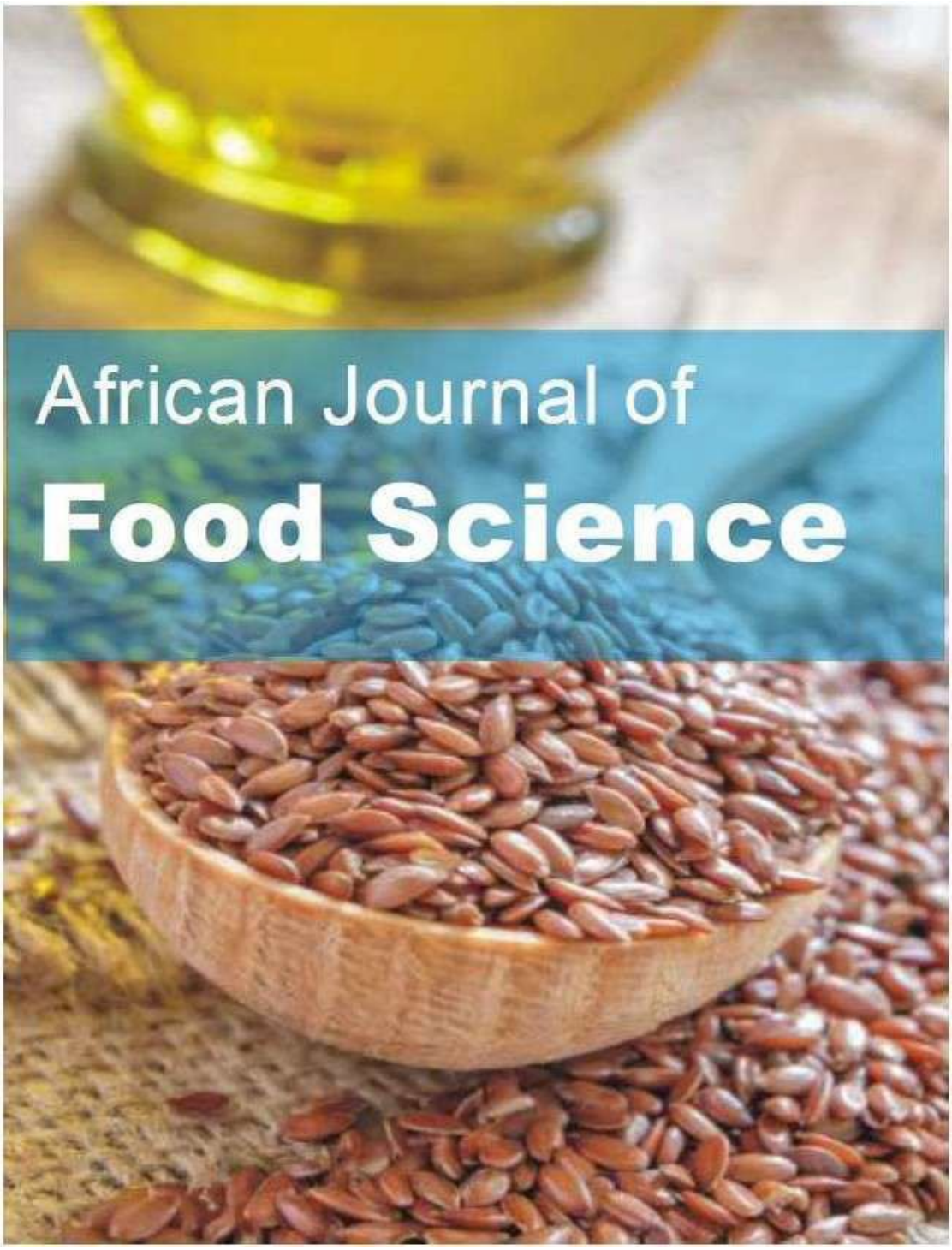

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Full Length Research Paper

Knowledge, practices and intention to consume omega 3 and omega 6 fatty acids among pregnant and breastfeeding women in Morogoro Municipality, Tanzania

Tesha, A. P.*, Mwanri, A. W. and Nyaruhucha, C. N.

Department of Human Nutrition and Consumer Sciences, Sokoine University of Agriculture, P. O. Box 3006, Morogoro, Tanzania.

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Omega 3 and omega 6 fatty acids are beneficial throughout the human life cycle. With regard to early child development, maternal fatty acid status influence cognitive and psychomotor development of the unborn child. Regardless of their importance in early childhood development they still receive less attention when compared to other nutrients. This paper aimed to assess knowledge, practices and intention to consume omega 3 and omega 6 fatty acids among pregnant and breastfeeding women in Morogoro Municipality and the study adopted the Theory of Planned Behavior (TPB). A cross-sectional survey which involved 318 randomly selected pregnant and breastfeeding women was conducted in three wards of Morogoro Municipality. Data were collected through face to face interview using a questionnaire. Statistical Package for Social Sciences (SPSS) version 20 was used for data analysis. Knowledge, Attitude and Practices (KAP) Scores were compared with demographic characteristics by Analysis of variance (ANOVA) with the level of significance set at $p<0.05$. The findings revealed limited knowledge on omega 3 and omega 6 fatty acids. However most of the participants showed positive attitude towards omega 3 and omega 6 fatty acid food sources and they intended to use them in the future if they are properly trained. Level of education had a significant influence on respondents' knowledge ($p = 0.003$) and attitudes ($p = 0.004$). It was concluded that, any attempt to increase consumption of omega 3 and omega 6 fatty acids among pregnant and breastfeeding women in Tanzania have to pay attention on their knowledge, attitudes and beliefs.

Key words: Omega 3 and omega 6 fatty acids, knowledge, attitude, intention, pregnant women, breastfeeding women.

INTRODUCTION

Fats in the diet mainly consist of triglyceride, a molecule composed of three fatty acids and a glycerol backbone (Mensink, 2016). Fatty acids (FA) consist of) carbon, hydrogen and oxygen, arranged as a linear carbon chain

*Corresponding author. E-mail: annietesha@gmail.com. Tel: +255 769 171458.

skeleton of variable lengths (Astrup, 2019). Depending on their degree of saturation/unsaturation in the carbon chain, they can be classified as saturated (no double bond), monounsaturated (one double bond) and polyunsaturated (two or more double bonds) fatty acids (Lund and Rustan, 2020).

Polyunsaturated fatty acids are very important for various metabolic processes that influence health and well-being (Forsyth et al., 2016). For example, the omega 3 polyunsaturated fatty acids such as docosahexaenoic acid (DHA) and alpha linolenic acid (ALA) are important for proper fetal development and they also influence cellular structure and function, brain development, neuronal, retinal and immune function of unborn babies (Kim et al., 2017). Omega 6 polyunsaturated fatty acids such as arachidonic acid (ARA), are also important for proper functioning of the central nervous system as well as regulation of various biological processes, particularly those related to cerebral, cardiovascular and immune functions (Calder, 2015).

Omega 3 and omega 6 fatty acids are called essential fatty acids and this is because they are not synthesized by the body and therefore, they must be obtained through diet or supplementation (Rocha et al., 2021). Dietary sources of long chain omega 3 fatty acids such as eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3) and omega 6 fatty acids such as arachidonic acid (C20:4n-6) include salmon, sardines, sword fish, tuna, shark and trout (Maurya et al., 2018). They can also be synthesized from alpha-linolenic acid found in vegetable oils, nuts and seeds such as flaxseeds and chia seeds (Maslova et al., 2018).

With regard to early child development, maternal fatty acid status particularly docosahexaenoic acid (DHA) helps in the development of the brain and retina of the unborn baby (Huffman et al., 2011). It can also influence the cognitive and psychomotor development of infants (Händel et al., 2021). Therefore maternal polyunsaturated fatty acid status plays an important role in early child development and it is important to ensure that women of reproductive age maintain a good polyunsaturated fatty acids (PUFAs) status prior, during and after conception (Stark et al., 2016).

In many developing countries, problems associated with inadequate intake of omega 3 and omega 6 fatty acids among pregnant and breastfeeding women have received little attention so far despite their potential major implications in terms early child development (Derbyshire, 2018). Several studies conducted in different areas have revealed positive association between maternal inadequate intake of omega 3 and omega 6 and attention deficiency hyperactive disorder (ADHD) (Händel et al., 2021), poor brain development (Shahidi and Ambigaipalan, 2018), gastrointestinal disorders (Mogensen, 2017), cardiovascular problems (Bird et al., 2018) as well as visual problems in infants (Shulkin et al., 2018). The study conducted by Jumbe et al. (2016) in Tanzania revealed a positive relationship between polyunsaturated fatty acid status and cognition, growth and executive function in children. Despite this developmental association which starts as soon as a mother conceives, it has been difficult to target this period because most of the mothers starts their first antenatal care visits in their second trimester (TDHS-MIS, 2016; WHO, 2016). In addition, provision of omega 3 and omega 6 fatty acids through supplements has been very expensive and questionable in terms of sustainability especially in developing countries.

In Tanzania, studies reporting knowledge, practices and intention to consume omega 3 and omega 6 fatty acid foods among pregnant and breastfeeding women are limited. Most of the previous studies have been focusing on micronutrient malnutrition such as those related to iron, zinc, iodine and vitamin A deficiency (Kinabo et al., 2019; Watts et al., 2019; Robert et al., 2021).

Therefore the findings from this study will be used by various food and nutrition stakeholders in Tanzania to initiate efforts to improve the nutritional status of pregnant and breastfeeding women and children. The overall objective of the study was to assess knowledge, practices and intention to consume omega 3 and omega 6 fatty acids among pregnant and breastfeeding women in Morogoro Municipality, Tanzania.

METHODOLOGY

Study design and setting

This study was cross sectional and it was conducted at Morogoro Municipality located in Morogoro region, Tanzania from May to June, 2021. Morogoro region lies between latitude 5º 58'' and 10º 0'' South of the Equator and longitude 35º 25'' and 35º 30'' East. The projected population size of Morogoro Municipality as of the year 2020/2021 is 409,565 (MMC, 2021). Administratively, Morogoro Municipality is composed of 25 wards. The most populated ward is Chamwino (n=35,699) and the least populated ward is Mzinga (n=1,748). Major economic activities include trade (wholesale, retail, food vending and petty trade), industrial activities as well as transport and communication. Food crops found in the area includes maize, beans, paddy, cassava, sorghum, potatoes and different types of fruits and vegetables (Kangile et al., 2020).

Sample size and sampling

The sample size was calculated based on the number of women aged between 15-49 years (63 807) since they are termed as women of reproductive age. Using 5% precision at 95% confidence level and 10% non-response rate, the obtained sample size was 318 respondents. Using Morogoro Municipal administrative structure, multistage cluster sampling was used to obtain the least administrative units which are the wards. Three out of the 25 wards were selected randomly and then with the assistance of the local leaders, the households were randomly selected based on the inclusion and exclusion criteria. Pregnant and breastfeeding women from three wards namely Mafiga, Kihonda and Mazimbu were involved in the study.

Inclusion and exclusion criteria

Pregnant and breastfeeding women who were aged between 18-49 years, have stayed in the study area for at least six months and were able to provide an informed consent were eligible to participate in the study. Pregnant or breastfeeding women who had special medical conditions, mentally ill and those who had not confirmed their pregnancy at the hospital were excluded from the study.

Data collection

Data collection was done through face to face interview using a questionnaire. The questionnaire was formulated through review of published literature to suit the research needs and then it was validated based on the comments of the nutrition experts. The Questionnaire was pretested in a randomly selected sample of 10 individuals who were not included in the study but had similar characteristics to the study sample. It was then corrected to avoid misleading information, ambiguous sentences and repeated questions.

The first part of the questionnaire inquired information regarding socio-demographic characteristics of the study participants such as age, education level, occupation, marital status and whether they were pregnant or breastfeeding. The second and third part of the questionnaire inquired information about participant's knowledge, practices and intention to consume omega 3 and omega 6 fatty acid foods sources.

There were 15 questions for assessing knowledge and 10 statements for assessing practices. The questions were administered in Kiswahili language and the terminologies such as saturated and unsaturated fatty acids were described in way that the respondents would understand what we were talking about. Also omega 3 and omega 6 terminologies were replaced by their food sources or metabolic functions. For example to know the sources of omega 3, the respondents were asked if they knew the foods that are important for brain development and if the answer was 'yes', then they were asked to mention them.

There were also 7 statements for measuring attitudes of the participants towards omega 3 and omega 6 fatty acids food sources with follow-up questions. The respondents were asked whether they 'agreed', 'neither agreed nor disagreed' or 'disagreed' with the statements. The last 16 questions measured participant's normative beliefs, control beliefs and general intention to consume omega 3 and omega 6 fatty acid rich foods.

In order to assess the socio demographic determinants of knowledge, attitude and practices; all the correct statements were scored as 'one' and the incorrect ones as 'zero' and then they were summed up independently. The total score for each respondent was calculated as (score/total score x 100). For example if a person scored 10 in knowledge assessment and the total number of questions were 15, then her score would be (10/15 x 100) = 66.7 which is approximately 67%.

Statistical analysis

Data were subjected to the descriptive analysis using the computer software Statistical Products and Service Solution (SPSS) version 20.0 after being cleaned. All the categorical variables were presented as frequencies and percentages and all the continuous variables were shown as Mean ± Standard Deviation. Cross tabulation and chi-square tests were used to determine associations. To compare knowledge, attitude and practice scores with social demographic characteristics, Analysis of Variance (ANOVA) were used. Turkeys HSD test was used was used for multiple mean comparison tests. Statistical significance was set at $p<0.05$.

Ethical considerations

The study protocol was approved by Sokoine University of Agriculture, Morogoro Regional Administrative Secretary, Morogoro District Administrative Secretary, Morogoro Municipal Council Director and Ward Executive Officers from Mafiga, Kihonda and Mazimbu. Also written informed consent was obtained from all the participants who took part in this study. All the participants were ensured of confidentiality and autonomy, the information obtained will not be misused and the outcomes of the research will be shared with them.

RESULTS

Social and demographic characteristics of the study participants

The age-range of the respondents was 18-44 years, with mean of 28.7 (SD 6.2). About half of the study subjects had primary school education and 42.8% (n=159) were home makers. In terms of marital status, most of them (42.1%, n=136) were married and majority (65.5%, n=173) had one or two children. About 32% (n=101) of all the study subjects were pregnant at the time of data collection and 43.2% (n=41) of those who were pregnant were in their second trimester (Table 1).

Knowledge regarding omega 3 and omega 6 fatty acid

Table 2 summarizes information about respondents' knowledge regarding omega 3 and omega 6 fatty acids. About half of the study participants (53.0%) were not able to differentiate between fat and oil. Most of them (63.5%) were able to correctly identify the sources of lipids. Only few (28.9%) heard about saturated and unsaturated fatty acids. Majority of the study participants could not correctly identify sources of saturated and unsaturated fatty acids. Only 20% of the participants had ever heard about omega 3 and omega 6 fatty acids. Respondents sought information regarding omega 3 and omega 6 fatty acids from a range of sources and some of them included school/college (37.5%), nutrition seminars, internet and media (31.2%). Majority of those who heard about omega 3 (92.2%, n=59) and omega 6 (95.3, n = 61) fatty acids couldn't correctly identify their sources. Most of them didn't know the benefits of omega 3 and omega 6 fatty acids prior to conception and to unborn baby (82.2%) or to the pregnant mother's body (71.9%). Only 18.8% knew the diseases that can be prevented by consuming foods rich in omega 3 and omega 6 fatty acids.

Practices regarding omega 3 and omega 6 fatty acids

Majority of the participants (97.5%) preferred plants as

Table 1. Socio-demographic characteristics of the study participants.

Variable	Frequency	Percent
Mean age 28.7±6.17		
Age (years)		
18-24	107	33.6
25-34	148	46.5
>35	63	19.8
Education level		
Never been to school	23	7.2
Primary school	159	50.0
Secondary school	90	28.3
Higher education	46	14.5
Occupation		
Home maker	136	42.8
Employed in a formal/informal sector	94	29.6
Self-employed	88	27.7
Marital status		
Never been married	97	30.5
Married	134	42.1
Separated/Divorced	21	6.6
Widowed	7	2.2
Living together	59	18.6
Have children		
Yes	262	82.4
No	56	17.6
Number of children		
1-2	172	65.6
3-4	75	28.7
5-6	12	4.6
>6	3	1.1
Pregnant		
Yes	101	31.8
No	217	68.2
Gestational age (trimesters)		
1st trimester	33	32.7
2nd trimester	41	40.6
3rd trimester	21	20.8
Doesn't know	6	5.9

their source of cooking oil and the main reason was the availability of the oils (45.7). Only half (50.0%) of the study participants used specific measurements for cooking oil. Only 37.4% of the subjects declared to have used supplements prior and during pregnancy and the frequently used supplements were iron and folic acid tablets (FEFO) (75.8%) and pregnancy care (15.0%) (Table 3).

Intention to consume omega 3 and omega 6 fatty acid food sources

Behavioural beliefs/attitudes

To determine behavioural beliefs, the study paid attention on what pregnant and breastfeeding women believed to be good for their health and that of their unborn and living babies. 58% of respondents agreed that consumption of omega 3 and omega 6 fatty acid rich foods prior and during pregnancy may result into having a healthy baby(s). Also 58.2% agreed that omega 3 and omega 6 fatty acid rich foods are good for their health and some of them (31.8%) believed that omega 3 and omega 6 fatty acid rich foods are good for weight management. Only few of the respondents (13.8%) had ever tasted fish oil itself or any food that has been made by fish oil. For those who have ever tasted it, most of them (52.3%) liked it. The main reasons for liking fish were good taste (67.9%), medicinal (21.4%), clear and doesn't clot (3.6%). The reasons given by those who disliked fish oil were being nauseous (23.5%), strong smell (70.0%) and bad taste (5.9%). Majority of the respondents (67.0%) didn't know whether consumption of fish oil during pregnancy was safe of not and their main concern was the presence of chemicals (53.3%). The reasons given by those who thought that fish oil was safe during pregnancy were being natural (23.1%), strengthening the body (14.3%), building the body (9.9%), growth of the baby (31.9%) and improving baby's intelligence quotient (17.6%) (Table 4).

Normative beliefs

Based on the results shown in Table 5, majority of the participants (57.9%) agreed that their close friends and family members thinks that they should consume healthy oils, expect them to consume healthy oils (53.8%) or advise them to consume healthy oils (46.7%). Also most of them (73.0%) agreed that their close friends and family members probably consume omega 3 and omega 6 fatty acids food sources especially from plants. Moreover 63.5% agreed that people who are in the same situation as the respondents (such as pregnant and breastfeeding women) probably consumes omega 3 and omega 6 fatty acids food sources.

Control beliefs

Based on the results from this study (Table 6), majority of the study participants (75.2%) agreed that they have

Table 2. Participants' knowledge on omega 3 and omega 6 fatty acids.

Knowledge	Mafiga n (%)	Kihonda n (%)	Mazimbu n (%)	Total n (%)	P-Value
Differentiate fats and oils					
Yes	44 (13.9)	56(17.7)	49(15.5)	149(47.0)	0.598
No	42 (13.2)	71 (22.4)	55 (17.4)	169(53.0)	
Know lipid sources					
Yes	66(20.8)	74(23.3)	62(19.5)	202(63.5)	0.011
No	20(6.3)	54(17.0)	42(13.2)	116(36.5)	
Heard saturated and unsaturated FA					
Yes	39(11.9)	29(9.1)	26(7.9)	94(28.9)	0.001
No	48(15.1)	99(31.1)	79(24.8)	226(71.1)	
Know sources of SFA					
Yes	14(14.9)	5(5.3)	6(6.4)	25(26.6)	0.468
No	25(26.6)	23(24.5)	21(22.3)	69(73.4)	
Know sources of UNSFA					
Yes	17(18.1)	6(6.4)	8(8.5)	31(33.0)	0.377
No	22(23.3)	21(23.4)	19(20.2)	63(67.0)	
Heard omega 3 and omega 6					
Yes	15(4.7)	27(8.5)	22(6.9)	64(20.1)	0.768
No	71(22.3)	101(31.8)	82(25.8)	254(79.9)	
Where heard about omega 3 and 6					
School	4(6.2)	12(18.8)	8(12.5)	24(37.5)	0.379
Hospital	4(6.2)	4(6.2)	4(6.2)	12(18.8)	
Parents/friends	3 (4.7)	3 (4.7)	2(3.1)	8(12.5)	
Seminars internet and media	4(6.2)	8(12.5)	8(12.5)	20(31.2)	
Know sources of omega 3					
Yes	2(3.1)	1(1.6)	2(3.1)	5(7.8)	0.631
No	13(20..4)	26(40.6)	20(31.3)	59(92.2)	
Know sources of omega 6					
Yes	0(0.0)	2(3.1)	1(1.6)	3(4.7)	0.714
No	15(23.4)	25(39.0)	21(32.8)	61(95.3)	
General benefits of EFA					
Yes	5(7.8)	6(9.4)	7(10.9)	18(28.1)	0.579
No	10(15.6)	21(32.8)	15(23.5)	46(71.9)	
Benefits of EFA prior to conception					
Yes	2(3.2)	4(6.5)	5(8.1)	11(17.8)	0.633
No	11(17.7)	23(37.1)	17(27.4)	51(82.2)	
Benefits of EFA to unborn baby					
Yes	2(3.1)	5(7.8)	4(6.2)	11(17.2)	0.703
No	13(20.1)	22(34.4)	18(28.2)	53(82.8)	
Disorders prevented by omega EFA					
Yes	4(6.2)	4(6.2)	4(6.2)	12(18.8)	0.586
No	11(17.2)	23(35.9)	18(28.2)	52(81.2)	

FA-Fatty Acid; SFA-Saturated Fatty Acids; UNSFA-Unsaturated Fatty Acids; EFA-Essential fatty acids.

Table 3. Practices on omega 3 and omega 6 fatty acids.

Practice	Mafiga n (%)	Kihonda n (%)	Mazimbu n (%)	Total n (%)	P-Value
Consider fat content in foods					
Yes	59(18.9)	79(24.8)	71(22.3)	209(65.7)	0.466
No	27(8.5)	49(15.4)	33(10.4)	109(34.3)	
Consider the type of fat to consume					
Yes	48(15.1)	72(22.6)	65(20.4)	185(58.2)	0.551
No	38(11.9)	56(17.6)	39(12.3)	133(41.8)	
Preferred lipid source					
Plant	82(25.8)	125(39.3)	103(32.4)	310(97.5)	
Animals	2(0.6)	2(0.6)	1(0.3)	5(1.6)	0.495
Fish	2(0.6)	1(0.3)	0(0.0)	3(0.9)	
Reasons for preference					
Availability	41(12.9)	57(18.0)	47(14.8)	145(45.7)	
Low price	12(3.8)	20(6.3)	21(6.6)	53(16.7)	
Good taste	6(1.9)	9(2.8)	4(1.3)	19(6.0)	
Good aroma	6(1.9)	12(3.8)	4(1.3)	22(6.9)	
Prevent diseases	4(1.3)	5(1.6)	2(0.6)	11(3.5)	0.169
Clear/refined	5(1.6)	5(1.6)	10(3.2)	20(6.3)	
Natural/fresh	7(2.2)	16(5.0)	14(4.4)	37(11.7)	
Healthy	4(1.3)	0(0.0)	0(0.0)	4(1.3)	
Low cholesterol	1(0.3)	4(1.3)	1(0.3)	6(1.9)	
Specific measurement for cooking oil					
Yes	45(14.2)	69(27.1)	45(14.2)	159(50.0)	0.240
No	41(12.6)	59(18.6)	59(18.8)	159(50.0)	
Use of margarine					
Yes	44(13.8)	64(20.1)	41(12.9)	149(46.9)	0.178
No	42(13.2)	64(20.1)	63(19.8)	169(53.1)	
Consume EFA sources prior to conceive					
Yes	26(8.2)	44(13.9)	27(8.5)	97(30.6)	0.361
No	60(18.9)	83(26.2)	77(24.3)	220(69.4)	
Consume fish oil					
Yes	13(4.1)	16(5.0)	12(3.8)	41(12.9)	0.624
No	73(23.0)	112(35.2)	92(28.9)	276(87.1)	
Use of supplements					
Yes	38(11.9)	53(16.7)	28(8.8)	119(37.4)	0.024
No	48(15.1)	75(23.6)	76(23.9)	199(62.6)	
Frequently used supplements during pregnancy					
FEFO	26(21.7)	43(35.8)	22(18.3)	91(75.8)	
Pregnancy care	2(1.7)	2(1.7)	3(2.5)	7(5.8)	0.727
MNP	8(6.7)	5(4.2)	5(4.2)	18(15.0)	
B Complex	1(0.8)	2(1.7)	1(0.8)	4(3.3)	

Table 4. Behavioural beliefs/attitudes.

Attribute	Mafiga n (%)	Kihonda n (%)	Mazimbu n (%)	Total n (%)	P-Value
EFA and healthy baby					
Agree	42(13.2)	80(25.2)	62(19.6)	184(58.0)	
Disagree	10(3.2)	19(6.0)	13(4.1)	42(13.2)	0.098
Neither	34(10.7)	28(8.8)	29(9.1)	91(28.7)	
EFAs are healthy					
Agree	41(12.9)	8(25.5)	63(19.8)	185(58.2)	
Disagree	14(4.4)	20(6.5)	15(4.7)	49(15.4)	0.142
Neither	31(9.7)	27(8.5)	26(8.2)	84(26.4)	
EFAs and weight management					
Agree	29(9.1)	41(12.9)	31(9.7)	101(31.8)	
Disagree	18(5.7)	41(12.9)	27(8.5)	86(27.0)	0.393
Neither	39(12.3)	46(14.5)	46(14.5)	131(41.2)	
Like using plant oil					
Agree	83(26.1)	122(38.4)	98(30.8)	303(95.3)	0.702
Disagree	3(0.9)	6(1.9)	6(1.9)	15(4.7)	
Tasted fish oil					
Agree	13(4.1)	18(5.7)	13(4.1)	44(13.8)	0.678
Disagree	73(23.0)	112(34.6)	91(28.6)	274(86.2)	
Like fish oil					
Agree	7(15.9)	9(20.5)	7(15.9)	23(52.3)	0.969
Disagree	6(13.6)	9(20.5)	6(13.6)	21(47.7)	
Reasons for liking fish oil					
Good taste	5(17.9)	7(25.0)	7(25.0)	19(67.9)	
Medicinal	2(7.1)	3(10.7)	1(3.6)	6(21.4)	0.677
Doesn’t clot	2(7.1)	0(0.0)	1(3.6)	1(3.6)	
No reason	1(3.6)	1(3.6)	0(0.0)	2(7.1)	
Reasons for disliking fish oil					
Nauseous	2(11.8)	2(11.8)	0(0.0)	4(23.5)	
Strong smell	2(11.8)	6(35.3)	4(23.5)	12(70.0)	0.277
Bad taste	1(5.9)	0(0.0)	0(0.0)	1(5.9)	
Is fish oil pregnancy safe					
Agree	27(8.5)	35(11.0)	29(9.1)	91(28.6)	
Disagree	4(1.3)	8(2.5)	2(0.6)	14(4.4)	0.547
Neither	55(17.3)	85(26.7)	73(23.0)	213(67.0)	
Reasons for being safe					
Natural	5(5.5)	9(9.9)	7(7.7)	21(23.1)	
Strong body	5(5.5)	4(4.4)	4(4.4)	13(14.3)	
Building body	4(4.4)	5(5.5)	0(0.0)	9(9.9)	0.487
Baby’s growth	6(6.6)	11(12.1)	12(13.2)	29(31.9)	
Genius babies	5(5.5)	5(5.5)	6(6.6)	16(17.6)	
No reason	2(2.2)	1(1.1)	0(0.0)	3(3.3)	
Reasons for fish oil being unsafe					
Chemicals	1(6.7)	5(33.3)	2(13.3)	8(53.3)	
Nauseous	1(6.7)	3(20.0)	0(0.0)	4(26.7)	0.695
Big babies	1(6.7)	1(6.7)	1(6.7)	3(20.0)	

EFAs=Omega 3 and omega 6 Fatty Acids.

Table 5. Normative beliefs.

Attribute	Mafiga n (%)	Kihonda n (%)	Mazimbu n (%)	Total n (%)	P-Value
Family/friends think I should consume EFAs					
Yes	56(17.6)	76(23.9)	51(16.7)	184(57.9)	0.100
No	30(9.4)	52(16.4)	53(16.1)	134(42.1)	
Family/friends expect me to consume EFAs					
Yes	50(15.7)	67(21.1)	54(17.0)	171(53.8)	0.635
No	36(11.3)	61(1.2)	50(15.7)	147(46.2)	
Family/friends advise me to consume EFAs					
Yes	39(12.3)	61(19.2)	48(15.1)	148(46.7)	0.946
No	47(14.8)	67(21.1)	55(17.4)	169(53.3)	
Family/friends probably consume EFAs					
Yes	71(22.3)	93(29.2)	68(21.4)	232(73.0)	0.030
No	15(4.7)	35(11.0)	36(11.3)	86(27.0)	
People like me probably consume EFAs					
Yes	60(18.9)	83(26.1)	59(18.6)	202(63.5)	0.164
No	26(8.2)	45(14.2)	45(14.2)	116(36.5)	

EFAs=Omega 3 and omega 6 Fatty Acids.

Table 6. Control beliefs.

Attribute	Mafiga n (%)	Kihonda n (%)	Mazimbu n (%)	Total n (%)	P-Value
I have control over essential fatty acids consumption					
Yes	72(22.6)	94(29.6)	73(23.0)	239(75.2)	0.084
No	14(4.4)	34(10.7)	31(9.7)	79(24.8)	
EFAs consumption is beyond my control					
Yes	15(10.7)	34(10.2)	30(8.5)	79(24.8)	0.072
No	74(16.4)	93(29.6)	72(24.2)	239(75.2)	
If it was up to me, I would consume EFAs					
Yes	73(23.0)	90(28.3)	70(22.0)	233(73.3)	0.015
No	13(4.1)	38(11.9)	34(10.7)	85(26.7)	
If want I can avoid using EFAs					
Yes	55(17.3)	74(23.3)	59(18.6)	188(59.1)	0.558
No	31(9.7)	54(17.0)	45(14.2)	130(40.9)	
I have the ability to consume EFAs					
Yes	74(23.3)	88(27.7)	69(21.7)	231(72.6)	0.004
No	12(3.8)	40(12.6)	35(11.0)	87(27.4)	
Presence of obstacles for consuming EFAs					
Yes	32(10.1)	37(11.7)	36(11.4)	105(33.1)	0.435
No	57(17.0)	90(28.4)	68(21.5)	212(66.9)	
Obstacles					
Money	19(17.9)	25(23.6)	21(19.8)	65(61.3)	
Partner	6(5.7)	11(10.4)	(8.5)	26(24.5)	0.433
Taboos	5(4.7)	3(2.8)	7(6.6)	15(14.1)	

EFAs=Essential fatty acids.

Table 7. General intention to consume omega 3 and omega 6 fatty acid food sources.

Attribute	Mafiga n (%)	Kihonda n (%)	Mazimbu n (%)	Total n (%)	P-Value
Intend to consume EFAs in the future					
Yes	53(16.7)	42(13.2)	37(11.6)	132(41.5)	0.001
No	33(10.4)	86(27.0)	66(20.8)	185(58.2)	
Intend to consume EFAs regularly					
Yes	51(16.0)	44(13.8)	42(13.2)	137(43.1)	0.001
No	35(11.0)	84 (26.4)	62(19.5)	181(56.1)	
Will consume after knowing health benefits					
Yes	82(25.8)	102(32.1)	77(24.2)	261(82.1)	0.002
No	4(1.3)	26(8.2)	27(8.5)	57(17.9)	

EFAs=Omega 3 and omega 6 Fatty Acids.

control over omega 3 and omega 6 fatty acids consumption and 24.8% said it was beyond their control. About 73.3% said that, if it was entirely up to them they would consume omega 3 and omega 6 fatty acid food sources. The main identified obstacles for consumption of omega 3 and omega 6 fatty acids were lack of money (61.3%), restrictions from the partners or parents (24.5%) as well as traditional beliefs (10.4%).

General intention

Information about general intention to consume omega 3 and omega 6 fatty acids is summarized in Table 7. A high proportion (58.2%) of respondents indicated that they were not intending to consume omega 3 and omega 6 fatty acid food sources and supplements. Most of them (56.1%) did not even agree to use them on regular basis. They only agreed to use them if they knew the health benefits of consuming omega 3 and omega 6 fatty acid food sources such as sardines for them and their unborn and born babies and they needed to be assured of their safety (82.1%).

Comparison of knowledge, attitude and practices (KAP) scores with demographic characteristics

This study compared knowledge, attitude and practices (KAP) scores with demographic characteristics as shown in Table 8. Results suggest no significant differences between participant's place of origin, age, occupation, marital and pregnancy status in terms of knowledge, attitude and practices. However, there was a significant difference between participants' education (never been to school, primary school, secondary and higher education) in terms of their knowledge ($p = 0.003$) and attitudes ($p = 0.004$). Those who have never been to school differ significantly with participants who had higher education in terms of knowledge and attitudes.

DISCUSSION

This is one of the few researches assessing knowledge, practices and intention to consume omega 3 and omega 6 fatty acids among Tanzanian pregnant and breastfeeding women. This will bring a new insight of what is known/not known before planning any nutritional intervention targeting improvement in consumption of omega 3 and omega 6 fatty acid sources among pregnant and breastfeeding women in Tanzania.

Knowledge represents the precondition to changing behaviour and if individuals do not have sufficient knowledge, they will have no reason to change old behaviours or adopt a new one (Reinholz and Andrews, 2020). In this study the authors assessed the current knowledge on omega 3 and omega 6 fatty acids food sources among pregnant and breastfeeding women in Tanzania. The respondents reported limited exposure to information regarding omega 3 and omega 6 fatty acids based on their food sources and functions. More than half of the respondents were not able to differentiate fats and oils which could probably be due to the presence of many brands of fats and oils. The sources of omega 3 and omega 6 were correctly identified may be due to the increased use of internet and the presence of different nutritional blogs and platforms. Knowledge regarding omega 3 and omega 6 fatty acids food sources, health benefits and consequences of deficiency was limited. Several studies conducted in other areas have also identified gaps in knowledge regarding omega 3 and omega 6 fatty acids (Thuppal et al., 2017; Hilleman et al., 2020). Low levels of knowledge could be attributed by dissemination of poor quality or even misleading nutritional information through the internet, social networks, and mass-media (Duarte et al., 2022). In order to improve omega 3 and omega 6 fatty acids' knowledge, it is important to increase efforts to educate people regarding the health benefits and consequences of consuming omega 3 and omega 6 fatty acid food sources prior, during and after pregnancy.

Table 8. Comparison of Knowledge, Attitude and Practices (KAP) Scores with Demographic Characteristics.

Variable	Number	Knowledge		Practice		Attitude	
		Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value
Age (Years)							
<18	2	15.00±7.07[a]		50.00±00.00[a]	0.068	42.86±20.20[a]	
18-24	105	17.24±16.82[b]	0.695	38.25±24.67[b]		45.03±21.68[b]	0.81
25-34	63	17.03±16.92[c]		46.28±26.78[c]		45.27±21.75[c]	
35-44	318	14.76±17.21[d]		38.62±28.53[d]		42.18±21.99[c]	
Education level							
Never been to school	23	14.35±17.01[abcd]		34.06±21.60[a]	0.18	36.02±18.71[ad]	
Primary school	159	13.40±13.21[bacd]	0.003	40.67±26.69[b]		43.67±20.47[b]	0.005
Secondary school	90	18.11±16.89[cabd]		44.07±26.48[c]		44.60±22.34[c]	
Higher education	46	26.09±23.52[dabc]		47.46±28.10[d]		51.80±24.35[da]	
Occupation							
Home maker	136	16.03±16.76[a]		38.85±25.25[a]	0.10	41.18±20.65[a]	
Employed in a formal/informal sector	94	17.23±18.80[b]	0.30	46.45±27.96[b]		50.46±21.94[b]	0.17
Self-employed	88	16.93±14.88[c]		42.61±26.73[c]		43.51±21.97[c]	
Marital status							
Single/never been married	97	15.05±14.59[a]		40.89±25.91[a]	0.06	41.24±20.71[a]	
Married	134	19.33±18.16[b]		46.45±26.64[b]		47.97±21.19[b]	
Separated/Divorced	21	13.33±20.33[c]	0.312	41.27±29.64[c]		45.58±24.59[c]	0.17
Widowed	7	7.14±9.51[d]		28.57±24.93[d]		42.86±29.73[d]	
Cohabiting	59	15.42±16.12[e]		35.88±23.33[e]		42.14±21.94[e]	
Have children							
Yes	262	16.26±16.56[a]	0.57	41.16±26.97[a]	0.16	43.24±21.68[a]	0.74
No	56	18.39±18.37[b]		46.73±24.49[b]		50.77±20.91[b]	
Pregnant							
Yes	101	17.23±17.78[a]	0.38	44.22±24.67[a]	0.14	45.54±21.07[a]	0.63
No	217	16.36±16.47[b]		41.17±27.45[b]		44.11±22.02[b]	

Values with different superscripts in a row differ significantly ($p<0.05$).

Other studies have given different suggestions on how to increase nutritional knowledge (Blondin and LoGiudice, 2018; Hussein et al., 2018; De Seymour et al., 2019).

Regarding practices on omega 3 and omega 6 fatty acids, most of the participants took into account the type of fat they consume since most of them preferred plant-based lipids. Fats have always been linked with increased risks for non-communicable diseases and therefore increased consciousness among the consumers (Hermann, 2018). Lower price was one of the reasons for choosing a certain brand of fat/oil. This is similar to what have been reported by Priyati and Tyers (2016) and FAO (2020). Other factors that influence fat choices are discussed by Klopčiča et al. (2020). In terms of consumption of omega 3 and omega 6 fatty acid food sources, more than half of the respondents declared not to consume. This may be contributed by the lack of information and knowledge regarding the available brands as well as health and nutritional benefits associated with it. Other studies have also reported lower consumption of omega 3 and omega 6 fatty acid food sources and the reasons given were lifestyle, socio-demographic issues as well as previous experiences (Supartini et al., 2018; Maciel et al., 2019; Rahman et al., 2020). Therefore there is a need to increase more efforts to educate women on the availability, food sources, preparation and consumption of omega 3 and omega 6 fatty acid foods among pre-pregnant, pregnant and breastfeeding women.

The present study also assessed women's attitude

towards omega 3 and omega 6 fatty acids. Attitudes are emotional, motivational, perceptive and cognitive beliefs that positively or negatively influence the behaviour or practice of an individual (Verplanken and Orbell, 2022). Most of the participants in this study agreed that consumption of omega 3 and omega 6 fatty acid food sources and supplements is good for their own health, for unborn babies and they can even be used for weight management. This is a good indication that any efforts targeting to improve intake of omega 3 and omega 6 fatty acids may succeed. Also most of the study participants had never tasted fish oil or any food or supplement made from fish oil and for those who happened to taste it, agreed that they liked it. These low levels of fish oil intake are in line with what was reported by Seymour et al. (2019) in Australia. The main reason given by those who liked it was a good taste and perceiving it as a medicine. Also, most of the study participants didn't know whether consumption of fish oil was safe or not during pregnancy and their main concern was the presence of chemicals that might harm the baby in the womb. Similarly (Judge, 2018) reported low intake of fish oil and fish oil-products and the main reason was lack previous experience and fear of potential health effects in the future.

An individual's behaviour may also be influenced by what is accepted or not accepted by specific people or groups and this may dictate whether behaving in a particular fashion is appropriate or not (Fang et al., 2017). Based on the results from this study, it seems that the people who are closer to a person may have a greater influence on the decision to consume omega 3 and omega 6 fatty acid food sources. This means that the more positive their peer groups are, the higher the probability of changing their behaviour. This agrees with what was reported by Risti et al. (2021) as well as Chen and Antonelli (2020) in Indonesia and Italy, respectively. Other factors that may influence an individual's behaviour include perceived health benefits, the use of media as well as the advice given by health professionals (Verplanken and Orbell, 2022).

Control belief refers to the presence of factors that may facilitate or impede performance of the behaviour and the perceived power of these factors (Nafaji et al., 2018). Based on the results from this study, it seems that most women have the ability to make decision over consumption of omega 3 and omega 6 fatty acid sources but their main concern is the cost of the products. This means that if low-cost, safe and user-friendly products are developed there is a chance for them to be accepted by the consumers. This finding is consistent with the findings of the previous research done by Wu et al. (2015) in China.

Conclusion

This study reveals limited knowledge and low consumption of omega 3 and omega 6 fatty acid food sources and supplements especially during pregnancy. Most of them showed positive attitude towards omega 3 and omega 6 fatty acid food sources and this gives hope of success for any attempt to improve consumption of omega 3 and omega 6 fatty acid food sources.

Level of education was reported as one of the factors that significantly influence consumer's knowledge and attitude. Therefore, attempts to increase consumption of omega 3 and omega 6 fatty acids among pregnant and breastfeeding women have to pay attention on their level of education.

Also based on this study, most of people are willing to consume omega 3 and omega 6 fatty acid food and other sources if they are aware of the health benefits associated with them. Therefore the findings from this study may contribute to the government and other stakeholders' efforts towards improving maternal and child health through development of low-cost and nutritious recipes using locally available ingredients.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

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Optimization of antioxidant activity of aqueous and alcoholic extracts of cashew apple bagasse by mean of experimental design

Adou Akpa Guy Blanchard GNAGNE[1,2]*, Doudjo SORO[2], Kablan Jean Ives GNOUMOU[2], N'guessan Jean Claude YAO[2] and Kouadio Ernest KOFFI[1]

[1]Laboratoire de Biotechnologie, Agriculture et Valorisation des Ressources Biologiques, Unité de Formation et de Recherche de Biosciences, Université Félix Houphouët-Boigny d'Abidjan, Côte d'Ivoire.
[2]Laboratoire des Procédés Industriels de Synthèse, de l'Environnement et des Energies Nouvelles, Institut National Polytechnique Houphouët-Boigny de Yamoussoukro, Côte d'Ivoire.

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Cashew apple bagasse (CAB) constitutes waste in cashew apple conversion in juice industries. This study focused on modelling an optimal condition of antioxidant activity of CAB, in view of valuing it. The response surface methodology through a Box-Behnken design was used to evaluate the effects of three factors influencing antioxidant component extraction. The factors are ethanol proportion of solvent (X1), sample/solvent ratio (X2) and time (X3). Non-toxic solvents (water and ethanol) were used for extraction. Results of the 15 runs show that polyphenol, flavonoid and tannin content varied from 3.12- 7.44 mg GAE/g CAB; 4.28- 14.99 mg QE/g CAB and 1.95- 9.25 TAE/g CAB respectively. The yield of ABTS radical scavenging varied from 22.9-75.9%. The DPPH 50% inhibiting concentration (IC50) varied from 2.28-26.97 mg/ml. Optimal polyphenol and tannin levels (7.44 mg GAE/g CAB and 9.25 mg TAE/g CAB respectively) were achieved using 50% ethanolic solvent. That of flavonoids was reached with the solvent 100% ethanol. Optimal antioxidant activity was reached using 50% ethanolic solvent for both ABTS and DPPH tests (75.9% and 2.28 mg/ml respectively). Statistical analysis showed that time has had no significant effect. Optimal condition of phenolic compounds extraction endowed with antioxidant property consists to macerate CAB powder in 46.1% ethanolic solvent at ratio of 9.5 g/100 ml for 6 h.

Key words: Cashew apple bagasse, non-toxic solvent, antioxidant activity, Box-Behnken design.

INTRODUCTION

Much pathology such as cancer, diabetes or cardiovascular diseases is caused by oxidative stress resulting from free radicals (Phaniendra et al., 2015). These latter are produced at the end of uncontrolled

*Corresponding author. E-mail: adoublanc@gmail.com. Tel: +225 0748639879.

metabolic processes of the body aerobic cells. These are among others superoxide ($O_2^{\bullet-}$), hydroxyl ($OH^{\bullet}$), alkoxyl ($RO^{\bullet}$) or peroxyl ($ROO^{\bullet}$) radicals (Sarr et al., 2015). During last recent decades, the prevalence of above-mentioned pathologies has been steadily increasing. In response, research has shown that the antioxidant property imparted by phenolic compounds to certain fruits is able to protect against oxidative stress (Oroian and Escriche, 2015). Thus, due to the high cost of care, ingestion of functional foods has found an important place in the diet of populations (Wani et al., 2016).

In fact, it has been reported that antioxidant compounds are associated with some properties particularly anticancer, cardio protective and anti-allergenic. Also, they have beneficial effects against chronic degenerative diseases such as cataract, macular degenerative, neurodegenerative disease, diabetes mellitus as well as aging process (Phaniendra et al., 2015). For this, synthetic antioxidant compounds like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) or tert-butyl hydroquinone (TBHQ) have long been used in food industries. They are used as additives and/or preservatives for their stability and lower cost, in order to retard oxidation reactions (Blasi and Cossignani, 2020). However, as mentioned by Lourenço et al. (2019), animal studies showed that BHA and BHT would be responsible for carcinogenesis and would cause undesirable effects on the liver. Use of natural antioxidants is therefore the privileged alternative. For this, many natural source have been reported among those is cashew apple (Andrade et al., 2015; Kaprasob et al., 2017). Cashew apple is the pseudo-fruit of cashew tree, a tropical fruit tree of Brazilian origin belonging to the Anacardiaceous family (Oliveira et al., 2019). Long devalued for its astringency and high perishability, it currently enjoys particular interest both in research and in the agro-industry because of its excellent functional properties. Moreover, scientific studies have focused on optimizing the extraction of its phenolic compounds which can be affected by several factors. The most important of them include the sample to solvent ratio, the type of solvent used, temperature, speed of agitation or time. Most of these work used toxic solvents such as methanol or acetone which are not suitable for human consumption (Andrade et al., 2015; Felix et al., 2018). The objective of this study consists in modelling an optimal antioxidant activity of CAB obtained after pressing apples for juice extraction, using non-toxic and environmentally friendly solvents.

MATERIALS AND METHODS

Biological material is constituted of CAB obtained after pressing apples for juice extraction. Thus, 580 kg of cashew apple were harvested in March 2021 in an orchard of N'dakouassikro, a village located to twenties kilometer from the city center of Yamoussoukro, political capital of Ivory Coast.

Solvents and reagents

All solvent and reagents used are analytical grade except technical ethanol (EtOH) 96%. There are: Folin-Ciocalteu and vanillin from Carlo Erba (France), methanol 99.6% from Honeywell (Germany), sodium nitrite ($NaNO_2$) from Merck (Germany), aluminum chloride ($AlCl_3$) from VWR International (Belgium), ABTS from Alfa Aesar (Germany), DPPH and gallic acid from Sigma Aldrich (USA), sodium carbonate (Na_2CO_3) from AppliChem (Germany), sodium hydroxide (NaOH) from Fischer Scientific (United Kingdom) and distilled water.

Equipment

Equipment used is composed of hydraulic press V. Stossier (Austria), electric dryer (China), Forplex hammer mill equipped with a sieve of 250 µm diameter (France), magnetic stirrer RS Lab 1C (France), ventilated oven Memmert UF55 (Germany), UV/Vis spectrophotometer Jasco V-530 (Japan), Radwag PS 750/X (Poland) and Sartorius Practum 213-1S (Germany) scales and a Merit water still W4000 (United Kingdom).

Production of CAB powder

During the harvest, apples were separated from the nuts *in situ*, and then convoyed to the school-factory located to the Centre site of *Institut National Polytechnique Houphouët-Boigny* (INPHB) of Yamoussoukro. Apples were washed five times in a row, the third time in 100 mg/L bleach for 10 min about. Juice was extracted by pressing apples using hydraulic press. The derived bagasse was dried at 55°C for 24 h. Then, dried bagasse was milled. Powder was conditioned in a glass bottle and preserved for further analysis.

Extraction of phenolic compounds

Extraction was carried out by maceration following the method described by Bohui et al. (2018) with modification. Water and EtOH were used as extraction solvents. A mass M of CAB powder was mixed with 100 ml of solvent whose proportion of ethanol is C. The mixture was kept stirring at 500 rpm for a time T. After stirring, extract was filtered twice through a mousseline cloth, then oven dried at 50°C for 24 ± 4 h. The dry extracts were kept in glass bottles until related analysis.

Experiment designing

To study the effects of three independent factors, extraction was carried out using a Box-Behnken type experimental design. The factors are EtOH proportion (X1), sample/solvent ratio (X2) and maceration time (X3). Tables 1 and 2 show the experimental range and matrix. The responses were expressed in function of independent factors by the second degree polynomial Equation 1 as enunciated by Patra et al. (2021).

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k=1} \sum_{j}^{k} \beta_{ij} X_i X_j + \sum_{i=1}^{k} \beta_{ii} X_i^2 \quad (1)$$

With: Y = response; Xi, Xj = independent factors affecting response; β_0, βi, βii, βij = regression coefficients of the model (intercept, linear, quadratic and interaction terms, respectively); k = number of factors (k = 3) (Table 1).

Table 1. Experimental domain.

Factor	Unit	Symbol	Level	Value
EtOH proportion	%	X1	(-1)	0
			0	50
			(+1)	100
Sample / solvent ratio	g/100 ml	X2	(-1)	1
			0	5.5
			(+1)	10
Time	Hours	X3	(-1)	6
			0	15
			(+1)	24

Source: Authors

Table 2. Experimental matrix.

Extract	Coded value			Real value		
	EtOH proportion	Samp/solv ratio	Maceration time	EtOH proportion	Samp/solv ratio	Maceration time
E1	0	(+1)	(-1)	50	10	6
E2	0	(-1)	(+1)	50	1	24
E3	(-1)	(-1)	0	0	1	15
E4	0	0	0	50	5.5	15
E5	(+1)	0	(-1)	100	5.5	6
E6	(-1)	(+1)	0	0	10	15
E7	0	(+1)	(+1)	50	10	24
E8	(+1)	(+1)	0	100	10	15
E9	(-1)	0	(-1)	0	5.5	6
E10	(-1)	0	(+1)	0	5.5	24
E11	0	0	0	50	5.5	15
E12	0	0	0	50	5.5	15
E13	0	(-1)	(-1)	50	1	6
E14	(+1)	0	(+1)	100	5.5	24
E15	(+1)	(-1)	0	100	1	15

Source: Authors

Determination of polyphenol content

The determination of polyphenol content was carried out following the colorimetric method using Folin-Ciocalteu's reagent as described by Wood et al. (2002). In a test tube, 2.5 ml of 10% diluted Folin-Ciocalteu reagent was added to 30 µl of extract (2.5 mg/ml). The mixture was let to react for 2 min in the dark. Then, 2 ml of 7.5% Na_2CO_3 were added. The tube was incubated in water bath set at 50°C for 15 min. The tube was rapidly cooled under tap water. The absorbance was measured spectrophotometrically at 760 nm against distilled water as blank. The contents were expressed as milligram of gallic acid equivalent per gram of CAB (mg GAE/g CAB).

Determination of flavonoid content

The flavonoid content determination was carried out by the colorimetric method using $AlCl_3$ reagent as described by Marinova et al. (2005). In 25 ml volumetric flasks containing 2.5 ml of extract (2.5 mg/ml), 0.75 ml of 5% $NaNO_2$ and 0.75 ml of 10% $AlCl_3$ were successively added. The mixture was let to react in the dark at laboratory temperature (22 ± 2°C) for 6 min. Then, 5 ml of NaOH 1N were added. The volume was completed up to the mark with distilled water. The solution was well mixed and the absorbance was measured spectrophotometrically at 510 nm using distilled water as blank. The flavonoid contents were expressed as milligram of quercetin per gram of CAB (mg QE/g CAB).

Determination of total tannin content

The tannin contents were measured following the spectrometric method using Folin-Ciocalteu's reagent as described by Ci and Indira (2016). 100 µl of extract were added to a test tube containing 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteu's reagent.

Then, 1 mL of 35% Na_2CO_3 was added. The volume was completed to 10 ml by adding 900 µl of distilled water. The tube was mixed and let to react for 30 min at laboratory temperature (22 ± 2°C). The absorbance was measured spectrophotometrically at 700 nm using distilled water as blank. Tannin contents were expressed as milligram of tannic acid equivalent per gram of CAB (mg TAE/g CAB).

Assessment of antioxidant activity

ABTS•+ cationic radical scavenging test

The ABTS•+ cationic radical scavenging test was carried out following the method described by Teow et al. (2007) with slight modification. ABTS•+ was generated by mixing at equal volume 3 mM of potassium persulfate to 8 mM of ABTS. Mixture was let to react in the dark for 16 h at ambient temperature (25-30°C). Before use, ABTS•+ solution was diluted with methanol to get an absorbance of 0.7 ± 0.02 at 734 nm. Then, 3.9 ml of the methanolic ABTS•+ working solution were added to 100 µl of extract and mixed thoroughly. The reaction mixture was kept in the dark for 6 min at laboratory temperature. Absorbance of extracts was thus measured at 734 nm. The ABTS+ scavenging activity was calculated following Equation 2:

$$Scav.Act._{ABTS}\ (\%) = \frac{A_{cont} - A_{ext}}{A_{cont}} \times 100 \quad (2)$$

With: Acont = absorbance of control, Aext = absorbance of extract.

DPPH radical scavenging test

The DPPH radical scavenging test was carried out following the method described by Sánchez-Moreno (2002). The DPPH working solution (C = 63.4 µM) was generated by dissolving DPPH in methanol to get an absorbance of 0.88 ± 0.02 at 517 nm. A concentration range of the extracts (0.25; 0.5; 1; 2.5; 5; 10 and 15 mg/ml) was prepared. In the test tubes containing 50 µl of each extract concentration, 1.95 ml of the DPPH working solution were added. The tubes were mixed and kept in the dark for 30 min at laboratory temperature. The absorbance of extracts was measured spectrophotometrically at 517 nm. The yield of DPPH radical scavenging was calculated following Equation 3:

$$Scav.Act._{DPPH}\ (\%) = \frac{A_{cont} - A_{ext}}{A_{cont}} \times 100 \quad (3)$$

The 50% inhibitory concentration (IC50) of DPPH was determined graphically by logarithmic regression. The lower the IC50 of an extract is, the more its antioxidant power is strong.

Statistical analysis

All tests were performed in triplicate and results expressed as mean. Analysis was performed using Excel 2013 (Microsoft, USA), Statistica 7.1 (Tulsa, OK, USA) and Design-Expert 11 (Stat-Ease, Minneapolis, MN, USA) software's. Excel software was used for tabulations. Design-Expert software was used to fit the model through analysis of variance (ANOVA) at 5% threshold ($p<0.05$). The determination coefficient or R squared (R^2), adjusted R squared (adj. R^2), predicted R squared (pred. R^2) and plots were also obtained from Design-Expert. Statistica software was used to test homogeneity by one way ANOVA via Newmann-Keuls test at 5% threshold.

RESULTS AND DISCUSSION

Table 3 presents the polyphenol, flavonoid and tannin contents, as well as the yield of ABTS radical scavenging and IC50's of DPPH. E2 got the highest polyphenol and tannin contents (7.44 mg GAE/g CAB and 9.25 mg TAE/g CAB respectively). The high flavonoid content (14.99 mg QE/g CAB) was observed with E15 extract. As for antioxidant activity, the high ABTS radical scavenging activity was observed with E11, while the high IC50 of DPPH was observed with E3; thus the less effective.

Inversely, the lower phenol and flavonoid contents (3.12 mg GAE/g CAB and 4.28 mg QE/g CAB) were observed with E8 and E1 respectively. E3 extract exhibited the lower tannin content. The lower ABTS scavenging yield comes to E15 extract, and the lower IC50 of DPPH was observed with E7 extract; thus the more effective.

Polyphenol

Polyphenol contents range from 3.12 (E8) to 7.44 mg GAE/g CAB (E2). Regression analysis showed that polyphenol extraction model is significant ($p < 0.0001$) with non-significant lack of fit ($p = 0.1125$). The R squared ($R^2 = 0.9157$) shows that there is a good correlation between the response and factors. The predicted R squared is in reasonable agreement with the adjusted R squared (adj. R^2 – pred. $R^2 < 0.2$). The ANOVA shows that X1 and X2 factors significantly impacted extraction. Effects of the factors on the linear plan of X2, quadratic of X1 and interaction between X1 and X2 are significant (Table 4). So, the lower the sample/solvent ratio is (level -1) and when ethanol proportion is around 50% (level 0), the more extraction is optimal; the effect of time being negligible (Figure 1). Predicted model is as given by Equation 4:

$$Y_1 = 6.37 - 0.0388X_1 - 0.5625X_2 - 0.6675X_1X_2 - 2.56X_1^2 \quad (4)$$

The polyphenol contents of our extracts are much higher than those obtained (0.1215 to 0.3685 mg GAE/g CAB) by Barretto et al. (2015) from the hydroethanolic extraction (30 to 70%) of CAB. This difference could be explained by the different treatments undergone by apples or the extraction technic used (Dirar et al., 2019). It could also be due to genetic variety, environmental parameters, ripening stage and/or harvesting condition as supported by Khorsand et al. (2022)(Figure 1).

Nevertheless, polyphenol contents of our extracts are lower compared to those of Andrade et al. (2015) (19.75 mg GAE/g CAB). These authors used a sequenced extraction process using 55% acetone followed by 50% methanol. The different results in these two studies could be explained by the solvents of different polarities used. On the one hand, our results show that the optimal extraction of polyphenol was reached with 50% ethanolic solvent (4.97- 7.44 mg GAE/g CAB) compared to

Table 3. Polyphenol, flavonoid and tannin contents, and antioxidant power through ABTS scavenging yield and IC50's of DPPH.

Extracts	Polyphenols (mg GAE/g CAB)			Flavonoids (mg QE/g CAB)			Tannins (mg TAE/g CAB)			ABTS scavenging yield (%)			IC50 (mg/ml)		
	Exper	Pred	Residual	Exper	Pred	Residual	Exper.	Pred	Residual	Exper.	Pred	Residual	Exper.	Pred	Residual
E1	5.42^{c}	5.81	-0.39	4.28^{g}	4.56	-0.27	6.42^{d}	6.57	-0.15	58.0^{d}	59.63	-1.63	2.4^{a}	2.46	-0.06
E2	7.44^{a}	6.94	0.50	9.38^{cd}	7.51	1.87	9.25^{a}	8.58	0.67	49.2^{ef}	47.58	1.62	4.73^{ab}	4.01	0.72
E3	3.33^{fg}	3.74	-0.41	6.47^{ef}	6.91	-0.44	1.95^{i}	3.11	-1.16	31.4^{h}	32.93	-1.53	26.97^{d}	19.8	7.17
E4	6.89^{ab}	6.37	0.52	8.70^{d}	7.70	1.00	8.01^{bc}	7.57	0.44	63.3^{c}	71.63	-8.33	2.61^{a}	2.52	0.09
E5	3.24^{fg}	3.77	-0.53	10.99^{b}	10.53	0.46	4.56^{fg}	4.88	-0.32	47.0^{f}	46.90	0.10	6.87^{bc}	4.46	2.41
E6	4.26^{de}	3.95	0.31	7.10^{e}	6.81	0.29	3.87^{gh}	3.71	0.16	38.0^{g}	39.48	-1.48	7.63^{c}	4.81	2.82
E7	4.97^{cd}	5.81	-0.84	4.44^{g}	4.66	-0.22	5.01^{de}	6.57	-1.56	71.6^{b}	70.03	1.57	2.28^{a}	2.46	-0.18
E8	3.12^{g}	2.54	0.58	10.12^{c}	9.45	0.67	3.23^{h}	2.58	0.65	50.4^{ef}	48.88	1.52	3.72^{a}	4.26	-0.54
E9	4.14^{def}	3.85	0.29	5.87^{f}	5.15	0.72	4.51^{fg}	3.41	1.10	53.3^{e}	50.20	3.10	4.16^{ab}	5.06	-0.9
E10	3.66^{efg}	3.85	-0.19	4.68^{g}	5.25	-0.57	3.31^{h}	3.41	-0.10	52.8^{e}	52.90	-0.10	4.08^{ab}	5.06	-0.98
E11	6.75^{ab}	6.37	0.38	6.93^{e}	7.70	-0.77	8.02^{bc}	7.57	0.45	75.9^{ab}	71.63	4.27	2.4^{a}	2.52	-0.12
E12	6.50^{b}	6.37	0.13	7.00^{e}	7.70	-0.70	7.46^{c}	7.57	-0.11	75.7^{a}	71.63	4.07	2.49^{a}	2.52	-0.03
E13	6.64^{ab}	6.94	-0.29	6.50^{ef}	7.41	-0.91	8.84^{ab}	8.58	0.26	46.5^{f}	48.08	-1.58	4.3^{ab}	4.01	0.29
E14	3.86^{efg}	3.77	0.09	9.56^{c}	10.64	-1.08	5.23^{ef}	4.88	0.35	51.0^{ef}	54.10	-3.10	4.98^{ab}	4.46	0.52
E15	4.86^{cd}	5.00	-0.14	14.99^{a}	15.05	-0.06	6.51^{d}	7.19	-0.68	22.9^{i}	21.43	1.47	6.89^{bc}	12.95	-6.06

Experimental values are means of runs performed in triplicate (n = 3). Means with different exponent letters in same column express a statistical difference ($p < 0.05$).
Source: Authors

100% ethanolic (3.12- 4.86 mg GAE/g CAB) and aqueous (3.33- 4.26 mg GAE/g CAB). This result could be explained by the fact that the use of mixture of water and organic solvents would be more effective than mono-solvents to extract phenolic due to polarity.

According to Jovanovic et al. (2017) and Lezoul et al. (2020), water adding increases the polarity index of solvent, thus increasing solubility of polyphenol, which helps to break hydrogen bonding facilitating their maximum extraction. In fact, 50 and 100% ethanolic solvents have respective polarity of 0.827 and 0.654. Therefore, polyphenols are more soluble in solvents of polarity in range of 0.8; that could explain the optimal content obtained by Andrade et al. (2015) when using a 50% methanolic solvent which has 0.88 polarity. Similar result was reported by El-Salam and Morsy (2019) who stated that optimal yields of polyphenols were reached using 40 and 50% EtOH solvents whose polarities are 0.862 and 0.827 respectively. Similarly, work of Dirar et al. (2019) conducted on Sudanese medicinal plants (*Cyperus rotundus* and *Guiera senegalensis*) revealed that polyphenol contents of 50 and 70% ethanolic solvents were maximized than those of mono-solvents (dichloromethane, acetone, 95% ethanol and water).

On the other hand, our result show that maceration time (X3) has had a negligible effect on polyphenol extraction. In fact, extract E13 and E2 were conducted in the same condition except for maceration time: 6 h and 24 h respectively. However, their phenolic contents were not statistically different ($p<0.05$). This result means that extension of the maceration time of extract E2 to 24 h has not help to maximize its polyphenol content. The same is true for extracts E5 and E14.

This result can be explain by the fact that longer extraction time could induce the polyphenol degradation by hydrolysis resulting sometimes in the decrease of the yield. This result is comforted by those of El-Salam and Morsy (2019) who report that phenolic content of *Malva parviflora* L. leaves had not significantly increased at over10 min; the extraction being extended till 45 min. Likewise, Wani et al. (2017) showed that extraction time had negligible effect when extracting

Table 4. ANOVA for reduced quadratic model of polyphenol extraction.

Source	Sum of squares	Df	Mean Square	F-value	p-value	
Model	28.87	4	7.22	27.17	< 0.0001*	Significant
X_1-EtOH prop.	0.0120	1	0.0120	0.0452	0.8359	
X_2- Samp/Solv ratio	2.53	1	2.53	9.53	0.0115*	
X_1X_2	1.78	1	1.78	6.71	0.0269*	
X_1^2	24.55	1	24.55	92.39	< 0.0001*	
Residual	2.66	10	0.2657			
Lack of fit	2.58	8	0.3223	8.26	0.1125	Not significant
Pure Error	0.0781	2	0.0390			
Cor total	31.53	14				
R^2	0.9157					
Adj. R^2	0.882					
Pred. R^2	0.7475					

Source: Authors

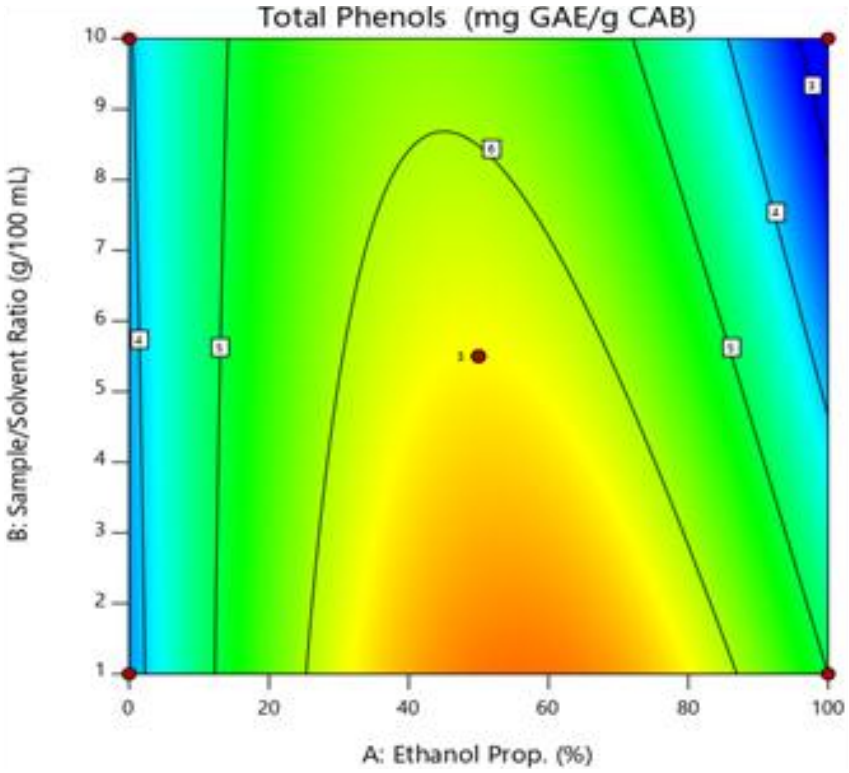

Figure 1. Response surface contours of EtOH proportion and Sample/solvent ratio effects at constant time (T = 15 h) on polyphenol extraction.
Source: Authors

polyphenols from dried apricot fruit extracts (*Prunus armeniaca* L.).

Flavonoid

Flavonoid contents range from 4.28 (E1) to 14.99 mg QE/g CAB (E15). The model is significant ($p = 0.0006$) and present no significant lack of fit ($p = 0.5109$). The R squared ($R^2 = 0.9184$) show that it exists good correlation between response and factors. The predicted R^2 is in reasonable agreement with adjusted R^2. The ANOVA shows that all the factors significantly impacted flavonoid extraction. Effects of the factors on the linear plan of X1

Table 5. ANOVA for reduced quadratic model of flavonoid extraction.

Source	Sum of squares	Df	Mean Square	F-value	p-value	
Model	106.67	6	17.78	15.02	0.0006*	Significant
X_1- EtOH prop.	58.00	1	58.00	48.98	0.0001*	
X_2- Samp/solv ratio	16.25	1	16.25	13.72	0.0060*	
X_3-Time	0.0220	1	0.0220	0.0186	0.8948	
X_1X_2	7.56	1	7.56	6.39	0.0354*	
X_1^2	12.80	1	12.80	10.81	0.0111*	
X_3^2	10.28	1	10.28	8.68	0.0185*	
Residual	9.47	8	1.18			
Lack of Fit	7.46	6	1.24	1.24	0.5109	Not significant
Pure Error	2.01	2	1.00			
Cor Total	116.14	14				
R^2	0.9184					
Adj. R^2	0.8573					
Pred. R^2	0.7186					

Source: Authors

and X2, quadratic of X1 and X3, and interaction between X1 and X2 are significant (Table 5). Thus, the higher the EtOH proportion is (level +1) and low is the sample/solvent ratio (level -1), the more flavonoid contents are optimal (Figure 2). The model is written as shown by Equation 5:

$$Y_2 = 7.7 + 2.69X_1 - 1.43X_2 + 0.0525X_3 - 1.37X_1X_2 + 1.86X_1^2 - 1.66X_3^2 \quad (5)$$

The flavonoid contents of our extracts are higher (9.56 to 14.99 mg QE/g dw) compared to those obtained by Krasnova and Seglina (2019) (1.45 to 5.59 mg CAE/g dw) in ultrasound assisted extraction of flavonoids from 11 cultivars of apple (*Malus domestica*) using 70% EtOH (Table 5 and Figure 2).

According to the results, it can be state that the use of a solvent with high EtOH concentration and a low sample/solvent ratio induces the extraction of high content of flavonoid. In fact, the E14 and E15 extracts were performed using 100% EtOH with sample/solvent ratio of 5.5 g/100 ml and 1 g/100 ml respectively. It was observed that E15 got the highest flavonoid content. This result can be explain on the one hand, by the fact that the use of lower sample/solvent ratio has been able to avoid solvent saturation, allowing well molecular movement; thus increasing the extraction of flavonoids (Ćujić et al., 2016). The same is true for E5 and E8 extracts. Our results are comforted by those of Pandey et al. (2021) who found high flavonoid contents when combining the high level of EtOH (90% EtOH) and low sample/solvent ratio (1:40 and 1:30, w:v). On the other hand, this result can be due to the predominant presence of some flavonoid compounds more soluble in ethanol such as quercetin, kaempferol or kaempferitrin (Cid-Ortega and Monroy-Rivera, 2018; Đorđević et al., 2018). Likewise, work of Dirar et al. (2019) show that use of high EtOH concentration solvent allowed to extract strong flavonoid contents from 4 Sudanese medicinal plants (*B. linariifolia*, *C. rotundus*, *M. pseudopetalosa* and *T. bakis*).

The ANOVA showed that time (X3) has had a significant effect on flavonoid extraction. In fact, E2 and E13 extracts were performed in the same conditions excepted for the time: 24 h and 6 h for E2 and E13 respectively. Results showed that E2 got the highest flavonoid content, indicating that extension of the maceration time of E2 extract allowed to increase its flavonoid content. Our findings are in convenience with those of Tranquilino-Rodríguez et al. (2020). These authors reported that flavonoid contents of 70, 83 and 96% ethanolic extracts of young cladodes of *Opuntia ficus-indica* were ranged from 3.095 to 10.392 mg QE/g dw proportionally with the increase of EtOH concentration and extraction time from 30 to 120 min.

Total tannin

Tannin contents range from 1.95 (E3) to 9.25 mg TAE/g CAB (E2). The model is significant ($p<0.0001$) and present no significant lack of fit ($p = 0.111$). The R squared ($R^2 = 0.8988$) show existence of a good correlation between the response and factors. ANOVA shows that X1 and X2 factors have significantly impacted tannin extraction. The linear effects of X1 and X2, interaction between X1 and X2 and quadratic of X1 are statistically significant (Table 6). The lower the sample/solvent ratio is (level -1) and the EtOH proportion turn around 60%, the more tannin extraction is optimal(Figure 3); the influence of time being negligible. Mathematic model is written following Equation 6:

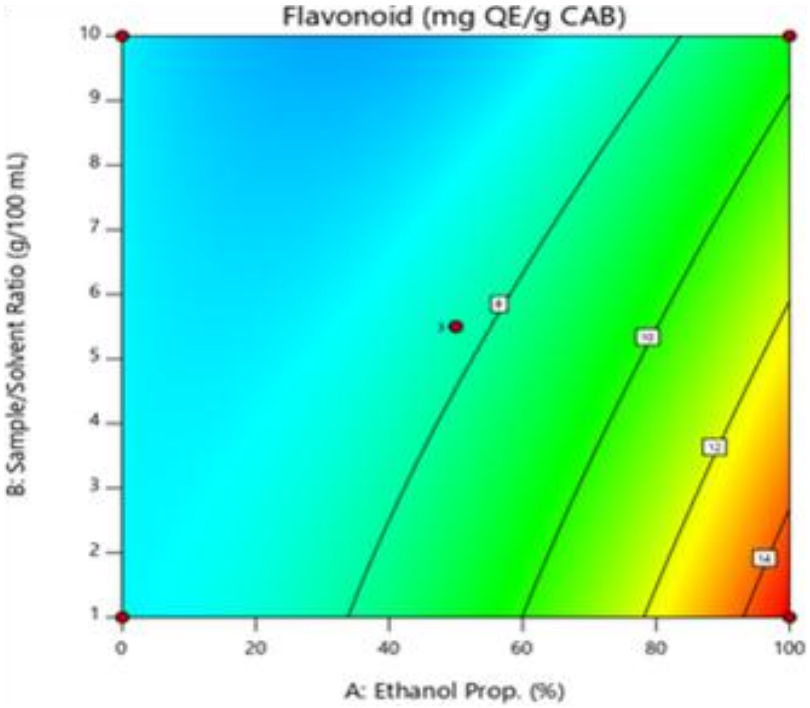

Figure 2. Response surface contours of EtOH proportion and Sample/solvent ratio effects at constant time (T = 15 h) on flavonoid extraction.
Source: Authors

$$Y_3 = 7.57 + 0.7362X_1 - X_2 - 1.3X_1X_2 - 3.43X_1^2 \quad (6)$$

The 50% ethanolic solvent induced the extraction of high tannin contents (5.01 to 9.25 mg TAE/g CAB) compared to those obtained using 100% EtOH (3.23 to 6.51 mg TAE/g CAB) and water (1.95 to 4.51 mg TAE/g CAB). The factors' effects in tannin extraction show similarities as in the case of polyphenol extraction. Same observation was reported by Detti et al. (2020) in their work on optimization of ultrasound assisted green extraction of polyphenols from *Pistacia lentiscus* leaves. This observation could be explained by the fact that tannins could be the most abundant components in the CAB, given that flavonoid and tannin are included into polyphenols.

Antioxidant activity

ABTS+ radical scavenging test

ABTS+ radical scavenging yields range from 22.9% (E15) to 75.9% (E11). Model is significant (*p = 0.0069*) and present no significant lack of fit (*p = 0.8599*). The R squared (R^2 = *0.9556*) show that it exists a good correlation between response and factors. Predicted R^2 is in reasonable agreement with adjusted R^2. ANOVA shows that X1 and X2 factors have impacted response. The effect on the linear plan of X1 and quadratic of X1 and X2 are significant (Table 7). ABTS+ scavenging yield is optimal when EtOH proportion is around 50% with sample/solvent ratio situated around 7 g/100 ml (Figure 4); time's effect being negligible. Mathematic model is as described in Equation 7:

$$Y_4 = 71.63 - 0.525X_1 + 8.5X_2 + 2.48X_3 + 5.23X_1X_2 + 1.13X_1X_3 + 2.72X_2X_3 - 20.63X_1^2 - 15.33X_2^2 + 0.0208X_3^2 \quad (7)$$

On the one hand, our results show that extraction solvent impacted antioxidant power. 50% ethanolic extracts exhibited the strong ABTS+ scavenging power (46.5 to75.9%) compared to those of aqueous (31.4 to 53.3%) and 100% ethanolic extracts (22.9 to 51%). Optimal ABTS radical scavenging yield was reached when EtOH proportion range from 40 to 60%. Under or above this interval, a drop of the ABTS scavenging power is observed. This result could be due to components extracted in solvent of EtOH proportion ranged in this interval, relative to its polarity. Work of Li et al. (2019) carried out on *Gordonia axillaris* fruit comfort this result.

Table 6. ANOVA for reduced quadratic model of tannin extraction.

Source	Sum of squares	Df	Mean square	F-value	p-value	
Model	62.97	4	15.74	22.21	< 0.0001*	Significant
X_1-EtOH prop.	4.34	1	4.34	6.12	0.0329*	
X_2- Samp/solv ratio	8.04	1	8.04	11.34	0.0071*	
X_1X_2	6.76	1	6.76	9.54	0.0115*	
X_1^2	43.84	1	43.84	61.85	< 0.0001*	
Residual	7.09	10	0.7087			
Lack of fit	6.88	8	0.8602	8.38	0.1110	Not significant
Pure Error	0.2054	2	0.1027			
Cor total	70.06	14				
R^2	0.8988					
Adj. R^2	0.8584					
Pred. R^2	0.6471					

Source: Authors

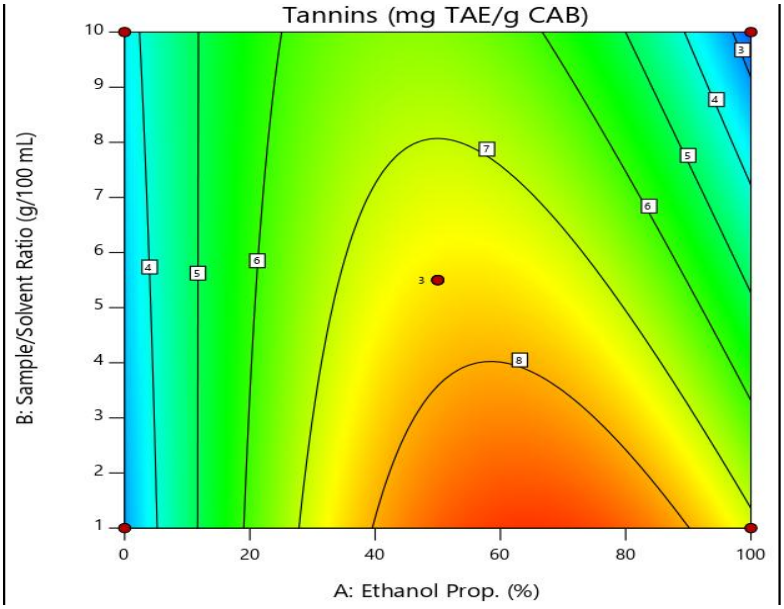

Figure 3. Response surface contours of EtOH proportio and Sample/solvent ratio effects at constant time (T = 15 h) on tannin extraction.
Source: Authors

Similarly, Tranquilino-Rodríguez et al. (2020) reported that a decrease in the ethanol concentration from 96 to 70% was inversely correlated to ABTS radical scavenging power. On the other hand, our results show that sample/solvent ratio is an important factor which influences ABTS scavenging power. In fact, ABTS scavenging yield was optimal at ratio ranging from 5 g/100 ml (that is, 1:20) to 8 g/100 ml (that is, 1:12.5). This result means that in this interval, the solvent saturation is avoided and compounds endowed with good ABTS scavenging are extracted (Figure 4). This statement is in convenience with that mentioned by Barretto et al. (2015) and Pandey et al. (2021) who state that sample to solvent ratio is the most important factor which influence ABTS radical scavenging.

DPPH radical scavenging test

The extract IC50's varied from 2.28 (E7) to 26.97 mg/ml (E3). The model is significant (p = 0.0001) and present no significant lack of fit (p = 0.0835). The R squared (R^2 =

Table 7. ANOVA for quadratic model of ABTS radical scavenging.

Source	Sum of squares	Df	Mean square	F-value	p-value	
Model	3070.96	9	341.22	11.96	0.0069*	Significant
X_1-EtOH prop.	2.20	1	2.20	0.0773	0.7922	
X_2-Samp/solv ratio	578.00	1	578.00	20.26	0.0064*	
X_3-Time	49.00	1	49.00	1.72	0.2470	
X_1X_2	109.20	1	109.20	3.83	0.1078	
X_1X_3	5.06	1	5.06	0.1774	0.6911	
X_2X_3	29.70	1	29.70	1.04	0.3544	
X_1^2	1571.31	1	1571.31	55.07	0.0007*	
X_2^2	867.63	1	867.63	30.41	0.0027*	
X_3^2	0.0016	1	0.0016	0.0001	0.9943	
Residual	142.67	5	28.53			
Lack of Fit	38.48	3	12.83	0.2463	0.8599	Not significant
Pure Error	104.19	2	52.09			
Cor Total	3213.63	14				
R^2	0.9556					
Adj. R^2	0.8757					
Pred. R^2	0.7354					

Source: Authors

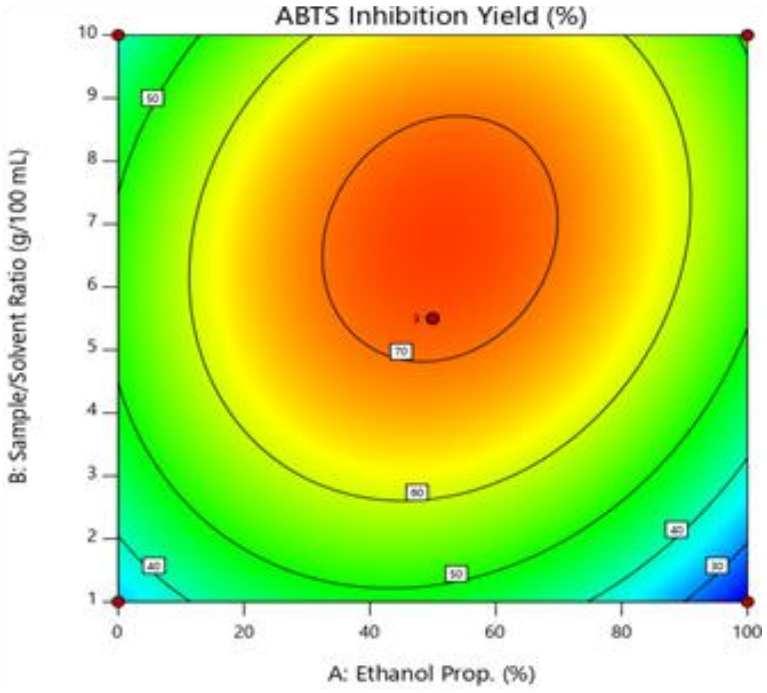

Figure 4. Response surface contours of EtOH proportion and Sample/solvent ratio effects at constant time (T = 15 h) on ABTS radical scavenging.
Source: Authors

0.879) reveals that a good correlation is established between response and factors. Predicted R^2 is in reasonable agreement with the adjusted R^2. The ANOVA shows that X2 and X1 factors impacted significantly the response. Linear effects of X2 and quadratic ones of X1 and X2 were statistically significant (Table 8). Figure 5

Table 8. ANOVA for reduced quadratic model of DPPH radical scavenging.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.1899	4	0.0475	18.16	0.0001*	Significant
X_1-EtOH prop.	0.0014	1	0.0014	0.5441	0.4777	
X_2-Samp/solv ratio	0.0494	1	0.0494	18.91	0.0014*	
X_1^2	0.1277	1	0.1277	48.81	< 0.0001*	
X_2^2	0.0174	1	0.0174	6.67	0.0273*	
Residual	0.0262	10	0.0026			
Lack of Fit	0.0256	8	0.0032	11.35	0.0835	Not significant
Pure Error	0.0006	2	0.0003			
Cor Total	0.2161	14				
R^2	0.879					
Adj. R^2	0.8306					
Pred. R^2	0.6829					

Source: Authors

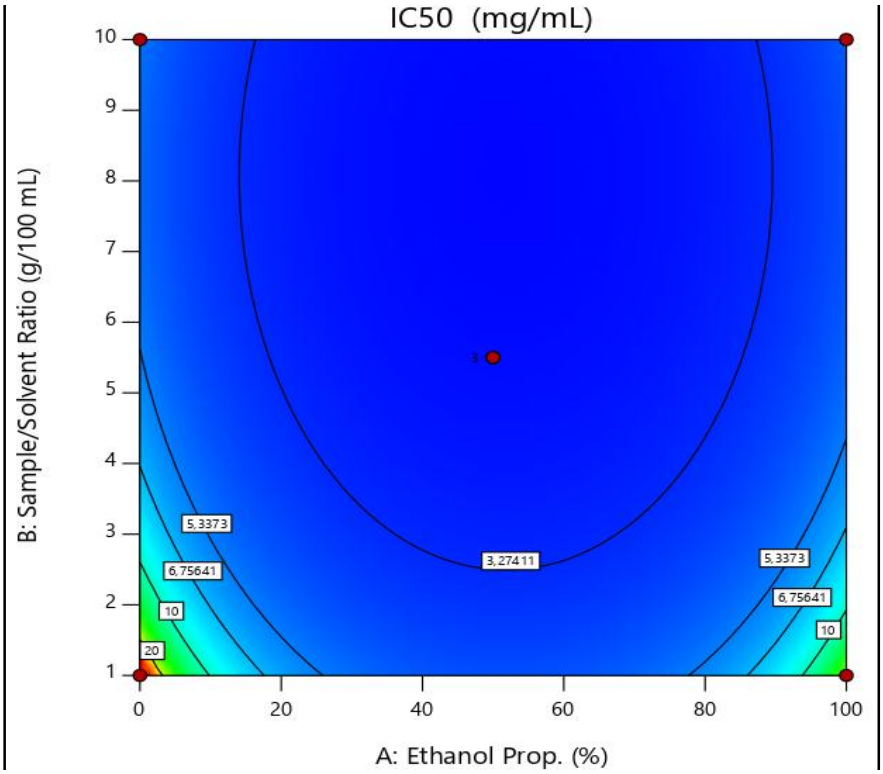

Figure 5. Response surface contours of EtOH proportion and Sample/solvent ratio effects at constant time (T = 15 h) on DPPH radical scavenging.
Source: Authors

shows that the lower the sample/solvent ratio (level -1) for a proportion of EtOH oscillating around 50% (level 0), the lower the IC50. Maceration time showed no significant effect. The mathematic model is as expressed in Equation 8:

$$Y_5 = \frac{1}{0.3964 + 0.0133X_1 + 0.0786X_2 - 0.1854X_1^2 - 0.0685X_2^2} \quad (8)$$

Similarly to the ABTS+ scavenging test, our results show that the 50% ethanolic extracts were more effective in

Table 9. Matrix of correlation.

	Polyphenol	Flavonoid	Tannin	ABTS	CI50
Polyphenol	1.00				
Flavonoid	-0.11	1.00			
Tannin	0.93	0.13	1.00		
ABTS	0.46	-0.51	0.37	1.00	
CI50	-0.43	0.04	-0.53	-0.59	1.00

Source: Authors

Table 10. Verification test of the model predictions.

Predicted Interval	Polyphenol (mg GAE/g CAB)		Flavonoid (mg QE/g CAB)		Tannin (mg TAE/g CAB)		ABTS (%)		IC50 (mg/mL)	
	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.
95% PI low	5.14		2.63		5.44		49.17		2.05	
Mean.	5.9	**6.62**	4.61	**5.31**	6.7	**5.45**	61.74	**74.06**	2.48	**2.5**
95% PI high	6.67		6.58		7.94		74.3		3.01	

Source: Authors

scavenging DPPH radical. Their IC50s ranged from 2.28 to 4.73 mg/mL, exhibiting stronger DPPH radical scavenging power than the 100% ethanolic (3.72 to 6.89 mg/ml) and aqueous extracts (4.08 to 26.97 mg/ml). This result is confirmed by that of Dirar et al. (2019) which shows that the 50% ethanolic extract of *Guiera senegalensis* leaves presented the strongest DPPH scavenging trapping power compared to those of mono-solvents (water, acetone, 95% EtOH and acetonitrile).

Optimization of antioxidant activity

The study of Pearson correlation (Table 9) shows that antioxidant activity via the ABTS scavenging test is positively correlated to polyphenol ($r = 0.46$; $p < 0.05$) and tannins ($r = 0.37$; $p < 0.05$). However, it is negatively correlated to flavonoids ($r = -0.51$; $p < 0.05$). Similarly, antioxidant activity via DPPH scavenging test is correlated to polyphenol ($r = -0.43$; $p < 0.05$) as well as tannin ($r = -0.53$; $p < 0.05$). The negative correlation coefficient ($r < 0$) indicates that the more polyphenol and tannin contents are optimal, the lower IC50s are; thus more effective. Flavonoids establish no correlation with DPPH scavenging. Nevertheless, ABTS and DPPH scavenging are correlated one to other ($r = -0.59$; $p < 0.05$).

Optimization of antioxidant activity with a desirability of 0.79 consists to macerate CAB powder at 9.5 g/100 ml ratio in a 46.1% ethanolic solvent for 6 h. A run replicated five times was carried out to verify this optimal condition. All values of the verification tests ranged in the predicted interval (PI) by the model (Table 10), meaning that the model fits well with the optimization of antioxidant power of CAB powder.

Conclusion

This study allows us to model optimal extraction condition of antioxidant phenolic compounds from CAB using non-toxic solvents. Evaluation of the effects of three factors showed that effects of EtOH proportion of solvent (X1) and sample to solvent ratio (X2) are significant. The determination coefficient ($R^2 > 0.8$) indicates that model adequately explain experimental data. Correlation study shows that flavonoids establish no correlation with ABTS and DPPH antioxidant tests. For a desirability of 0.79, the model to reach an optimal antioxidant power is as: EtOH proportion (46.1%), sample/solvent ratio (9.5 g/100 ml) and maceration time (6 h). To this condition, ABTS scavenging yield reaches 74% and the IC50 of DPPH is 2.5 mg/ml, conceding with model predictions. Using non-toxic solvents to reach optimal antioxidant bioactive compound extraction allows envisaging its use in food industry due to their benefits for health.

ABBREVIATIONS

CAB, cashew apple bagasse; **GAE,** gallic acid equivalent; **QE,** quercetin equivalent, **TAE,** tannic acid

equivalent; **EtOH,** ethanol; **ABTS,** 2,2'-azinobis (3-ethyl-benzothiazoline-6-sulphonic acid; **DPPH,** 1,1-diphenyl-2-picryl-hydrazyl.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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Effect of *Citrus aurantifolia* juice on bodyweight and haematological indices of wistar rats

Obiajulu Christian Ezeigwe*, Chukwuemeka Obumneme Okpala, Michael Okey Enemali, Ebele Lauretta Iloanya, Chidiebere Malachy Chigbo, Chisom Miracle Okeke, Chioma Blessing Okeke and Michael Chinonso Okafor

Department of Applied Biochemistry, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

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Citrus aurantifolia **(Lime) is a citrus fruit and an excellent source of vitamin C and flavonoids which have unique antioxidant properties. This study determined the effects of *C. aurantifolia* on the bodyweight and hematological indices of rats. A total of 20 rats of both sex weighing between 120 and 130 g were randomized into 4 groups of five rats each and used. Group A: Normal Control; Group B: 2 ml/kg of *C. aurantifolia* fruit juice; Group C: 4 ml/kg of *C. aurantifolia* fruit juice; Group D: 8 ml/kg of *C. aurantifolia* fruit juice. The *C. aurantifolia* fruit was freshly squeezed and the juice was administered to the test groups for a period of three months. The haematological parameters were analysed using standard methods. The results revealed that administration of *C. aurantifolia* juice caused a significant difference ($p<0.05$) in the bodyweights of the experimented groups from week 3 up to week 12 compared with the normal control group. After three months of the administration, only the platelet counts showed a significant difference ($p>0.05$) in group B compared with other groups throughout the three months of administration. Therefore, it is then concluded that bioactive substances present in lime like lycopene and vitamin C induces the proliferation of white blood cells in the blood circulation. Hence, it was proven that lime has a protective effect which may serve as an alternative treatment option in patients with leukopenia.**

Key words: Lime, three months, administration, platelet, fruit, groups.

INTRODUCTION

Food is thought about as a basic need for human. However, unhealthy food consumption degrades the quality of life instead of a balance diet. In this regard, Slavin and Lloyd (2012) stated that vegetables and fruits contain essential nutrients such as dietary fiber, carotenoids, vitamins, minerals, folate, plant sterols, and various phytochemicals are required for human daily diet. According to Wang et al. (2014); Crowe (2015) and Aune et al. (2017), these nutrients help to reduce mortality and prevent chronic diseases, including various cardiovascular

*Corresponding author. E-mail: oc.ezeigwe@unizik.edu.ng. Tel: +2347034432437.

diseases, cancers and even mental illnesses which is ascribed to the presence of different phytochemicals on those fruits and vegetables, functioning as anti-inflammatory agents, phytoestrogens, and antioxidants (Gouegni and Abubakar, 2013; Rodriguez-Casado 2016).

Enejoh et al. (2015) reported that the fruit juice of *Citrus aurantifolia* (Rutaceae), commonly known as lime, is added to sugar and palm oil or honey to relief cough and considered as tonic for libido and as antidote for poison. Aibinu et al. (2007) reported that the diluted form of the *C. aurantifolia* fruit juice is used for mouth wash to treat sore mouth and sore throat. The fruits of *C. aurantium* are sources of flavonoid-type compounds with diverse biological effects (Kang et al., 2011; Hamada et al., 2017; Liu et al., 2008) besides the essential oil and its components (Moraes et al., 2009; Barceloux, 2008). Additionally, Zhang et al. (2017) reported the isolation of flavonoid glycosides from the plant and the determination of biogenic amine and flavanone contents by Pellati et al. (2004) and Bagatela et al. (2015). Akhtar (2013) reported the use of the juice in treating irritation, diarrhoea and swelling due to mosquito bites, and is sometimes used as vermifuge when mixed with oil and also incorporated into weight management diet. Medicinal plants play important role in the management of diseases due to their rich antioxidants, phytochemicals, easy accessibility and affordability by the populace (Alaebo et al., 2020; Onochie et al., 2020; Iloanya et al., 2021).

Phytopharmaceuticals have shown an outstanding role in new drug discovery (Khan and Amin, 2016; Khan et al., 2016) in the last years and a large population globally are getting therapeutic benefits from them (Marya et al., 2018) both in the crude form as well as pure chemical entities. Kuding leaves known to be a medicinal plant has been observed in some studies to affect bodyweight and packed cell volume (Alisa et al., 2015; Ezeigwe et al., 2018). Some herbs may be beneficial in bodyweight reduction but may have adverse effect on the blood parameters. There is always needed to investigate the effect of medicinal plants on biochemical and haematological parameters before recommending them to those that need them for bodyweight reduction. One of our earlier studies investigated the effect of *C. aurantifolia* fruit juice on biochemical parameters of Wistar rats (Ezeigwe et al., 2022). There is paucity of information on the effects of *C. aurantifolia* on the blood parameters of Wistar rats, thus, the present study aimed to evaluate the effect of *C. aurantifolia* juice on bodyweight and hematological indices of rats.

MATERIALS AND METHODS

Sample collection and identification

The *C. aurantifolia* fruits were purchased from Eke Market, Awka, Awka South Local Government Area, Anambra State, Nigeria. The sample was identified by a taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka. The voucher number as deposited in the herbarium of Nnamdi Azikiwe University, Awka is NAUH 196[A].

Test animals

A total of 20 Wistar albino rats of both sex weighing between 120-130 g were purchased from Chris Experimental Animal Farm and Research Laboratory, Awka, Anambra State and used for the experiment. They were maintained and housed in cages under standard environmental conditions (27±3°C, 12 h light/dark cycle) in the Department of Applied Biochemistry Laboratory, Nnamdi Azikiwe University, Awka. They were allowed to acclimatize with the environment for one week before use. The animals were fed vital grower's mash pellets purchased from Vital Feed Distributor at Awka, Anambra state and fed *ad libitum*. At the end of the one-week acclimatization period, the animals were weighed, grouped, and labeled.

Study design

A total of 20 rats of both sex weighing between 120 and 130 g were randomized into 4 groups of five rats each and used. The *C. aurantifolia* fruit was freshly squeezed and the juice was administered to the test groups for a period of three months.

Group A: Normal Control
Group B: 2 ml/kg bodyweight of *C. aurantifolia* fruit juice
Group C: 4 ml/kg bodyweight of *C. aurantifolia* fruit juice
Group D: 8 ml/kg bodyweight of *C. aurantifolia* fruit juice

Determination of bodyweight

The weight of the experimental subjects was checked using an electronic weighing scale. The bodyweights of the rats were monitored before, during, and after the experiment to know whether the continuous administration of lime juice caused a noticeable increase or decrease in bodyweight. Percentage bodyweight was also calculated using the formular below:

$$\text{Percentage weight} = \frac{Weekly\ \ weig\,ht - Initial\ \ weig\,ht}{Initial\ \ weig\,ht} \times \frac{100}{1}$$

Haematological analysis

Haematological parameters were determined using automated haematology analyzer (Mindray-BC-5300). The haematological parameters that were analysed include haemoglobin (HGB), packed cell volume (PCV), red blood cells (RBC), platelets (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), neutrophils (NEUT), lymphocytes (LYMPH), monocytes (MON), eosinophils (EOS), basophils (BAS).

Statistical analysis of results

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences software for windows version 23 (SPSS Inc., Chicago, Illinois, USA). All the data collected were expressed as Mean ± SEM. Statistical analysis of the results obtained were performed by using ANOVA Tests to determine if significant difference exists between the mean of the test and control groups. The limit of significance was set at $p<0.05$.

Table 1. Weekly Bodyweight of rats administered different doses of *C. aurantifolia* fruit juice.

Groups	Weight (g) Week 0	Weight (g) Week 1	Weight (g) Week 2	Weight (g) Week 3	Weight (g) Week 4	Weight (g) Week 5	Weight (g) Week 6	Weight (g) Week 7	Weight (g) Week 8	Weight (g) Week 9	Weight (g) Week 10	Weight (g) Week 11	Weight (g) Week 12
Normal control	123.72±2.59	130.11±2.33	139.06±1.86[a]	146.64±1.32[a]	154.20±1.65[a]	162.71±1.18[a]	170.63±1.06[a]	181.57±1.30[a]	190.61±2.13[a]	197.74±2.57[a]	205.12±1.32[a]	211.41±2.46[a]	219.35±2.68[a]
2 ml/kg bw. of *C. aurantifolia* fruit juice	125.41±1.23	131.73±3.99	134.31±2.02	138.53±1.93[a]	143.20±0.91[a]	149.82±2.21[a]	151.34±2.07[a]	146.12±2.12[a]	140.45±2.87[a]	137.02±1.14[a]	136.37±1.50[a]	132.14±1.31	126.82±2.64
4 ml/kg bw. of *C. aurantifolia* fruit juice	121.91±1.07	126.42±1.53	129.76±1.61	133.23±2.03	136.52±1.35[a]	138.51±2.75[a]	137.23±1.57[a]	134.67±1.34[a]	130.24±1.37	128.52±2.29	125.16±1.34	124.78±2.18	120.30±1.39
8 ml/kgbw. of *C. aurantifolia* fruit juice	123.23±2.62	130.65±1.11	134.32±2.72	139.21±2.16[a]	140.98±1.83[a]	140.25±0.65[a]	138.72±1.52[a]	136.32±1.26[a]	135.90±1.14[a]	130.64±2.06	127.02±1.46	126.23±1.77	121.86±1.43

[a]Significant increase with respect to week 0; [b]Significant decrease with respect to week 0.
Source: Authors

Table 2. Percentage increase/decrease in bodyweight of rats administered different doses of *C. aurantifolia* fruit juice.

Groups	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
Normal Control	-	5.17	12.37	18.51	24.66	31.53	37.91	46.89	54.08	59.82	65.80	70.90	77.28
2 ml/kg bw. of *C. aurantifolia* fruit juice	-	5.02	7.10	10.45	14.19	19.46	20.65	16.51#	11.96#	9.25#	8.69#	5.34#	1.12#
4 ml/kg bw. of *C. aurantifolia* fruit juice	-	3.69	6.40	9.27	11.98	13.62	12.55#	10.42#	6.81#	5.41#	2.63#	2.30#	1.31#
8 ml/kg bw. of *C. aurantifolia* fruit juice	-	6.01	9.01	12.99	14.37	13.80#	12.58#	10.63#	10.31#	6.01#	3.08#	2.44#	1.14#

*increase; #decrease.
Source: Authors

RESULTS

Effect on bodyweight

The body weight of rats administered different doses of *C. aurantifolia* fruit was recorded as shown in Table 1. Group A (Normal control) showed an increase in bodyweight from the baseline, which steadily increased up to the 12th week. Group B showed an increased-decreased pattern in bodyweight. It increased from the baseline, which increased steadily up to week 6, followed by a sharp decrease in week 7, which continued till the 12th week. Group C and D followed the same increased-decreased pattern, with a decrease in bodyweight manifesting from week 6 (Table 1). The percentage increase/ decrease in bodyweight of rats was calculated and shown in Table 2. It showed a significant ($p<0.05$) increase in the bodyweight of the normal control (Group A) compared with the test groups (Groups B, C and D).

Haematological analysis

Table 3 showed the Hemoglobin (HGB) concentration of rats administered with *C. aurantifolia*. HGB of the subjects from Group A (Normal control) and Group B decreased from their baselines after 1st month, followed by a further decrease in the 2nd month but increased in the 3rd month. Group C showed a decrease from its baseline after the 1st month, peaked in the 2nd month and even higher in the3rd month. Group D

Table 3. Haemoglobin concentration of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	HGB (g/dl)			
	Initial	1st Month	2nd Month	3rd Month
Normal Control	14.93±0.07	13.81±0.03	13.30±0.09	14.75±0.01
2 ml/kg bw. of *C. aurantifolia* fruit juice	14.63±0.03	14.15±0.02	13.03±0.05	14.12±0.06
4 ml/kg bw. of *C. aurantifolia* fruit juice	13.75±0.10	13.62±0.03	13.69±0.03	14.32±0.03
8 ml/kg bw. of *C. aurantifolia* fruit juice	14.02±0.15	13.23±0.02	13.96±0.02	13.85±0.03

Source: Authors

Table 4. Haematocrit of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	HCT (%)			
	Initial	1st Month	2nd Month	3rd Month
Normal control	47.83±2.20	46.12±2.51	43.54±2.80	47.27±1.32
2 ml/kg bw. of *C. aurantifolia* fruit juice	45.22±1.93	46.04±1.14	42.81±1.43	45.04±2.12
4 ml/kg bw. of *C. aurantifolia* fruit juice	42.35±0.58	43.67±1.83	43.93±3.11	45.01±0.52
8 ml/kg bw. of *C. aurantifolia* fruit juice	46.96±1.97	43.13±0.93	44.02±1.07	43.27±0.17

Source: Authors

Table 5. Mean Corpuscular Volume of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	MCV (fl)			
	Initial	1st Month	2nd Month	3rd Month
Normal Control	60.76±3.08	60.35±1.82	60.57±3.36	59.54±1.81
2 ml/kg bw. of *C. aurantifolia* fruit juice	60.86±1.60	60.32±1.30	59.64±1.83	60.20±3.03
4 ml/kg bw. of *C. aurantifolia*fruit juice	59.23±0.98	60.21±1.23	58.53±2.42	58.63±0.71
8 ml/kg bw. of *C. aurantifolia*fruit juice	58.87±2.76	59.49±2.94	58.41±0.96	56.12±1.63

Source: Authors

Table 6. Mean Corpuscular Haemoglobin of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	MCH (pg)			
	Initial	1st Month	2nd Month	3rd Month
Normal control	19.01±0.02	18.73±0.18	18.76±0.03	18.51±0.01
2 ml/kg bw. of *C. aurantifolia* fruit juice	18.57±0.09	18.36±0.03	18.51±0.12	18.74±0.03
4 ml/kg bw. of *C. aurantifolia*fruit juice	18.91±0.03	18.72±0.01	18.63±0.03	18.79±0.01
8 ml/kg bw. of *C. aurantifolia* fruit juice	18.75±0.11	18.22±0.03	18.95±0.02	18.63±0.25

Source: Authors

followed the same pattern as in Group C with a decrease in the 3rd month. Table 4 showed the Hematocrit concentration of rats administered with *C. aurantifolia*. HCT of the subject from Group A (Normal control) decreased from its baseline after 1st month, followed by a further decrease in the 2nd month but increased in the 3rd month. HCT of the subject from Group B increased from its baseline after 1st month, followed by a sudden decrease in the 2nd month but shot up in the 3rd month. Group C increased from its baseline after 1st month, which steadily increased in the 2nd month, up till the 3rd month. Group D showed a decreased and increased pattern after 1st month, 2nd month and 3rd month, respectively.

MCV results were shown in Table 5. Although there was a decrease in all the groups in the 3rd month of administration from their baselines, no significant differences ($p>0.05$) were observed throughout the three months of administration with Lime Juice. MCH results were shown in Table 6. There were no significant

Table 7. Mean Corpuscular Haemoglobin Concentration of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	MCHC (g/dl)			
	Initial	1st Month	2nd Month	3rd Month
Normal control	31.22±0.01	32.64±0.32	31.83±0.03	32.05±0.07
2 ml/kg bw. of *C. aurantifolia*fruit juice	31.62±0.03	33.20±0.03	31.73±0.04	31.01±0.03
4 ml/kg bw. of *C. aurantifolia*fruit juice	33.14±0.15	30.82±0.07	32.57±0.03	31.97±0.02
8 ml/kg bw. of *C. aurantifolia* fruit juice	32.71±0.02	31.66±0.01	33.25±0.05	33.19±0.02

Source: Authors

Table 8. Red Blood Cell count of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	RBC (× $10^{12/L}$)			
	Initial	1st Month	2nd Month	3rd Month
Normal Control	7.84±0.28	7.53±0.21	6.91±0.04	7.62±0.04
2 ml/kg bw. of *C. aurantifolia* fruit juice	7.61±0.06	7.50±0.06	7.78±0.02	7.53±0.01
4 ml/kg bw. of *C. aurantifolia* fruit juice	7.41±0.07	7.26±0.03	7.63±0.03	7.68±0.61
8 ml/kg bw. of *C. aurantifolia* fruit juice	7.23±0.13	7.15±0.09	7.32±0.03	7.49±0.03

Source: Authors

Table 9. Platelet count of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	PLT (× $10^{9/L}$)			
	Initial	1st Month	2nd Month	3rd Month
Normal Control	869.56±3.18	756.57±3.14	796.20±2.81	823.13±2.15
2mL/kg bw. of *C. aurantifolia*fruit juice	696.30±5.62	730.12±3.12[c]	826.35±4.92[e]	933.75±3.26[g]
4mL/kg bw. of *C. aurantifolia*fruit juice	811.32±2.87	813.63±5.49	792.57±2.28	627.16±5.10[h]
8mL/kg bw. of *C. aurantifolia* fruit juice	720.18±5.30	652.92±3.72[d]	691.71±2.23	585.41±3.22[h]

[c]Significant increase with respect to 1st Month; [d]Significant decrease with respect to 1st Month; [e]Significant increase with respect to 2nd Month; [f]Significant decrease with respect to 2nd Month; [g]Significant increase with respect to 3rd Month; [h]Significant decrease with respect to 3rd Month.
Source: Authors

differences (p>0.05) observed throughout the three months of administration with lime Juice as the same level was maintained in all the groups. The results in Table 7 showed the MCHC of rats administered with different doses of *C. aurantifolia* fruit juice. There were no significant differences (p>0.05) observed throughout the three months of administration with lime Juice.

Red blood cell count (RBC) of rats administered with *C. aurantifolia* is shown in Table 8. RBC of the subject from Group A (Normal control) decreased from its baseline after 1st month, followed by a further decrease in the 2nd month but peaked in the 3rd month. Group B showed a decreased and increased pattern after 1st month, 2nd month and 3rd month, respectively. Group C and D decreased from their baselines after 1st month, which steadily increased in the 2nd month, up till the 3rd month.

Platelet count (PLT) of rats administered with *C. aurantifolia* is shown in Table 9. PLT of the subject from Group A (Normal control) decreased from its baseline after 1st month, followed by a sharp increase in the 2nd month up till the 3rd month. Group B increased from its baseline after 1st month, which steadily increased in the 2nd month, up till the 3rd month. Group C increased from its baseline after 1st month, followed by a sharp decline in the 2nd month, which further decreased in the 3rd month. Group D showed a decreased and increased pattern after 1st month, 2nd month and 3rd month, respectively. All platelet counts in both Normal control and experimented groups are within the normal range (NV=500-1300 × 10^9/L) according to Barrientos et al. (2020).

White blood cell count (WBC) of rats administered with *C. aurantifolia* is shown in Table 10. WBC of the subject from Group A (Normal control) decreased from its baseline after 1st month, followed by a further decrease in the 2nd month and peaked in the 3rd month. Group B displayed an increased and decreased pattern after 1st month, 2nd month and 3rd month, respectively. Group C decreased from its baseline after 1st month, followed by a

Table 10. White Blood Cell count of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	WBC (× $10^{9/L}$)			
	Initial	1st Month	2nd Month	3rd Month
Normal control	10.91±0.11	10.54±0.30	8.62±0.51	10.35±2.12
2 ml/kg bw. of *C. aurantifolia*fruit juice	6.82±0.66	8.93±1.21	7.21±0.29	11.29±3.04g
4 ml/kg bw. of *C. aurantifolia*fruit juice	10.15±0.83	8.38±1.82	10.54±1.32	11.10±1.71
8 ml/kg bw. of *C. aurantifolia* fruit juice	8.65±0.91	9.13±1.26	10.62±0.67	13.29±1.38g

[c]Significant increase with respect to 1st Month; [d]Significant decrease with respect to 1st Month; [e]Significant increase with respect to 2nd Month; [f]Significant decrease with respect to 2nd Month; [g]Significant increase with respect to 3rd Month; [h]Significant decrease with respect to 3rd Month.
Source: Authors

Table 11. Neutrophils of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	NEU (%)			
	Initial	1st Month	2nd Month	3rd Month
Normal Control	4.86±1.03	3.89±0.31	4.12±0.32	3.70±0.29
2 ml/kg bw. of *C. aurantifolia* fruit juice	4.63±0.37	4.33±1.24	4.65±1.32	3.14±0.25
4 ml/kg bw. of *C. aurantifolia* fruit juice	4.37±0.31	4.10±0.12	3.23±1.00	3.32±0.15
8 ml/kg bw. of *C. aurantifolia*fruit juice	4.90±0.35	4.73±0.01	3.97±0.26	4.83±0.11

Source: Authors

Table 12. Lymphocytes of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	LYM (%)			
	Initial	1st Month	2nd Month	3rd Month
Normal Control	94.83±2.31	95.31±1.62	94.20±2.90	97.16±1.32
2 ml/kg bw. of *C. aurantifolia*fruit juice	92.67±2.57	96.53±1.14	93.04±3.21	96.72±1.52
4 ml/kg bw. of *C. aurantifolia*fruit juice	94.51±1.98	93.75±1.67	95.16±1.64	94.37±1.91
8 ml/kg bw. of *C. aurantifolia* fruit juice	96.14±2.02	95.83±1.39	96.39±1.54	94.13±2.46

Source: Authors

sudden increase in the 2nd month, up till the 3rd month. Group D increased steadily from its baseline after the 1st month and continued till the 3rd month. All white blood cell counts in both Normal control and experimented groups are within the normal range (NV=6-18 × 10^9/L) according to Barrientos et al. (2020).

Neutrophils of rats administered with *C. aurantifolia* were shown in Table 11. Group A (Normal control) and Group B displayed a decreased and increased pattern after the 1st month, 2nd month and 3rd month. Groups C and D increased from their baselines in the 1st month, followed by a further decrease in the 2nd month and peaked in the 3rd month.

Lymphocytes of rats administered with *C. aurantifolia* were shown in Table 12. Group A (Normal control) and Group B displayed an increased and decreased pattern after the 1st month, 2nd month and 3rd month. Group C and Group D displayed a decreased and increased pattern after the 1st month, 2nd month and 3rd month.

Monocytes of rats administered with *C. aurantifolia* were shown in Table 13. Group A (Normal control) revealed an absence of the cell throughout the testing time. Group B revealed an absence of the cell in its baseline, 1st, and 3rd month except in the 2nd month. Group C revealed a decrease from its baseline after 1st month, which peaked in the 2nd month, with an absence of the cell in the 3rd month. Group D revealed the presence of the cell after the 3rd which increased significantly ($p<0.05$) from its baseline.

Eosinophils of rats administered with *C. aurantifolia* were shown in Table 14. A decreased pattern of the cell and an increased pattern of the cell throughout the experiment were observed in Group A (Normal control) and Group D, respectively. Group B decreased from its baseline after 1st month, followed by a further decrease in the 2nd month, and peaked in the 3rd month. Group C showed a decreased and increased pattern after the 1st month, 2nd month and 3rd month, respectively. There were

Table 13. Monocytes of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	MON (%)			
	Initial	1st Month	2nd Month	3rd Month
Normal Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2 ml/kg bw. of *C. aurantifolia* fruit juice	0.00±0.00	0.00±0.00	0.13±0.00[e]	0.00±0.00
4 ml/kg bw. of *C. aurantifolia* fruit juice	0.20±0.00	0.12±0.00[d]	0.25±0.00	0.00±0.00
8 ml/kg bw. of *C. aurantifolia* fruit juice	0.13±0.00	0.00±0.00	0.00±0.00	0.40±0.00[g]

[c]Significant increase with respect to 1st Month; [d]Significant decrease with respect to 1st Month; [e]Significant increase with respect to 2nd Month; [f]Significant decrease with respect to 2nd Month; [g]Significant increase with respect to 3rd Month; [h]Significant decrease with respect to 3rd Mont.
Source: Authors

Table 14. Eosinophils of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	EOS (%)			
	Initial	1st Month	2nd Month	3rd Month
Normal Control	0.46±0.00	0.40±0.00	0.35±0.00	0.21±0.00[h]
2 ml/kg bw. of *C. aurantifolia*fruit juice	0.33±0.00	0.22±0.00[d]	0.16±0.00[f]	0.25±0.00[h]
4 ml/kg bw. of *C. aurantifolia* fruit juice	0.51±0.00	0.36±0.00[d]	0.43±0.00[f]	0.42±0.00[h]
8 ml/kg bw. of *C. aurantifolia* fruit juice	0.43±0.00	0.44±0.00	0.65±0.00[e]	0.99±0.01[g]

[c]Significant increase with respect to 1st Month; [d]Significant decrease with respect to 1st Month; [e]Significant increase with respect to 2nd Month; [f]Significant decrease with respect to 2nd Month; [g]Significant increase with respect to 3rd Month; [h]Significant decrease with respect to 3rd Month.
Source: Authors

Table 15. Basophyls of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	BAS (%)			
	Initial	1st Month	2nd Month	3rd Month
Normal Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2 ml/kg bw. of *C. aurantifolia*fruit juice	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4 ml/kg bw. of *C. aurantifolia*fruit juice	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
8 ml/kg bw. of *C. aurantifolia* fruit juice	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Source: Authors

no observed changes in the basophyls levels of the different groups of rats starting from the normal control to the experimental groups (Table 15). Table 15 revealed the absence of basophyls in rats adiministered with different doses of *C. aurantifolia* fruit juice.

DISCUSSION

A reduction in bodyweight and food consumption was observed following the intake of *C. aurantifolia* fruit juice in rats after 12 weeks (Table 1). However, this decrease was only observed in the group of rats administered 4 and 8 ml/kg bodyweight of *C. aurantifolia* after 12 weeks of administration. This entails that lime juice can be a good regimen in the management of bodyweight at certain doses. However, some medicinal plants such as *Justicia secunda* Vahl leaves (Onochie et al., 2020) do not have the capacity to cause observable changes in the bodyweight of experimental animals. It is possible that phytoestrogens of *C. aurantifolia* inhibit serotonin re-uptake and thereby increase the levels of serotonin in synaptic clefts as in the case of *C. cyminum L*. which was reported by (Amin and Nagy, 2009), which in turn would result in enhanced satiety. Additionally, bodyweight may be reduced by limonene through increased lipolysis by a histaminergic response and reduced appetite (Shen et al., 2005).

The HGB concentration results obtained and represented in Table 3 were all within the normal range (NV=11-19.2 g/dL) according to Barrientos et al. (2020), and no significant difference ($p > 0.005$) was recorded

throughout the three months of administration with lime juice. It suggests that administration with lime juice at different dosages used does not have any negative impact on HGB concentration and could be effective in the treatment of anaemia. Blood indices (MCV, MCH, and MCHC) results as shown in Tables 5, 6 and 7 respectively showed no significant differences ($p>0.05$) throughout the three months of administration with lime Juice. There was no significant difference ($p>0.05$) observed in RBC counts of both normal control and groups administered with lime juice (Table 8), and the values were within the normal range (NV= 6.76-9.75 × 10^{12}/L) according to Barrientos et al. (2020). The steady values of RBC could be attributed to the rich milieu of antioxidants that protects the RBC from any oxidative damage.

Platelets are small, colorless cell fragments in our blood that form clots and stop or prevent bleeding. The result in table 9 revealed a significant increase ($p<0.05$) in group B relative to other administered groups and Normal control (Group A) after 3rd month of lime juice administration. The lowest value was found in group D after 3rd month of lime juice administration. The results although either showing a decreasing or increasing patterns in the different groups administered with lime juice were all with the normal range (NV=500-1300 × 10^{9}/L) according to Barrientos et al. (2020), hence neither thrombocytosis nor thrombocytopenia can be implied.

Eyong et al. (2004) reported that rapid increase in WBC counts following a foreign attack on the system by pathogens gives rise to a boost in the body's defense mechanisms as the normal physiological response. After 3rd month of administration of lime juice, it was observed that the administered groups showed significant ($p<0.05$) increases relative to their baselines (Table 10). This could imply that lime juice could serve as an immune booster. The effects of the prolonged administration of lime fruit juice on the white blood cells differentials were also investigated. Following the results obtained at monthly intervals, there was no significant difference ($p<0.05$) in the neutrophil and lymphocyte levels of the administered groups. However, monocytes and eosinophils revealed an inconsistent increase and decrease at different doses analysed at monthly intervals (Tables 13 and 14). The white blood cell and its differentials are vital for the protection of the body against foreign invaders and help to stimulate cytokine erythropoietin which subsequently stimulates blood cell synthesis (Oladejo and Osukoya, 2021).

Conclusion

The domestic and ethnomedical use of *C. aurantifolia* cannot be overemphasized. The observations from this study revealed that fruit juice of *C. aurantifolia* not only could serve as immune booster but can also be used in bodyweight management. There should be a move from pre-clinical screening of the plant's fruit juice to the isolation of active compounds and the actual development of useful drugs from the plant.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effect of storage period and boiling time of fresh shea nuts on physico-chemical characteristics of kernels and butter

Fernande G. HONFO[1]*, Anita R. LINNEMANN[2], Mohamed M. SOUMANOU[3], Noel AKISSOE[1] and Martinus A. J. S. van BOEKEL[2]

[1]Faculté des Sciences Agronomiques, Université d'Abomey-Calavi, Benin.
[2]Food Quality and Design, Department of Agrotechnology and Food Sciences, Wageningen University, the Netherlands.
[3]Ecole Polytechnique d'Abomey-Calavi, Université d'Abomey-Calavi, Benin.

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Two traditional treatments for shea (*Vitellaria paradoxa*) butter processing namely storage of fresh nuts and duration of subsequent boiling were studied by using the response surface methodology (RSM) to determine best treatment. Experimental treatments influenced several kernel parameters, such as fat content (38-52% dw), redness (*a values between 6.3 and 11.7), and butter parameters, viz. yield (24 to 36% wet weight of kernel mass), brightness (*L** values between 70-80), yellowness (*b** values between 16-23), and free fatty acid (FFA) percentage (0.5-2%). On the other hand, the moisture content (6-8%) of the kernels and the peroxide values (2.3 - 3.8 meq O_2/kg) of the butter were not affected. Storage for 3 days and boiling for 28 ± 3 min gave the best results, that is, kernels with a moisture content of 7% and a fat content of 50% dw. Butter extracted by traditional technique from these kernels yielded 32% on wet weight of kernel mass with 0.8% of FFA, and 2.5 meq O_2/kg of peroxide. This butter can be used for food and cosmetic purposes without refining. Furthermore, the microstructure of fresh shea nuts, studied with Laser Scanning Confocal Microscopy, showed large and small fat globules with some free spaces inside.**

Key words: *Vitellaria paradoxa*, kernels, storage, boiling, physico-chemical characteristics, shea butter.

INTRODUCTION

The shea tree (*Vitellaria paradoxa*) is a forest food resource with a significant contribution to the diet of local people in extensive parts of Africa (Honfo et al., 2014). Shea kernels are also an important export commodity at international level due to a great percentage of butter that it contained (Ajala et al., 2015; Bello-Bravo et al., 2015). Shea butter is one of the most important vegetable fats due to its various uses over the years: Locally, the butter is used for human consumption, in soap, pomade and traditional pharmacology. At global level it is in great

*Corresponding author. E-mail: fernandeh@yahoo.fr.

demand among chocolate, cosmetic and pharmaceutical industries as well as biodiesel production (Bello-Bravo et al., 2015). Shea fat is the main component of the kernel and it is principally composed of triglycerides and a large fraction (5-17%) of unsaponifiable compounds that make this fat useful for cosmetic purposes (Maranz et al., 2004; Nahm, 2011). Among shea fatty acids, stearic fatty acid and oleic fatty acid represent 90% of the total fatty acids (Honfo et al., 2014; Maranz et al., 2004). The unsaponifiable fraction of shea butter is mainly composed of triterpene alcohols, tocopherol, phenols, and sterols (Maranz et al., 2003; Nahm, 2011). Shea fat distribution and structure in the kernel are important in determining its physical and chemical properties as well as its extractability. Hence, knowledge of the microstructure, in relation to the macroscopic properties, may offer options to improve the existing processing methods and to design new ones (Borchers et al., 2003).

The harvesting period of shea fruits often coincides with the start of the rainy season, a period of many competing farm activities. Consequently, the processors, women only, have limited time, and therefore, need to store the fresh nuts for several days (commonly 3 to 15 days) before further processing (Honfo et al., 2012). In general, storage conditions are inappropriate and not suited to keep the nuts fresh. All these conditions often lead to the germination of the nuts and exposed the nuts to external agents such as microorganisms, moisture and insects, which affected the quality of the final products (kernel and butter). Germination of nuts also led to the reduction of butter yield, affected the quality and gave the butter a bitter taste (Nahm, 2011).

The preservation and processing of fresh shea nuts varied across shea regions and generally involved fruit gathering, depulping, and boiling of fresh nuts, followed by sun drying, shelling, roasting, milling, churning, and oil separation. Some processing unit operations, such as boiling and sun drying, have been reported as critical operations for kernel quality (Womeni, 2004; Kapseu et al., 2007; Jasaw et al., 2015). The boiling operation during shea processing facilitates the shelling of kernels and inactivates the enzymes, lipases, responsible for triglyceride hydrolysis (Womeni et al., 2006; Abdul-Mumeen et al., 2013). In Benin, boiling is traditionally done for 15 to 60 min and the end of this unit operation is generally determined by a colour change of the boiling water (Honfo et al., 2012). Variations in the duration of boiling could influence shea butter quality. For instance, excessive boiling results in cellular damage, leading sometimes to discolouration of the shea nut (Aculey et al., 2012), while improper lipase inactivation may be occurred due to insufficient boiling, leading to a high free fatty acid (FFA) content in the kernels (Bup et al., 2011). In addition, mouldy kernels may be come from nuts that have been boiled and were not dried well. Among many other challenges with this traditional method are also the technological limits of the process, which did not enable a full separation of liquid and solid phases, with no adequate monitoring of the extraction conditions (Alenyorega et al., 2015).

However, several studies on shea fruits assessed the impact of processing on quality indicators like the FFA, peroxide value, unsaponifiable fraction, and tocopherol content of kernels and butter (Kapseu et al., 2007; Bup et al., 2011; Aculey et al., 2012; Alenyorega et al., 2015; Honfo et al., 2017). To date, the impact of the storage duration of fresh shea nuts on kernel and butter quality characteristics is not well documented. Such an investigation combined with assessing the effect of the duration of boiling is important to determine how long the fresh nuts can be stored and how long they should be boiled to obtain shea products with optimal quality attributes. The main objective of this study was therefore to assess the effect of storage of fresh nuts and boiling time on some physico-chemical quality attributes of shea kernels and butter. Additionally, the microstructure of fat distribution in shea kernels was visualized to improve the understanding of the processes used for butter extraction.

MATERIALS AND METHODS

Experimental design

The response surface methodology (RSM) is a statistical method that used quantitative data derived from an appropriate experimental design with quantitative factors to estimate the relationship between a response and the factors in order to optimize processes or products (Giovanni, 1983; Stroescu et al., 2013). In this study, a central composite face-centered design (CCFD) with two factors was used to assess the simultaneous effect of storage periods (3-21 days) and boiling time (10-60 min) of fresh shea nuts on certain quality characteristics of derived kernels and butter and to determine the optimum storage period and boiling time that will give kernels and butter that comply to export requirements. This design is usually used to study linear interactions and the quadratic effects between factors (Montogomery, 2001; Akinoso et al., 2011). Ranges of storage duration and boiling time were chosen according to the processing practices in Benin (Honfo et al., 2012). The design generated 13 combinations (Table 1) and each of them was duplicated, giving a total of 26 combinations. For each parameter investigated, the design gave the following regression formula:

$$Y = I + aX_1 + bX_2 + cX_1^2 + dX_2^2 + eX_1X_2 \quad (1)$$

Where: Y is the response, I is a constant; a and b are linear effect coefficients; c and d are quadratic effect coefficients; and e is an interaction effect coefficient. X_1 and X_2 are the variables storage duration and boiling time, respectively.

Experimental processing

Fresh shea fruits were collected from different shea trees in Arbonga village at Banikoara (11° 4'N and 2° 25'E), a location in Alibori Department, North-Benin. The fruits were depulped on the same day. After-day the fresh nuts were transported to the University of Abomey-Calavi, where the experiments took place.

Table 1. Different combinations of storage period and boiling time of shea nuts generated by the CCFD and the different responses.

Treatment	Storage period (day)	Boiling time (Minute)	Moisture content (%)	Kernel redness (*a**)	Fat content (% dw)	Butter yield (%)	Butter brightness (*L**)	Butter yellowness (*b**)	FFA (%)	Peroxide (meq O_2/kg)
1	12	35	7.6±0	7.5±0.3	47.1±2.2	30.7±0.7	75.4±1.0	20.7±0.9	1.4±0	2.7±0.1
2	3	10	7.2±0.7	6.5±0.2	49.0±1.7	30.9±1.1	78.7±2.0	22.1±0.6	1.2±0.4	2.3±0.1
3	12	35	7.3±0.4	7.8±0.5	44.2±1.7	30.8±0.7	74.1±3.0	21.2±1.8	1.4±0.6	2.8±0.2
4	12	35	7.5±0.2	7.8±0.5	45.4±0.1	31.6±0.3	72.8±3.0	20.2±2.0	1.5±0	2.7±0.2
5	12	35	7.0±0.7	7.7±0	46.2±1.2	33.7±2.0	75.2±0.9	20.4±1.8	1.4±0	2.9±0.6
6	21	60	7.4±0.1	11.6±0.1	40.6±0.8	28.2±2.9	71.5±0	21.1±0.9	1.9±0.1	3.5±0.5
7	21	35	6.8±0.4	7.6±0.4	44.5±0.6	28.6±1.4	72.0±1.5	17.1±1.0	1.7±0.2	3.3±0.6
8	12	60	7.4±0.3	10.8±0.7	42.8±2.7	29.9±3.8	74.3±1.9	20.5±3.1	1.4±0	2.7±0
9	21	10	6.8±0.6	6.9±0.1	40.2±2.5	26.3±1.1	70.9±0	17.5±0	1.7±0	2.7±0.5
10	12	10	7.1±0.6	6.8±0.2	40.4±1.0	29.3±0.8	71.9±1.7	20.9±1.7	1.4±0	2.9±0.2
11	3	60	6.9±0.4	10.9±0.2	52.1±0.5	32.8±0.4	74.9±1.1	18.9±1.1	1.0±0.1	2.8±0.2
12	3	35	6.4±0.1	7.2±0.1	49.7±0.8	33.6±0.6	76.5±1.5	20.8±1.5	0.6±0.1	2.7±0.1
13	12	35	7.3±0	7.7±0.2	48.0±5.3	31.8±1.3	73.9±0.8	20.5±0.8	1.4±0.1	2.6±0.2

[1]: Mean ± Standard deviation.

Three kilogram of fresh shea nuts were used for each treatment. The fresh shea nuts were stored in a room at 28 ± 1°C with a relative humidity of 81 ± 2%. The nuts were just piled on the floor until the end of storage period according to treatment. Boiling was done in water that was three times the volume of the nuts (Honfo et al., 2012) during the experimental time for each treatment. At the end of the boiling time, the nuts were placed in a basket for draining. The drained nuts were subsequently oven-dried at 38-40°C for 5 days, within the temperature range commonly used for sun-drying in Benin. The drying reduced the moisture content of the nuts from 75 to 10%, which facilitated the shelling operation. The dried nuts were shelled manually using a metal rod and the kernels were further oven dried at the same temperature for another 5 days. Next, the butter was extracted from the dried kernels according to the traditional process by grinding with electric grinder machine, roasting for 20 min at 130°C in an oven, milling with an electric milling machine (Kenwood blender, UK), manual churning and heating. The oil was washed and heated to remove particles and mucilage from the first stage of heating. The resulting oil was then filtered and left to cool. Samples of dried kernels and butters were packed in plastic containers and stored at 4°C until analyses.

Microstructural observation of shea kernels

Cross-sections of fresh kernels were studied using a Laser Scanning Confocal Microscopy (LSCM) (510 META Carl Zeiss Germany). The excitation wavelength was 543 nm, and the emission was recorded between 420 and 590 nm. For LSCM observations, pieces of fresh kernels (that is, kernels that had not been treated) were cut into slices of 10 µm thickness by a Reichert-Jung cryostat (Microtome) at -15°C. The samples were then mounted on glass slides, coloured with deionized water containing lipid-soluble Nile Red to stain the fat and covered for observation. Samples were also directly observed under the lenses of the digital microscope (Nikon Eclipse 80i, Nikon Corporation, Tokyo, Japan).

Physico-chemical characteristics of shea kernels and butter

Moisture contents of kernels were determined according to AOAC (2002) as well as the fat content of kernels using a Soxhlet apparatus with petroleum ether as a solvent for 4 h at 70°C. The yield of shea butter was expressed on wet weight as the percentage of the mass of filtered oil (butter) on the mass of the kernels used. Colour was determined by measuring the Hunter parameters *L**, *a** and *b** values using a chromameter (Konica Minolta CR 410, Japan). The parameter a* explains the redness of kernels, while *L** and *b** express the brightness and the yellowness of butters, respectively. FFA percentages were determined by titration and calculated as the oleic acid percentage (NB ISO 660, 2006) and the peroxide values of butter samples were determined by titration (NB ISO 3960, 2006). All reagents used for laboratory analysis were from Sigma-Aldrich, St. Louis, USA.

Statistical analyses

The experimental design and the different statistical analyses as well as the optimization conditions were determined with Minitab 16.0 software as well as the different contour plots for each treatment. Analysis of

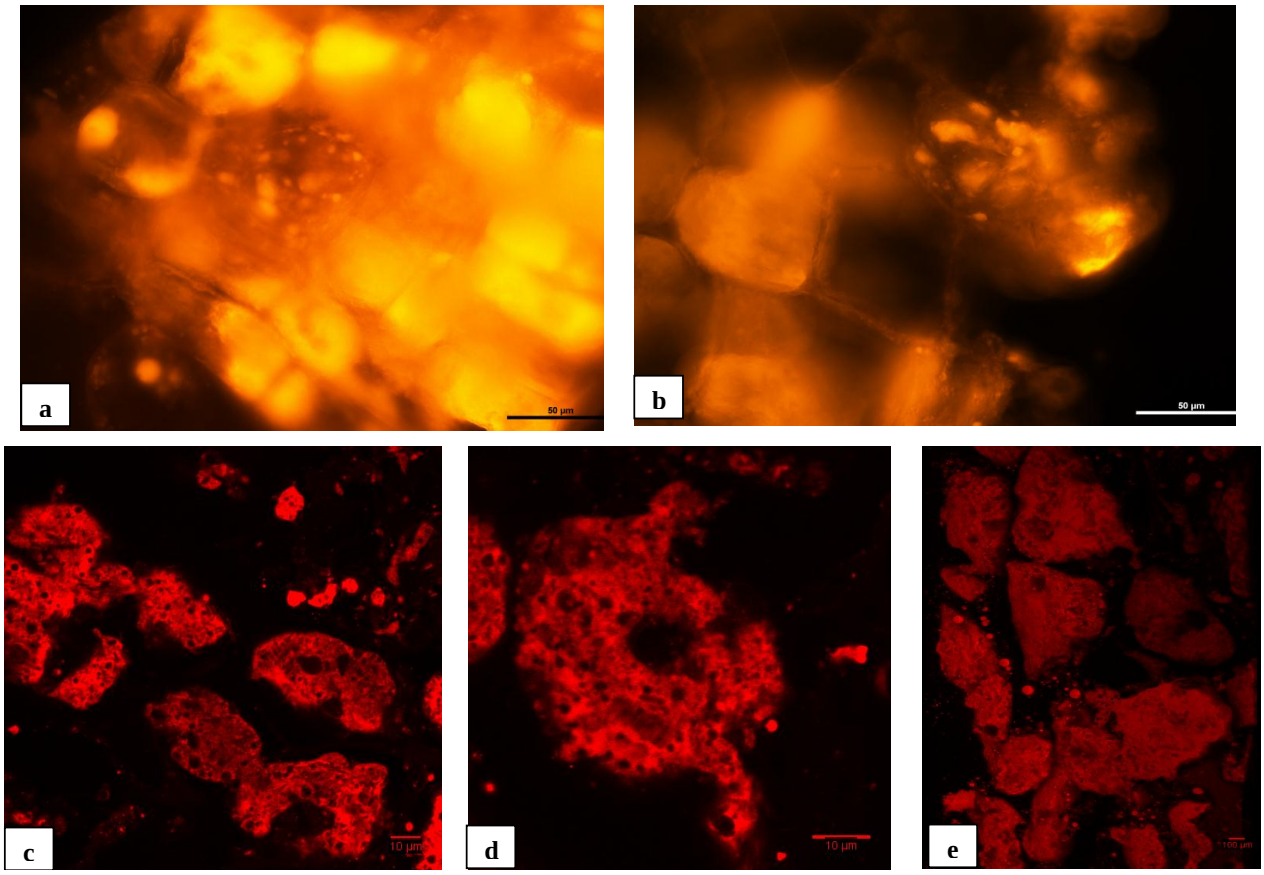

Figure 1. a and b microscope images; a: cross-section of non oxidized kernel; b: flesh of oxidized kernel; c, d and e: Laser Confocal Scanning micrograph of the flesh of shea kernel; c and d: micrographs showing the periphery of seed endosperm; e: micrograph of endomembrane localization, fat is stained with Nile Red.

variance (ANOVA) tables were generated and the effect of independent variables and regression coefficients of individual linear, quadratic and interaction terms were calculated. Based on sequential and lack-of-fit *P* values the best fitting significant model ($p<0.05$) was selected.

RESULTS AND DISCUSSION

Microstructure of shea kernel

Figures 1a and b showed the micrographs of non-oxidized and oxidized kernel sections taken by the digital microscope. Both figures showed similar cell sizes and cell structures. However, some cells of the cross-section of the oxidized kernel are black, showing the absence of lipid inside. In the micrograph of the non-oxidized kernels, all cells contained lipid. Micrographs 1c and 1d showed the fat structure of the periphery of the seed endosperm and Figure 1e presented the endoplasmic reticulum containing lipid fraction in cells. As the figures illustrated, fat can be recognized under LSCM as oval, polygonal or irregular shapes with various sizes. The dark areas represented the serum pores including water, protein and others nutrients. Most fat globules were large with some free space inside; they were organized into aggregates. However, small fat globules were also dispersed in the serum pores. Lopez et al. (2007) found that small fat globules in milk had a higher stability against rupture of the milk fat globule membrane and a greater resistance to deformation and coalescence under pressure than large fat globules. A similar behaviour might be occurred in plant fat. In addition, rupture of the fat globule membrane to release fat could be enhanced by the closer proximity of the fat globules and may also be depended on several parameters including the temperature and time during different processing operations (Lopez et al., 2007). Investigation of changes in the microstructure in relation to storage and boiling treatments is recommended to gain a better insight in the way in which processing affects the quality of shea products.

Moisture content and colour characteristics of shea kernels

Moisture content *(MC)* gave the following regression

Table 2. Regression coefficients (*RC*), constant (*I*), coefficient of determination (R^2) and lack-of-fit *P* values (*P*) values describing relations between moisture content, redness, and fat content of shea kernels, yield of butter, FFA percentage and peroxide value of different butters according to the central composite design.

Parameter	Moisture content (%)	Kernel redness (*a**)	Fat content (% dw)	Butter yield (%)	Butter brightness (*L**)	Butter yellowness (*b**)	FFA (%)	Peroxide (meq O_2/kg)
Storage: X_1	0.1113	0.04454	-1.1479**	-0.1604	-0.5790*	-0.1333	0.0698*	-0.0306
Boiling: X_2	-0.0254	-0.06767*	0.4175**	0.2363**	0.0204	-0.498	-0.044	0.0062
X_1^2	-0.0058*	-0.00126	0.0345**	-0.0042	0.0050	-0.0102	-0.0016	0.0020
X_2^2	0.0003	0.00215**	-0.0048**	-0.0029**	-0.0012	0.0014	0.0002	-0.0001
X_1X_2	0.0012	0.00042	-0.0036	-0.0000	0.0048	0.007**	0.0004	0.0005
Constant	6.9117	6.84458	42.397	27.5162	79.1155	24.6282	1.0237	2.5049
R^2	35.6	97.3	84.6	71.5	61.5	42.5	74.1	48.4
Lack of fit	0.68	0.22	0.69	0.51	0.73	0.84	0.35	0.12

*Significant at P <0.05; ** Significant at P <0.01.

equation for the effect of storage period (X_1) and boiling time (X_2) of nuts:

$$MC\ (\%) = 6.9117 + 0.113X_1 - 0.0254X_2 - 0.0058X_1^2 + 0.0003X_2^2 + 0.0012X_1X_2$$

This model explained only 36% of the variation in moisture content (6.3-7.7%) of shea kernels (Table 1). Both factors did not significantly influence the moisture content of the kernels (Table 2). The linear term of the model had a positive value whereas its quadratic term expresses negative influence on moisture content. The contour plots show the variations of moisture content with the storage period and the boiling time (Figure 2a). These different variations could be due to the integrity of the nuts shell as well as their permeability to water. Shells are sometimes cracked and this might facilitate the water absorption by kernel. However, the moisture content of shea kernels complies with the export requirements for this parameter (7-8%) (UEMOA, 2011). In general, moisture content represented one of the most important characteristics of agricultural materials as it affects their physical, mechanical and chemical properties (Sitkei, 1986). This assumption is also valid for shea kernels; besides, some studies found that the moisture content of shea kernels influenced their storability, handling and processing (Aviara et al., 2005; Jasaw et al., 2015).

Colour is one of the quality characteristics of shea kernels that is often taken into account during purchase, and the desired colour is red/brown (Lovett, 2004). Values of *a** indicated the red colour of kernels. Values of *a** were significantly influenced by the boiling time and the model explained 97% of these variations (Table 2); they varied from 6.3 to 11.7 (Table 1). The contour plots of the variations of *a** showed an increase of a* values with increasing boiling time, irrespective of the storage duration (Figure 2b). Additionally, long boiling times resulted in darker nuts and kernels. The darker colour of nuts might be due to the release of tannin of the shell with the increasing of boiling time. However, it was noticed that a long storage duration of shea nuts resulted in more infested, black and germinated nuts (Figure 3), than when stored for short times.

Fat content of shea kernels and butter yield

The regression model explained 85% of the variations in fat content of the kernels, which ranged from 38 to 52% dw (Table 1). The linear terms of both factors as well as their quadratic terms were significant for the fat contents of the shea kernels (Table 2). This effect can be described as a decrease in the fat content with increasing storage duration, which might be due to the germination occurring during prolonged storage (Figure 4a). However, as shown in Figure 4a, fat content increased with boiling time until it reached a maximum of 48 to 52% around 32 min after 3 days of storage. This could be caused by the rupture of the membranes of fat globules membrane to release fat with the increasing of boiling time and the size of the fat globules.

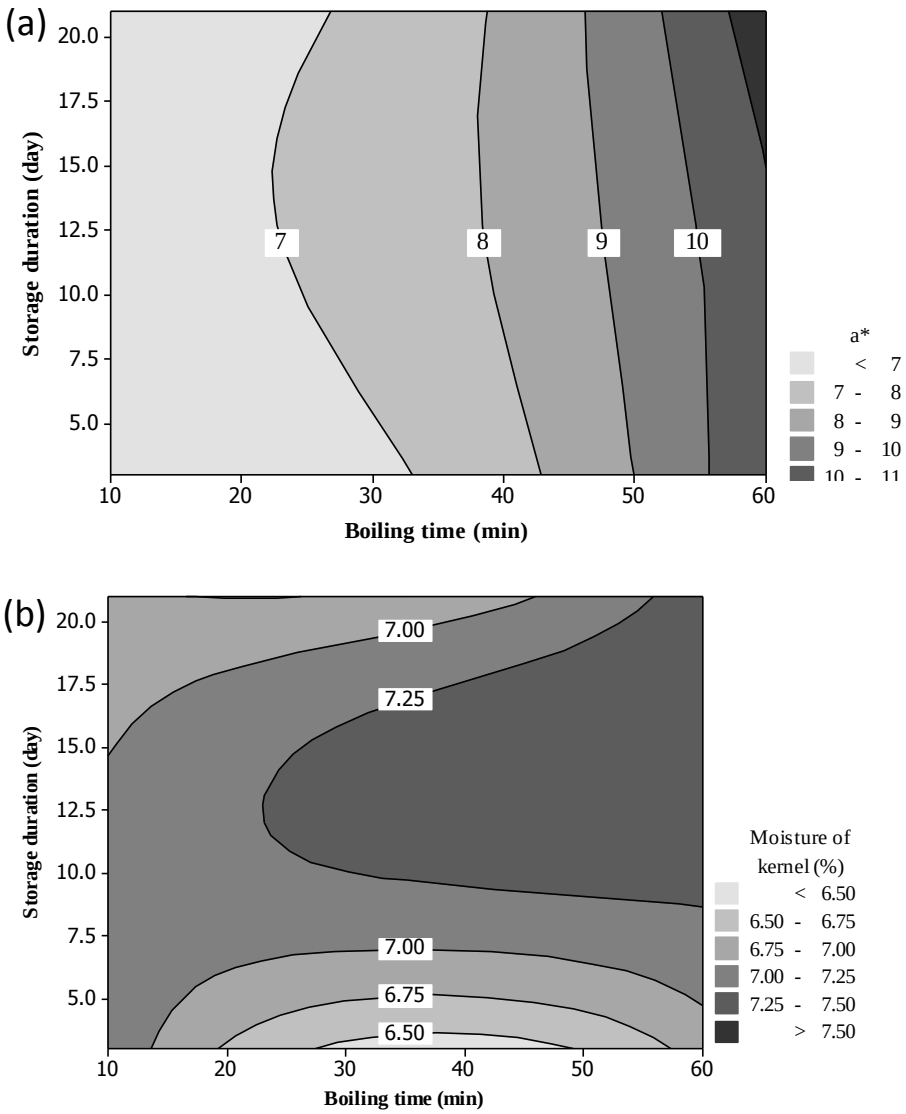

Figure 2. Contour plots showing the effect of storage period and the boiling time on moisture content and redness of kernels. a: Moisture content (%) of kernels; b: Redness (*a**) of kernels.

During boiling, small fat globules coagulated, promoting fat released as it does in the case of milk (Lopez, 2002). Additionally, the coagulation of proteins during cooking, resulting in free space for the diffusion of oil, may be increased the amount of oil extracted as cooking time increases (Board, 2002).

The butter yield varied from 24 to 36% on wet weight of kernel mass (Table 1); the highest values were obtained for nuts stored for 3 days and boiled for 30 to 35 min. Compared with the yields (25-30%) generally found for traditional processing (CNUCED, 2006), most butter yields were relatively high. Butter yield was significantly and positively linked to the linear effect of boiling time; for its quadratic term, significant negative effects were also observed (Table 2). This result is corroborated by the variation in the crude fat content of the kernels. The linear and quadratic effects of storage period as well as the interaction effect of the two factors were not significant. The regression model explained 73% of the variation and the contour plots showed the same trend as observed for the fat content (Figure 2b). Thus, irrespective of the boiling time, the butter yield decreases with increasing storage duration. Besides, Ajala and others found that extraction methods and extraction time have significant effect on the yield of shea butter (Ajala et al., 2015).

Colour characteristics of butter

Brightness (*L**) values express the level of lightness or

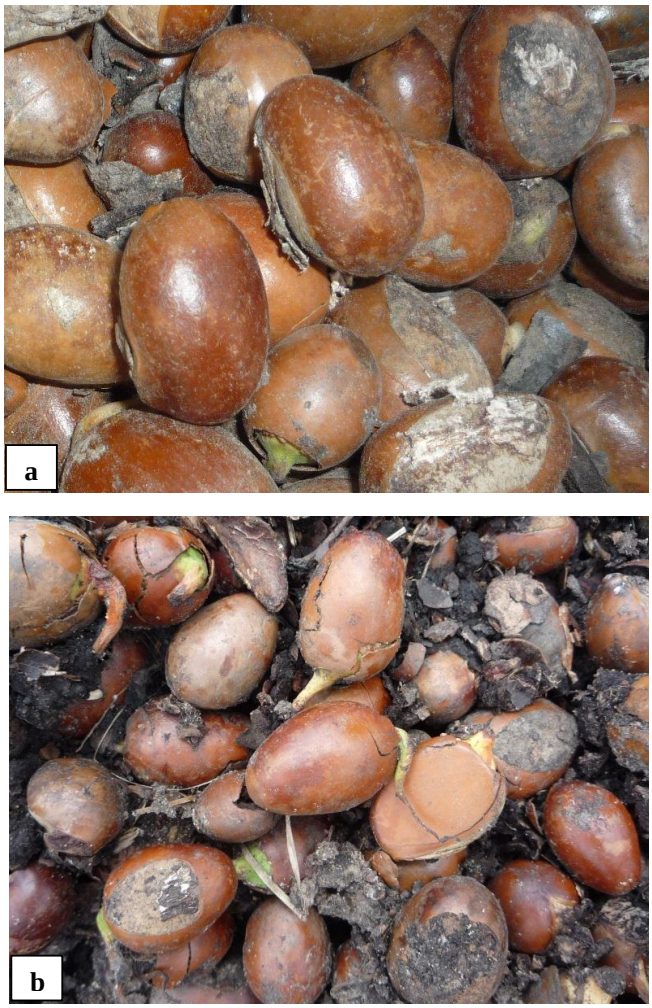

Figure 3. Fresh shea nuts after (a) 3 days and (b) 21 days of storage.

brightness. *L** values for the butter were significantly and negatively affected by the storage period of nuts (Table 2). *L** values varied from 70.5 to 80.1 (Table 1) and the regression model based on the storage period and boiling time explained 62% of the variation. The contour plots show a decrease of *L** values due to the storage, irrespective of the boiling time (Figure 5a). This decrease might be due to the damage of fat by nut germination during the storage, resulting in a change of butter colour, in particular, the brightest of butter.

Yellowness (*b**) was positively related to the interactive effect of both storage period and boiling time (Table 2). The parameter *b** expressed the intensity of the yellow colour of the butter and varied from 15.8 to 22.7 (Table 1). Only 43% of the variation was explained by the regression model related to the two factors. The contour plots showed a drop of the *b** values with the increasing of storage duration from the beginning until 47 min of boiling time (Figure 5b). A decrease of *b** values was also noticed with an increase of the boiling time at the beginning of storage period.

The highest values for *L** and *b** were found for the shortest storage period in combination with the shortest boiling treatment, namely 10 min. Colour is of great importance in many food products, including shea butter, as consumers use it as a cue to determine product attributes such as safety, texture and flavour (Clydesdale, 1998). A yellow colour is based on the pigments such as carotenoids and corresponds to the natural state of shea butter; when the butter is also bright, it is most attractive (Lovett, 2004; Megnanou and Niamke, 2015).

FFA percentage and peroxide value of shea butter

The FFA percentages varied from 0.5 to 2% (Table 1) and were significantly and positively related to the linear effect of storage period while the opposite was found for

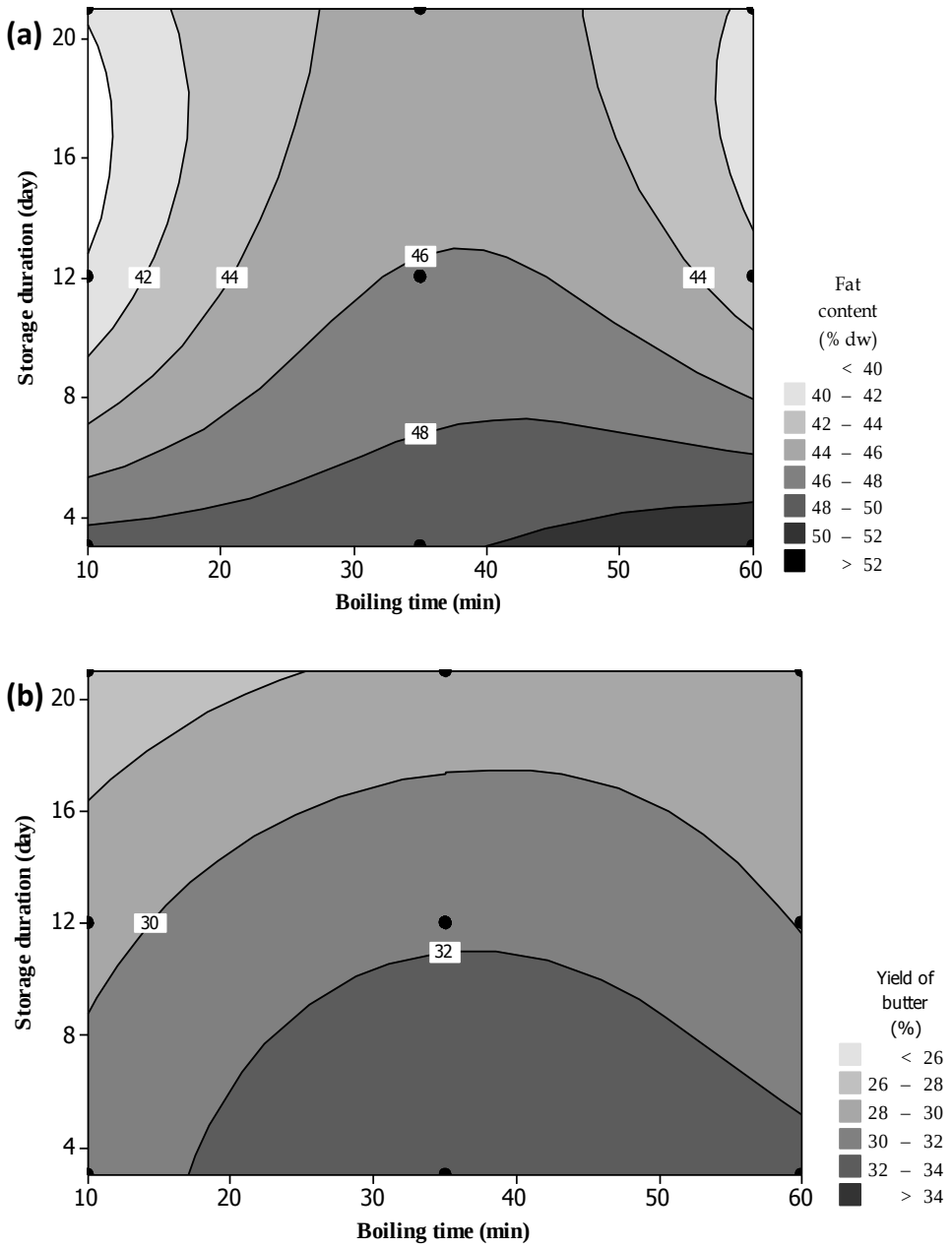

Figure 4. Contour plots showing the effect of storage period and the boiling on fat content of shea kernel and yield of butter. (a) Fat content (% dw) of kernels; (b) Yield (%) of butter.

the boiling time (Table 2). The regression model explained 74% of the variation. Low FFA values were found at the beginning of the storage period. However, some of the FFA percentages were higher than the threshold of 1% tolerated for cosmetic purposes (NB 04 02 001, 2006), but all of them were lower than the maximum value of 3 and 4% approved by shea production countries for international trade and by the Codex Alimentarius, respectively for food purposes (Codex Alimentarius, 2006; NB 04 02 001, 2006; UEMOA, 2011). An increase in FFA is commonly associated with a decrease in the hardness of the butter, thereby reducing the commercial value in the case of cocoa butter (Calliauw et al., 2008). This phenomenon is likely to apply to shea butter too as both butters are similar in terms of composition and uses. The contour plots presented in Figure 4a showed a gradual increase of the FFA percentage during the storage of nuts, irrespective of the boiling time. This increase may be due to the germination of the nuts during storage. Indeed, shea fruits fall from the tree and may start to germinate, which led to the activation of the lipases, responsible for

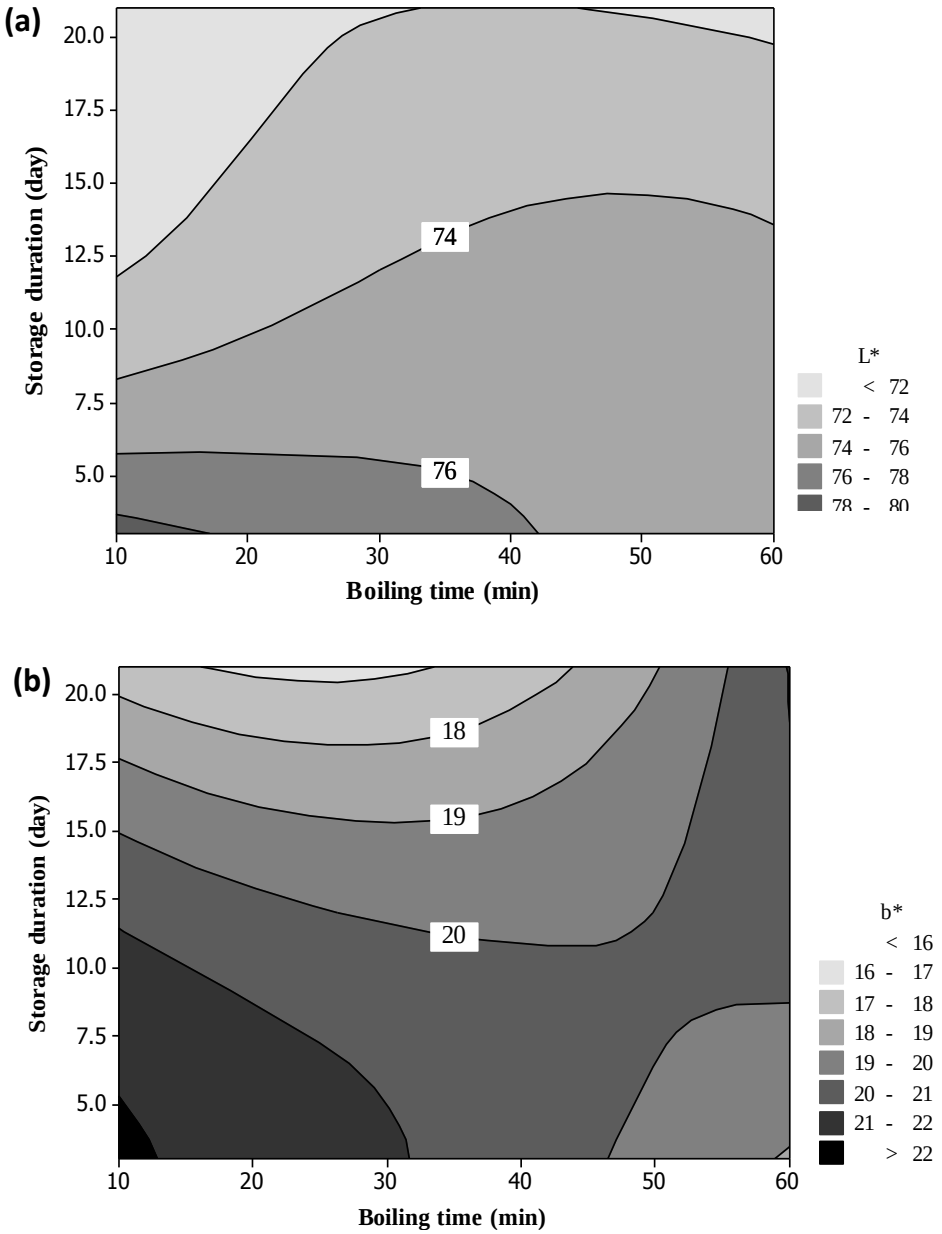

Figure 5. Contour plots showing the effect of storage period and the boiling time on colour parameters of shea butter. a: Brightness (*L**); b: Yellowness (*b**).

triglyceride hydrolysis (Kim and Min, 2008; Megnanou and Niamke, 2015) as FFA are carboxylic acids released from triglycerides through the effect of a lipase or oxidation. Many studies have shown detectable lipase activity, mostly during seed germination (Wanasundara et al., 2001). Furthermore, storage conditions may be promoted the growth of micro-organisms particularly moulds which could be extended the FFA percentage (Allal et al., 2013).

The different peroxide values obtained 2.3 to 3.8 meq O_2/kg (Table 1) were lower than the maximum value of 10 meq O_2/kg set for cosmetic uses (NB 04 02 001, 2006). The regression model of the peroxide values explained 48% of the variation (Table 2). The simultaneous effects of the two factors on the peroxide value were not significant. The contour plots showed a gradual increase of the peroxide value with increasing storage period and boiling time (Figure 6b). The increase in the amount of peroxide with the boiling time was also found by Womeni et al. (2006), when they investigated the effect of cooking time and oven temperature on the peroxide value. The peroxide value is usually used to quantify the primary oxidative products in oils and fat. The rise in the peroxide value during prolonged cooking of shea nuts may be due

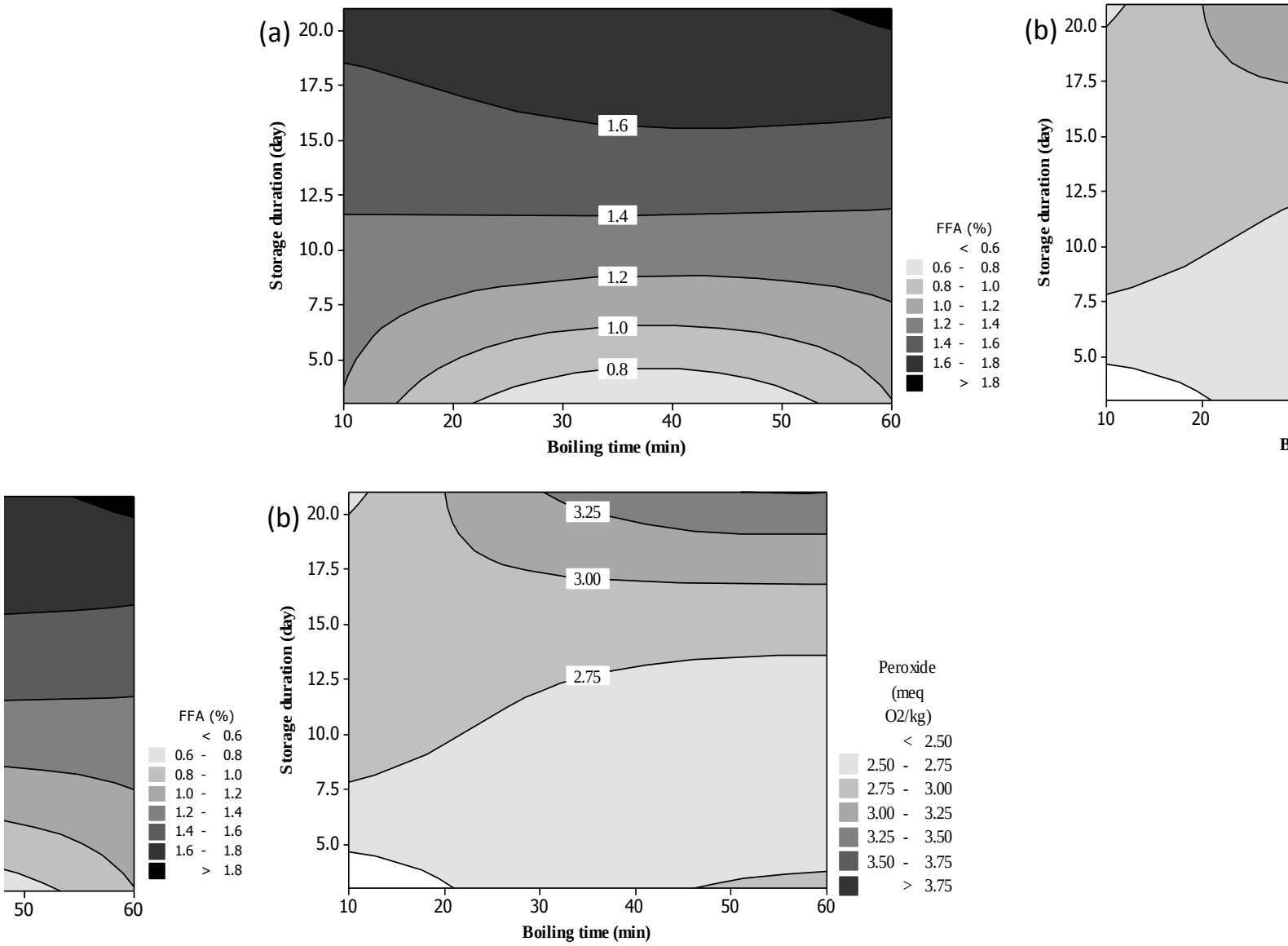

Figure 6. Contour plots showing the effect of storage period and the boiling time on FFA percentage and peroxide value of shea butter. (a) FFA percentage; (b) Peroxide value.

to the breakdown of water soluble anti-oxidants such as the monomers and polymers of catechins during the process (Maranz et al., 2004; Honfo et al., 2014). This led to oxidation of fat inside the nuts and subsequently increased the peroxide value.

Since the models have shown non-significant lack-of-fit *P* values for all investigated parameters, the different regression equations sufficiently explained most of the variability of the responses. In general, a lack-of-fit is used to check the fitness of the regression models and a non-significant lack-of-fit *P* value indicates the adequacy of the model and the accuracy of the predictions (Womeni et al., 2006).

Optimisation of storage period and boiling time

Optimum conditions for the storage period and boiling time are predicted by the models. A condition is considered optimal if the desirability value associated to the response is 1 or close to 1. The desirability value is generally between 0 and 1 and explained the level of validity of the predicted optimum condition. From the results, optimum conditions were obtained for each parameter investigated. Since, the desirability was not the same for all responses with a unique optimal condition to get kernels with low moisture and high fat content, and butter with low FFA and peroxide values, a

range of desirability (0.8-1) was then used. This resulted in an optimum storage period of 3 days and a boiling time of 28 ± 3 min at which the following values were obtained: A moisture content of the kernels of 7%, a fat content of 50% dw, a butter yield of 32%; butter extracted from these kernels may have a FFA content of 0.8% and a peroxide value of 2.5 meq O_2/kg and could be used for cosmetic and food purposes without refining (NB 04 02 001, 2006; USAID/WATH, 2005).

Conclusion

In shea kernels, the fat is organized in large globules with some free spaces inside, and in small globules. The preservation of shea nuts for further use is very critical for end product quality. Handling alternatives of shea nuts such as storage and boiling applied separately or in combination had significant effects on colour and fat content of kernels and the FFA percentage of butter. Longer storage reduced the fat content of the kernels and increased the FFA percentage of the butter. Increasing the boiling time might allow the extraction of more fat from the kernels. The optimum value was found around 32 min. Based on the predicted optimal conditions, the storage of fresh nuts 3 days and their boiling for 28 ± 3 min should be recommended for shea nuts processing. In addition to this study, further investigations should be taken up on other critical processing operations (*viz.* roasting, churning) to upgrade each step of the traditional technique and consequently to improve the yield and quality of butter.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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