KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF SCIENCE

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

PASTING OF G-IRRADIATED PROTEINS FROM VIGNA SUBTERRANEA IN NATIVE STARCH MODELS AND THE SURFACE FUNCTIONAL PROPERTIES OF THE PROTEINS

THIS THESIS IS SUBMITTED TO THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY IN PARTIAL FULFILMENT FOR THE REQUIREMENT OF THE MASTER OF SCIENCE (MSc.) DEGREE IN FOOD SCIENCE AND TECHNOLOGY

BY

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ABSTRACT

This research was carried out to evaluate the surface functional properties of gamma irradiated Bambara groundnut protein isolates and to study their performance in modeled starch systems using the Brabender Viscoamylograph. Irradiation was done at five levels: 2.50, 5.00, 7.50, and 10.00kGy; while protein-starch admixtures in three combinations: 30P:70S, 50P:50S, 70P:30S were pasted. The results showed significant ($p<0.05$) effects of increasing irradiation doses on some protein related functional properties, while pasting characteristics of admixtures showed no dose-dependent significant ($p<0.05$) changes. Protein solubility decreased following irradiation, even though there were no significant differences ($p<0.05$) among samples. All samples readily solubilized at pH 8.00 with the non-irradiated (0.00kGy) showing the highest solubility value of 0.173g/ml. Increases in Water and Oil Absorption Capacities (WAC and OAC) were dose-dependent, with samples showing significant differences ($p<0.05$). The 10.00kGy samples recorded the highest values of 18.45% and 10.09% for WAC and OAC respectively. Foaming Properties increased across irradiation doses with some significant differences ($p<0.05$) among samples. However the 10.00kGy irradiated samples compared to Egg White, recorded lower values for foaming properties. Significant decreases ($p<0.05$) in Emulsifying Properties were recorded after irradiation, with the 2.50kGy sample recording the highest values of 45.83% and 73.33% for Emulsifying Activity and Emulsion Stability respectively. Pasting characteristics again increased significantly ($p<0.05$) with increasing starch:protein ratios. Correlation studies showed that the pasting properties were solely dependent on the starch concentration within the admixtures, indicating the insignificant contribution of modified Bambara groundnut proteins to the pasting properties of the blends. Enhanced surface functional properties of the gamma irradiated proteins makes them potential foaming, emulsifying, shelf life extension, and flavour retention agents. Admixtures may also serve as thickening agents for foods that require various degrees of viscosities.

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Last but not the least, my special thanks goes to all family and friends who supported me with prayers and words of encouragement throughout my entire study.

DEDICATION

I dedicate this Thesis to

God, who gave me life and strength throughout my study

and

My family and friends, who encouraged and supported me.

APPENDIX 10: VISCOGRAPHIC ANALYSIS OF PROTEIN - STARCH ADMIXTURES

APPENDIX 10A: Pasting characteristics of 30% Protein: 70%Starch blend

30P:70S (w/w)													
					Beginning of Gelatinization			Maximum Viscosity			Start of Holding Period		
Irrad. Doses Of Proteins (kGy)	Moisture (%)	Weight (G)	Volume (ml)	Measuring Range	Time (Sec)	Torque (BU)	T°C	Time (Sec)	Torque (BU)	T°C	Time (Sec)	Torque (BU)	T°C
0.00 + starch	11.35	38.80	421.20	250.00	15.00	80.00	74.40	15.00	537.00	94.40	1800.00	625.00	94.20
0.00 + starch	11.35	38.80	421.20	250.00	15.00	80.00	78.40	15.00	545.00	94.40	1800.00	612.00	94.20
AVERAGE	**11.35**	**38.80**	**421.20**	**250.00**	**15.00**	**80.00**	**76.40**	**15.00**	**541.00**	**94.40**	**1800.00**	**618.50**	**94.20**
2.50 + starch	10.75	39.70	420.20	250.00	1090.00	39.00	76.20	2430.00	557.00	94.60	1800.00	522.00	94.20
2.50 + starch	10.75	39.70	420.20	250.00	1090.00	39.00	76.20	2430.00	557.00	94.60	1800.00	522.00	94.20
AVERAGE	**10.75**	**39.70**	**420.20**	**250.00**	**1090.00**	**39.00**	**76.20**	**2430.00**	**557.00**	**94.60**	**1800.00**	**522.00**	**94.20**
5.00 + starch	11.14	38.70	421.20	250.00	1100.00	13.00	76.50	2695.00	580.00	94.60	1800.00	457.00	94.30
5.00 + starch	10.68	38.50	421.50	250.00	1080.00	59.00	76.20	2680.00	570.00	94.60	1800.00	501.00	94.40
AVERAGE	**10.91**	**38.60**	**421.35**	**250.00**	**1090.00**	**36.00**	**76.35**	**2687.50**	**575.00**	**94.60**	**1800.00**	**479.00**	**94.35**
7.50 + starch	10.96	38.60	421.50	250.00	1080.00	36.00	76.20	2690.00	754.00	94.60	1800.00	535.00	94.30
7.50 + starch	9.79	38.10	421.80	250.00	1100.00	17.00	76.70	2685.00	486.00	94.60	1800.00	407.00	94.40
AVERAGE	**10.38**	**38.35**	**421.65**	**250.00**	**1090.00**	**26.50**	**76.45**	**2687.50**	**620.00**	**94.60**	**1800.00**	**471.00**	**94.35**
10.00+ starch	10.51	38.40	421.60	250.00	1090.00	18.00	76.30	2685.00	554.00	94.60	1800.00	461.00	94.20
10.00+ starch	11.00	38.60	421.30	250.00	1090.00	62.00	76.40	2690.00	529.00	94.60	1800.00	461.00	94.40
AVERAGE	**10.76**	**38.50**	**421.45**	**250.00**	**1090.00**	**40.00**	**76.35**	**2687.50**	**541.50**	**94.60**	**1800.00**	**461.00**	**94.30**

Pasting characteristics of 30% Protein: 70%Starch blend

Irrad. Doses (kGy)	Start of Cooling Period			End of Cooling Period			End of Final Holding Period			BV	SV
	Time (Sec)	Torque (BU)	T°C	Time (Sec)	Torque (BU)	T°C	Time (Sec)	Torque (BU)	T°C	Torque (BU)	Torque (BU)
0.00 + starch	2700.00	536.00	94.20	4500.00	862.00	50.80	5400.00	876.00	50.00	4.00	290.00
0.00 + starch	2700.00	529.00	94.20	4500.00	862.00	50.80	5400.00	878.50	50.00	4.00	287.00
AVERAGE	**2700.00**	**532.50**	**94.20**	**4500.00**	**862.00**	**50.80**	**5400.00**	**877.25**	**50.00**	**4.00**	**288.50**
2.50 + starch	2700.00	554.00	94.60	4500.00	840.00	50.00	5400.00	821.00	49.90	4.00	286.00
2.50 + starch	2700.00	554.00	94.60	4500.00	840.00	50.00	5400.00	821.00	49.90	4.00	286.00
AVERAGE	**2700.00**	**554.00**	**94.60**	**4500.00**	**840.00**	**50.00**	**5400.00**	**821.00**	**49.90**	**4.00**	**286.00**
5.00 + starch	2700.00	581.00	94.60	4500.00	1031.00	50.50	5400.00	994.00	49.90	0.00	448.00
5.00 + starch	2700.00	570.00	94.60	4500.00	917.00	51.20	5400.00	904.00	50.00	2.00	347.00
AVERAGE	**2700.00**	**575.50**	**94.60**	**4500.00**	**974.00**	**50.85**	**5400.00**	**949.00**	**49.95**	**1.00**	**397.50**
7.50 + starch	2700.00	755.00	94.60	4500.00	1180.00	50.50	5400.00	1092.00	49.90	0.00	424.00
7.50 + starch	2700.00	486.00	94.60	4500.00	815.00	50.50	5400.00	795.00	50.00	0.00	327.00
AVERAGE	**2700.00**	**620.50**	**94.60**	**4500.00**	**997.50**	**50.50**	**5400.00**	**943.50**	**49.95**	**0.00**	**375.50**
10.00+ starch	2700.00	553.00	94.60	4500.00	938.00	50.60	5400.00	921.00	49.90	2.00	386.00
10.00+ starch	2700.00	529.00	94.60	4500.00	854.00	51.10	5400.00	843.00	50.00	3.00	326.00
AVERAGE	**2700.00**	**541.00**	**94.60**	**4500.00**	**896.00**	**50.85**	**5400.00**	**882.00**	**49.95**	**2.50**	**356.00**

BV= *Breakdown viscosity*
SV= *Setback viscosity*

APPENDIX 10B: Pasting characteristics of 50% Protein: 50%Starch blend

50P:50S (w/w)													
					Beginning of gelatinization			Maximum viscosity			Start of holding period		
Irrad. Doses of Proteins (kGy)	Moisture (%)	Weight (g)	Volume (ml)	M. range	time (sec)	Torque (BU)	T°C	time (sec)	Torque (BU)	T°C	time (sec)	Torque (BU)	T°C
0.00 + starch	10.51	38.40	421.60	250.00	1160.00	51.00	78.10	1840.00	314.00	95.10	1800.00	311.00	94.30
0.00 + starch	10.51	38.40	421.60	250.00	1160.00	51.00	78.10	1840.00	314.00	95.10	1800.00	311.00	94.30
AVERAGE	**10.51**	**38.40**	**421.60**	**250.00**	**1160.00**	**51.00**	**78.10**	**1840.00**	**314.00**	**95.10**	**1800.00**	**311.00**	**94.30**
2.50 + starch	9.82	38.10	421.80	250.00	15.00	10.00	50.40	1950.00	287.00	95.40	1800.00	276.00	94.20
2.50 + starch	9.97	38.20	421.70	250.00	1150.00	14.00	77.90	1920.00	289.00	95.60	1800.00	278.00	94.20
AVERAGE	**9.90**	**38.15**	**421.75**	**250.00**	**582.50**	**12.00**	**64.15**	**1935.00**	**288.00**	**95.50**	**1800.00**	**277.00**	**94.20**
5.00 + starch	9.86	38.10	421.80	250.00	570.00	8.00	63.80	1920.00	278.00	95.60	1800.00	271.00	94.30
5.00 + starch	9.86	38.10	421.80	250.00	570.00	8.00	63.80	1920.00	278.00	95.60	1800.00	271.00	94.30
AVERAGE	**9.86**	**38.10**	**421.80**	**250.00**	**570.00**	**8.00**	**63.80**	**1920.00**	**278.00**	**95.60**	**1800.00**	**271.00**	**94.30**
7.50 + starch	10.18	38.20	421.70	250.00	1180.00	22.00	78.70	1820.00	286.00	94.60	1800.00	281.00	94.20
7.50 + starch	10.40	38.30	421.70	250.00	1115.00	46.00	77.20	2240.00	272.00	94.60	1800.00	260.00	94.20
AVERAGE	**10.29**	**38.25**	**421.70**	**250.00**	**1147.50**	**34.00**	**77.95**	**2030.00**	**279.00**	**94.60**	**1800.00**	**270.50**	**94.20**
10.00+ starch	9.95	38.20	421.70	250.00	1115.00	21.00	77.20	2060.00	275.00	94.50	1800.00	258.00	94.20
10.00+ starch	10.48	38.40	421.60	250.00	1135.00	64.00	77.60	1990.00	309.00	95.40	1800.00	291.00	94.20
AVERAGE	**10.22**	**38.30**	**421.65**	**250.00**	**1125.00**	**42.50**	**77.40**	**2025.00**	**292.00**	**94.95**	**1800.00**	**274.50**	**94.20**

Pasting characteristics of 50% Protein: 50%Starch blend

	Start of cooling period			End of cooling period			End of final holding period			BV	SV
Irrad. Doses (kGy)	**time (sec)**	**Torque (BU)**	**T°C**	**time (sec)**	**Torque (BU)**	**T°C**	**time (sec)**	**Torque (BU)**	**T°C**	**Torque (BU)**	**Torque (BU)**
0.00 + starch	2700.00	272.00	94.30	4500.00	376.00	50.80	5400.00	349.00	50.00	42.00	104.00
0.00 + starch	2700.00	272.00	94.30	4500.00	376.00	50.80	5400.00	349.00	50.00	42.00	104.00
AVERAGE	**2700.00**	**272.00**	**94.30**	**4500.00**	**376.00**	**50.80**	**5400.00**	**349.00**	**50.00**	**42.00**	**104.00**
2.50 + starch	2700.00	254.00	94.60	4500.00	364.00	50.70	5400.00	334.00	49.80	34.00	109.00
2.50 + starch	2700.00	252.00	94.60	4500.00	349.00	50.50	5400.00	322.00	50.00	40.00	100.00
AVERAGE	**2700.00**	**253.00**	**94.60**	**4500.00**	**356.50**	**50.60**	**5400.00**	**328.00**	**49.90**	**37.00**	**104.50**
5.00 + starch	2700.00	255.00	94.60	4500.00	344.00	50.60	5400.00	323.00	49.90	21.00	88.00
5.00 + starch	2700.00	255.00	94.60	4500.00	344.00	50.60	5400.00	323.00	49.90	21.00	88.00
AVERAGE	**2700.00**	**255.00**	**94.60**	**4500.00**	**344.00**	**50.60**	**5400.00**	**323.00**	**49.90**	**21.00**	**88.00**
7.50 + starch	2700.00	250.00	94.60	4500.00	335.00	50.60	5400.00	305.00	49.80	36.00	83.00
7.50 + starch	2700.00	259.00	94.60	4500.00	346.00	50.50	5400.00	325.00	50.00	12.00	85.00
AVERAGE	**2700.00**	**254.50**	**94.60**	**4500.00**	**340.50**	**50.55**	**5400.00**	**315.00**	**49.90**	**24.00**	**84.00**
10.00+ starch	2700.00	254.00	94.60	4500.00	330.00	50.70	5400.00	310.00	50.00	20.00	87.00
10.00+ starch	2700.00	285.00	94.60	4500.00	373.00	51.00	5400.00	353.00	50.00	23.00	87.00
AVERAGE	**2700.00**	**269.50**	**94.60**	**4500.00**	**351.50**	**50.85**	**5400.00**	**331.50**	**50.00**	**21.50**	**87.00**

APPENDIX 10C: Pasting characteristics of 70% Protein: 30%Starch blend

70P:30S (w/w)													
	Moisture	**Weight**	**Volume**		**Beginning of gelatinization**			**Maximum viscosity**			**Start of holding period**		
Irrad. Doses (kGy)	**(%)**	**(g)**	**(ml)**	**M. range**	**time (sec)**	**Torque (BU)**	**T°C**	**time (sec)**	**Torque (BU)**	**T°C**	**time (sec)**	**Torque (BU)**	**T°C**
0.00 + starch	10.07	38.20	421.70	250.00	5.00	2.00	86.00	1625.00	94.00	94.80	1800.00	105.50	94.00
0.00 + starch	10.07	38.20	421.70	250.00	5.00	2.00	90.40	1625.00	92.00	94.20	1800.00	105.50	94.00
AVERAGE	**10.07**	**38.20**	**421.70**	**250.00**	**5.00**	**2.00**	**88.20**	**1625.00**	**93.00**	**94.50**	**1800.00**	**105.50**	**94.00**
2.50 + starch	8.95	37.70	422.20	250.00	1460.00	8.00	85.80	2555.00	43.00	94.60	1800.00	40.00	94.10
2.50 + starch	8.87	37.70	422.20	250.00	1525.00	61.00	87.40	2605.00	85.00	94.60	1800.00	68.00	94.20
AVERAGE	**8.91**	**37.70**	**422.20**	**250.00**	**1492.50**	**34.50**	**86.60**	**2580.00**	**64.00**	**94.60**	**1800.00**	**54.00**	**94.15**
5.00 + starch	8.90	37.70	422.20	250.00	1695.00	51.00	91.50	1745.00	57.00	92.70	1800.00	24.00	94.00
5.00 + starch	8.90	37.70	422.20	250.00	1695.00	51.00	91.50	1745.00	57.00	92.70	1800.00	24.00	94.00
AVERAGE	**8.90**	**37.70**	**422.20**	**250.00**	**1695.00**	**51.00**	**91.50**	**1745.00**	**57.00**	**92.70**	**1800.00**	**24.00**	**94.00**
7.50 + starch	8.90	37.70	422.20	250.00	1435.00	11.00	85.10	2360.00	5.00	94.60	1800.00	27.00	94.00
7.50 + starch	8.90	37.70	422.20	250.00	1435.00	11.00	85.10	2360.00	5.00	94.60	1800.00	26.50	94.00
AVERAGE	**8.90**	**37.70**	**422.20**	**250.00**	**1435.00**	**11.00**	**85.10**	**2360.00**	**5.00**	**94.60**	**1800.00**	**26.75**	**94.00**
10.00+ starch	9.44	37.90	422.10	250.00	1505.00	10.00	86.80	2545.00	34.00	94.50	1800.00	23.00	93.90
10.00+ starch	9.44	37.90	422.10	250.00	1505.00	10.00	86.80	2545.00	34.00	94.50	1800.00	23.00	93.90
AVERAGE	**9.44**	**37.90**	**422.10**	**250.00**	**1505.00**	**10.00**	**86.80**	**2545.00**	**34.00**	**94.50**	**1800.00**	**23.00**	**93.90**

Pasting characteristics of 70% Protein: 30%Starch blend

Irrad. Doses (kGy)	Start of cooling period			End of cooling period			End of final holding period			BV	SV
	time (sec)	Torque (BU)	T°C	time (sec)	Torque (BU)	T°C	time (sec)	Torque (BU)	T°C	Torque (BU)	Torque (BU)
0.00 + starch	2700.00	70.00	94.60	4500.00	127.00	50.70	5400.00	116.00	50.00	1.00	56.00
0.00 + starch	2700.00	71.00	94.60	4500.00	125.00	50.70	5400.00	121.00	50.00	1.00	58.00
AVERAGE	**2700.00**	**70.50**	**94.60**	**4500.00**	**126.00**	**50.70**	**5400.00**	**118.50**	**50.00**	**1.00**	**57.00**
2.50 + starch	2700.00	40.00	94.50	4500.00	94.00	50.30	5400.00	82.00	50.00	3.00	51.00
2.50 + starch	2700.00	83.00	94.60	4500.00	124.00	50.80	5400.00	116.00	50.00	1.00	40.00
AVERAGE	**2700.00**	**61.50**	**94.55**	**4500.00**	**109.00**	**50.55**	**5400.00**	**99.00**	**50.00**	**2.00**	**45.50**
5.00 + starch	2700.00	39.00	94.60	4500.00	82.00	50.60	5400.00	75.00	50.00	17.00	42.00
5.00 + starch	2700.00	39.00	94.60	4500.00	82.00	50.60	5400.00	75.00	50.00	17.00	42.00
AVERAGE	**2700.00**	**39.00**	**94.60**	**4500.00**	**82.00**	**50.60**	**5400.00**	**75.00**	**50.00**	**17.00**	**42.00**
7.50 + starch	2700.00	2.00	94.60	4500.00	12.00	50.60	5400.00	10.00	50.00	1.00	9.00
7.50 + starch	2700.00	2.00	94.60	4500.00	12.00	50.60	5400.00	10.00	50.00	1.00	9.00
AVERAGE	**2700.00**	**2.00**	**94.60**	**4500.00**	**12.00**	**50.60**	**5400.00**	**10.00**	**50.00**	**1.00**	**9.00**
10.00+ starch	2700.00	33.00	94.50	4500.00	78.00	50.40	5400.00	72.00	50.00	1.00	44.00
10.00+ starch	2700.00	33.00	94.50	4500.00	78.00	50.40	5400.00	72.00	50.00	1.00	44.00
AVERAGE	**2700.00**	**33.00**	**94.50**	**4500.00**	**78.00**	**50.40**	**5400.00**	**72.00**	**50.00**	**1.00**	**44.00**

APPENDICES

APPENDIX 1- FORMULAE USED FOR CALCULATIONS

1. % Moisture = $\frac{\text{(Wet weight – dry weight)} \times 100}{\text{Initial weight of sample}}$

2. % Foam Capacity = $\frac{\text{(Volume after whipping – volume before whipping)} \times 100}{\text{Volume before whipping}}$

3. % Foam Stability = $\frac{\text{foam volume after time (t)} \times 100}{\text{Initial foam volume}}$

4. % Emulsifying Activity = $\frac{\text{height of emulsion} \times 100}{\text{Height of whole layer}}$

5. % Emulsion stability = $\frac{\text{height of emulsion after heating} \times 100}{\text{Height of whole layer}}$

6. % Water Absorption Capacity = $\frac{\text{volume of bound water} \times 100}{\text{Initial volume of water}}$

7. % Oil Absorption Capacity = $\frac{\text{volume of bound oil} \times 100}{\text{Initial volume of oil}}$

8. Equation for protein solubility: $Y = 6139X – 41.80$

   Y= absorbance

   X= soluble protein per gram of Bradford's solution

APPENDIX 2 – DATA ON SOLUBILITY

APPENDIX 2A: Composite design table for protein solubility at optimum conditions

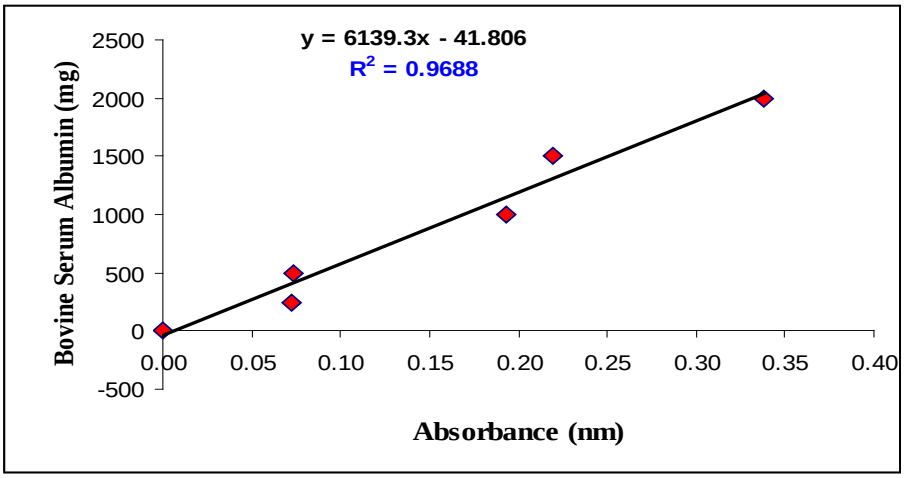
OPTIMUM CONDITIONS FOR PROTEIN SOLUBILITY			SAMPLE CODE
Buffer pH	**Protein slurry (g/20ml)**	**Vortexing Time (hrs)**	
Blank	0.00	0.00	**Blank**
6.50	3.50	2.00	**A1**
3.98	3.50	2.00	**B**
5.00	2.00	3.00	**C**
8.00	5.00	1.00	**D**
6.50	3.50	4.00	**E**
6.50	6.02	2.00	**F**
5.00	5.00	3.00	**G**
6.50	3.50	2.00	**A2**
6.50	1.00	2.00	**H**
8.00	2.00	3.00	**I**
6.50	3.50	2.00	**A3**
6.50	3.50	2.00	**A4**
5.00	2.00	1.00	**J**
9.02	3.50	2.00	**K**
8.00	5.00	3.00	**L**
6.50	3.50	2.00	**A5**
6.50	3.50	2.00	**A6**
5.00	5.00	1.00	**M**
8.00	2.00	1.00	**N**
6.50	3.50	0.50	**O**

** Replicate combinations are for estimation of the standard error*

APPENDIX 2B: A STANDARD ABSORBANCE TABLE FOR BSA

BSA standard	Mass of BSA(microgram)	Volume of BSA(ml)	Volume of water(ml)	Volume of Bradford reagent(ml)	Absorbance A	Absorbance B	Average Absorbance
Blank	**0**	1.000	0.000	2.000	0.000	0.000	**0.000**
1	**250**	1.000	32.000	2.000	0.073	0.071	**0.072**
2	**500**	1.000	16.000	2.000	0.075	0.073	**0.074**
3	**1000**	1.000	8.000	2.000	0.193	0.193	**0.193**
4	**1500**	1.000	5.300	2.000	0.219	0.219	**0.219**
5	**2000**	1.000	4.000	2.000	0.336	0.340	**0.338**

APPENDIX 2C: A STANDARD REGRESSION GRAPH FOR BOVINE SERUM ALBUMIN



APPENDIX 2D: Protein solubility of 0.00kGy irradiated Bambara protein sample

Optimum conditions	PROTEIN SOLUBILITY AT 0.00kGY					
	ABSORBANCE at 595.00nm				SOLUBLE PROTEINS (ml)	
CODE	REP 1	REP 2	REP 3	Average	X1	X2
A1	0.262000	0.261000	0.270000	0.264333	0.006852	0.171300
B	0.412000	0.395000	0.398000	0.401667	0.006874	0.171859
C	0.386000	0.404000	0.398000	0.396000	0.006873	0.171836
D	0.349000	0.351000	0.351000	0.350333	0.006866	0.171650
E	0.610000	0.584000	0.587000	0.593667	0.006906	0.172641
F	0.456000	0.429000	0.449000	0.444667	0.006881	0.172034
G	0.554000	0.544000	0.544000	0.547333	0.006898	0.172452
A2	0.285000	0.270000	0.273000	0.276000	0.006854	0.171347
H	0.482000	0.480000	0.502000	0.488000	0.006888	0.172210
I	0.398000	0.388000	0.397000	0.394333	0.006873	0.171829
A3	0.269000	0.247000	0.255000	0.257000	0.006851	0.171270
A4	0.285000	0.287000	0.292000	0.288000	0.006856	0.171396
J	0.389000	0.404000	0.409000	0.400667	0.006874	0.171855
K	0.182000	0.195000	0.187000	0.188000	0.006840	0.170989
L	0.604000	0.616000	0.594000	0.604667	0.006907	0.172686
A5	0.229000	0.240000	0.238000	0.235667	0.006847	0.171183
A6	0.257000	0.227000	0.235000	0.239667	0.006848	0.171199
M	0.483000	0.518000	0.486000	0.495667	0.006890	0.172242
N	0.288000	0.302000	0.300000	0.296667	0.006857	0.171431
O	0.226000	0.227000	0.206000	0.219667	0.006845	0.171118
BLANK	0.000000	0.000000	0.000000	0.000000	0.006809	0.170223

** X1 = Average absorbance + 41.81*
6139.30
**X1 values are the amount (mg) of soluble proteins in 0.04ml protein solution.*

**X2 = (Average absorbance + 41.81) / 6139.30*
0.04

**X2 values are the amount (mg) of soluble proteins in 1.00ml protein solution*

APPENDIX 2E: Protein solubility of 2.50kGy irradiated Bambara protein sample

Optimum conditions CODED	**PROTEIN SOLUBILITY AT 2.50kGY**					
	ABSORBANCE at 595.00nm				**SOLUBLE PROTEINS (ml)**	
	REP 1	**REP 2**	**REP 3**	**Average**	**X1**	**X2**
A1	0.147000	0.173000	0.175000	0.165000	0.006836	0.170895
B	0.092000	0.103000	0.103000	0.099333	0.006825	0.170628
C	0.176000	0.180000	0.187000	0.181000	0.006838	0.170960
D	0.085000	0.097000	0.104000	0.095333	0.006824	0.170611
E	0.079000	0.090000	0.088000	0.085667	0.006823	0.170572
F	0.276000	0.284000	0.290000	0.283333	0.006855	0.171377
G	0.276000	0.304000	0.308000	0.296000	0.006857	0.171429
A2	0.081000	0.112000	0.113000	0.102000	0.006826	0.170639
H	0.153000	0.165000	0.175000	0.164333	0.006836	0.170892
I	0.211000	0.223000	0.226000	0.220000	0.006845	0.171119
A3	0.110000	0.121000	0.123000	0.118000	0.006828	0.170704
A4	0.101000	0.115000	0.120000	0.112000	0.006827	0.170679
J	0.065000	0.071000	0.076000	0.070667	0.006820	0.170511
K	0.342000	0.362000	0.359000	0.354333	0.006867	0.171666
L	0.344000	0.337000	0.374000	0.351667	0.006866	0.171655
A5	0.075000	0.089000	0.090000	0.084667	0.006823	0.170568
A6	0.141000	0.162000	0.166000	0.156333	0.006834	0.170860
M	0.158000	0.157000	0.165000	0.160000	0.006835	0.170875
N	0.195000	0.216000	0.220000	0.210333	0.006843	0.171080
O	0.082000	0.082000	0.083000	0.082333	0.006822	0.170558
BLANK	0.000000	0.000000	0.000000	0.000000	0.006809	0.170223

APPENDIX 2F: Protein solubility of 5.00kGy irradiated Bambara protein sample

Optimum conditions	PROTEIN SOLUBILITY AT 5.00kGY					
	ABSORBANCE at 595.00nm				SOLUBLE PROTEINS (ml)	
CODE	REP 1	REP 2	REP 3	Average	X1	X2
A1	0.120000	0.130000	0.128000	0.126000	0.006829	0.170736
B	0.134000	0.136000	0.139000	0.136333	0.006831	0.170778
C	0.202000	0.213000	0.207000	0.207333	0.006843	0.171067
D	0.082000	0.080000	0.081000	0.081000	0.006822	0.170553
E	0.275000	0.282000	0.286000	0.281000	0.006855	0.171367
F	0.312000	0.316000	0.316000	0.314667	0.006860	0.171505
G	0.382000	0.388000	0.390000	0.386667	0.006872	0.171798
A2	0.082000	0.092000	0.092000	0.088667	0.006823	0.170584
H	0.244000	0.246000	0.245000	0.245000	0.006849	0.171221
I	0.304000	0.318000	0.313000	0.311667	0.006860	0.171492
A3	0.150000	0.162000	0.162000	0.158000	0.006835	0.170867
A4	0.128000	0.131000	0.137000	0.131000	0.006830	0.170757
J	0.233000	0.239000	0.240000	0.237333	0.006848	0.171190
K	0.247000	0.247000	0.244000	0.246000	0.006849	0.171225
L	0.359000	0.402000	0.402000	0.387667	0.006872	0.171802
A5	0.219000	0.219000	0.222000	0.220000	0.006845	0.171119
A6	0.133000	0.142000	0.145000	0.140000	0.006832	0.170793
M	0.290000	0.266000	0.291000	0.282333	0.006855	0.171373
N	0.304000	0.313000	0.312000	0.309667	0.006859	0.171484
O	0.119000	0.143000	0.145000	0.135667	0.006831	0.170776
BLANK	0.000000	0.000000	0.000000	0.000000	0.006809	0.170223

** X1 = $\frac{\text{Average absorbance} + 41.81}{6139.30}$*

**X1 values are the amount (mg) of soluble proteins in 0.04ml protein solution.*

**X2 = $\frac{(\text{Average absorbance} + 41.81) / 6139.30}{0.04}$*

**X2 values are the amount (mg) of soluble proteins in 1.00ml protein solution*

APPENDIX 2G: Protein solubility of 7.50kGy irradiated Bambara protein sample

Optimum conditions	PROTEIN SOLUBILITY AT 7.50kGY					
	ABSORBANCE at 595.00nm				SOLUBLE PROTEINS (ml)	
CODE	REP 1	REP 2	REP 3	Average	X1	X2
A1	0.242000	0.282000	0.279000	0.267667	0.006853	0.171313
B	0.215000	0.206000	0.219000	0.213333	0.006844	0.171092
C	0.526000	0.563000	0.549000	0.546000	0.006898	0.172447
D	0.268000	0.285000	0.289000	0.280667	0.006855	0.171366
E	0.272000	0.314000	0.298000	0.294667	0.006857	0.171423
F	0.330000	0.334000	0.320000	0.328000	0.006862	0.171559
G	0.442000	0.459000	0.462000	0.454333	0.006883	0.172073
A2	0.266000	0.277000	0.296000	0.279667	0.006854	0.171362
H	0.274000	0.276000	0.286000	0.278667	0.006854	0.171358
I	0.337000	0.368000	0.383000	0.362667	0.006868	0.171700
A3	0.249000	0.297000	0.304000	0.283333	0.006855	0.171377
A4	0.209000	0.199000	0.196000	0.201333	0.006842	0.171043
J	0.255000	0.306000	0.307000	0.289333	0.006856	0.171401
K	0.392000	0.407000	0.402000	0.400333	0.006874	0.171853
L	0.393000	0.424000	0.422000	0.413000	0.006876	0.171905
A5	0.296000	0.290000	0.274000	0.286667	0.006856	0.171391
A6	0.339000	0.373000	0.361000	0.357667	0.006867	0.171680
M	0.292000	0.313000	0.309000	0.304667	0.006859	0.171464
N	0.332000	0.351000	0.369000	0.350667	0.006866	0.171651
O	0.143000	0.173000	0.165000	0.160333	0.006835	0.170876
BLANK	0.000000	0.000000	0.000000	0.000000	0.006809	0.170223

$$* X1 = \frac{Average\ absorbance + 41.81}{6139.30}$$

**X1 values are the amount (mg) of soluble proteins in 0.04ml protein solution.*

$$*X2 = \frac{(Average\ absorbance + 41.81) / 6139.30}{0.04}$$

**X2 values are the amount (mg) of soluble proteins in 1.00ml protein solution*

APPENDIX 2H: Protein solubility of 10.00kGy irradiated Bambara protein sample

Optimum conditions	PROTEIN SOLUBILITY AT 10.00kGY					
	ABSORBANCE at 595.00nm				SOLUBLE PROTEINS (ml)	
CODE	REP 1	REP 2	REP 3	Average	X1	X2
A1	0.324000	0.348000	0.360000	0.344000	0.006865	0.171624
B	0.338000	0.349000	0.380000	0.355667	0.006867	0.171672
C	0.575000	0.522000	0.570000	0.555667	0.006899	0.172486
D	0.408000	0.413000	0.430000	0.417000	0.006877	0.171921
E	0.539000	0.550000	0.566000	0.551667	0.006899	0.172470
F	0.463000	0.468000	0.457000	0.462667	0.006884	0.172107
G	0.746000	0.881000	0.886000	0.837667	0.006945	0.173634
A2	0.522000	0.496000	0.517000	0.511667	0.006892	0.172307
H	0.321000	0.321000	0.307000	0.316333	0.006860	0.171511
I	0.420000	0.445000	0.457000	0.440667	0.006881	0.172018
A3	0.497000	0.511000	0.500000	0.502667	0.006891	0.172270
A4	0.396000	0.402000	0.413000	0.403667	0.006875	0.171867
J	0.379000	0.382000	0.365000	0.375333	0.006870	0.171752
K	0.512000	0.525000	0.532000	0.523000	0.006894	0.172353
L	0.403000	0.411000	0.409000	0.407667	0.006875	0.171883
A5	0.414000	0.419000	0.431000	0.421333	0.006878	0.171939
A6	0.367000	0.388000	0.369000	0.374667	0.006870	0.171749
M	0.407000	0.403000	0.403000	0.404333	0.006875	0.171870
N	0.370000	0.382000	0.397000	0.383000	0.006871	0.171783
O	0.209000	0.262000	0.288000	0.253000	0.006850	0.171253
BLANK	0.000000	0.000000	0.000000	0.000000	0.006809	0.170223

$$* X1 = \frac{Average\ absorbance + 41.81}{6139.30}$$

**X1 values are the amount (mg) of soluble proteins in 0.04ml protein solution.*

$$*X2 = \frac{(Average\ absorbance + 41.81) / 6139.30}{0.04}$$

**X2 values are the amount (mg) of soluble proteins in 1.00ml protein solution*

APPENDIX 3 – DATA ON THE FUNCTIONAL PROPERTIES OF THE IRRADIATED BAMBARA PROTEIN SAMPLES

APPENDIX 3A: Moisture, Emulsifying activity (EA) and Emulsion stability

Irradiation Doses	Moisture	EA	ES
0.00	5.81(0.44)	38.33(2.98)	42.50(2.50)
2.50	5.78(0.21)	45.83(1.44)	73.33(2.89)
5.00	5.81(0.48)	44.17(1.44)	65.00(5.00)
7.50	5.77(0.14)	41.67(1.44)	61.67(5.77)
10.00	5.81(0.04)	35.00(0.00)	49.17(1.44)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 3B: Foam capacity (FC), Foam stability at 30seconds and Foam stability at 10 minutes

Irradiation Doses	FC	FS at 30 sec	FS at 10 minutes
0.00	53.00(1.41)	92.16(1.78)	43.14(0.40)
2.50	65.00(1.41)	92.12(0.79)	52.73(0.41)
5.00	69.00(1.41)	89.94(0.92)	43.79(0.37)
7.50	77.00(4.24)	92.66(0.62)	51.98(1.95)
10.00	80.00(0.00)	96.67(0.00)	58.89(1.57)
EW	95.00(1.41)	97.44(0.71)	68.72(0.22)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 3C: Foam stability at 30 minutes, Foam stability at 60 minutes, and Foam stability at 120 minutes

Irradiation Doses	FS at 30 minutes	FS at 60 minutes	FS at 120 minutes
0.00	23.53(1.63)	15.03(2.91)	7.52(0.53)
2.50	27.88(2.82)	17.27(0.28)	12.12(0.10)
5.00	18.93(1.52)	17.16(1.82)	10.06(0.75)
7.50	40.68(4.17)	19.21(3.66)	11.86(1.08)
10.00	44.44(1.57)	19.44(3.93)	13.06(1.18)
EW	55.90(2.58)	44.10(0.32)	38.97(0.28)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 3D: Water absorption capacities (WAC) and Oil absorption capacities (OAC) for irradiated samples

Irradiation Doses	WAC	OAC
0.00	16.86(1.03)	9.01(0.02)
2.50	17.78(0.07)	9.11(0.05)
5.00	17.82(0.69)	9.31(0.15)
7.50	17.82(0.12)	9.58(0.24)
10.00	18.45(0.38)	10.09(0.21)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 4 – ANALYSIS OF VARIANCE RESULTS FOR PROTEIN SOLUBILITY FOR IRRADIATED BAMBARA PROTEINS UNDER OPTIMUM CONDITIONS

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	0.070	0.0175	0.01	1.000
Residual	5	8.175	1.636		
Total	9	8.247			

APPENDIX 5 - ANALYSIS OF VARIANCE RESULTS FOR FUNCTIONAL PROPERTIES OF IRRADIATED BAMBARA PROTEIN SAMPLES

APPENDIX 5A: percent moisture

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	0.00314	0.00079	0.01	1.000
Residual	5	0.48670	0.09734		
Total	9	0.48984			

APPENDIX 5B: Percent emulsifying activity (%EA)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	230.833	57.708	19.79	<.001
Residual	10	29.167	2.917		
Total	14	260.000			

APPENDIX 5C: Percent emulsion stability (%ES)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	1861.73	465.43	30.99	<.001
Residual	10	150.17	15.02		
Total	14	2011.90			

APPENDIX 5D: Percent Foam capacity (% FC)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	5	2057.667	411.533	94.97	<.001
Residual	6	26.000	4.333		
Total	11	2083.667			

APPENDIX 5E: Percent Foam stability (% FS at 30 seconds)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	5	85.2245	17.0449	18.55	0.001
Residual	6	5.5136	0.9189		
Total	11	90.7381			

APPENDIX 5F: Percent Foam stability (% FS at 10 minutes)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	5	961.714	192.343	170.22	<.001
Residual	6	6.780	1.130		
Total	11	968.494			

APPENDIX 5G: Percent Foam stability (% FS at 30 minutes)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	5	1981.530	396.306	60.32	<.001
Residual	6	39.423	6.570		
Total	11	2020.952			

APPENDIX 5H: Percent Foam stability (% FS at 60 minutes)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	5	1193.449	238.690	35.13	<.001
Residual	6	40.766	6.794		
Total	11	1234.215			

APPENDIX 5I: Percent Foam stability (% FS at 120 minutes)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	5	1349.7679	269.9536	462.29	<.001
Residual	6	3.5037	0.5839		
Total	11	1353.2716			

APPENDIX 5J: Percent Oil absorption capacity (% OAC)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	2.25599	0.56400	22.42	<.001
Residual	10	0.25160	0.02516		
Total	14	2.50759			

APPENDIX 5K: Percent water absorption capacity (% WAC)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	3.9012	0.9753	2.86	0.081
Residual	10	3.4098	0.3410		
Total	14	7.3109			

APPENDIX 6 – GRAPHICAL REPRESENTATION OF COGELLED PROTEIN-STARCH ADMIXTURES

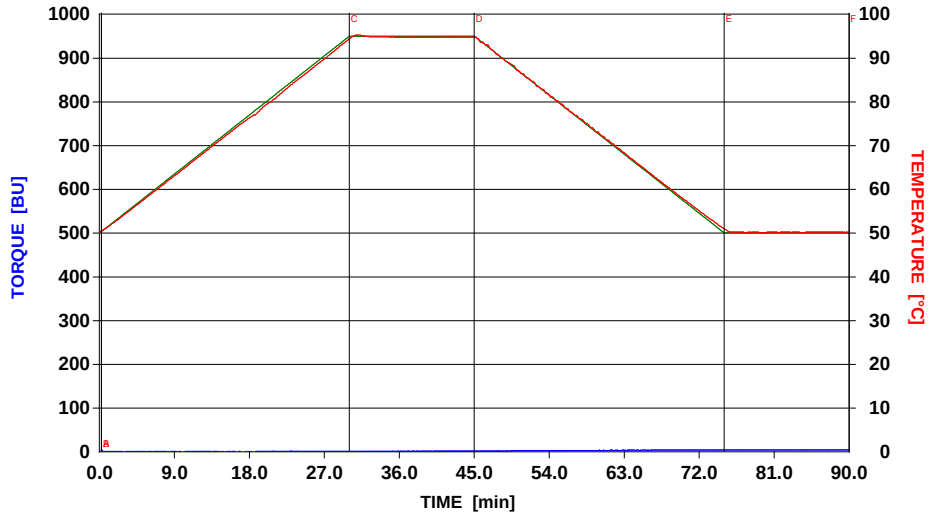
APPENDIX 6A: Cogelation at 30P:70S using 0.00kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	MR NAJAH		Date	:	7/17/2008	
Sample	:	0.0KGY 30P:70S		Method	:		
Moisture	:	11.35	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	38.8	[g]
Water	:	420	[ml]	Corr. to 14%	:	421.2	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	1000	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 1000 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:00:15	5	50.4
B	Maximum viscosity	00:00:15	5	50.4
C	Start of holding period	00:30:00	0	94.2
D	Start of cooling period	00:45:00	1	94.6
E	End of cooling period	01:15:00	3	50.8
F	End of final holding period	01:30:00	3	50.0
B-D	Breakdown		4	
E-D	Setback		2	

File : Measurement V: 2.3.16

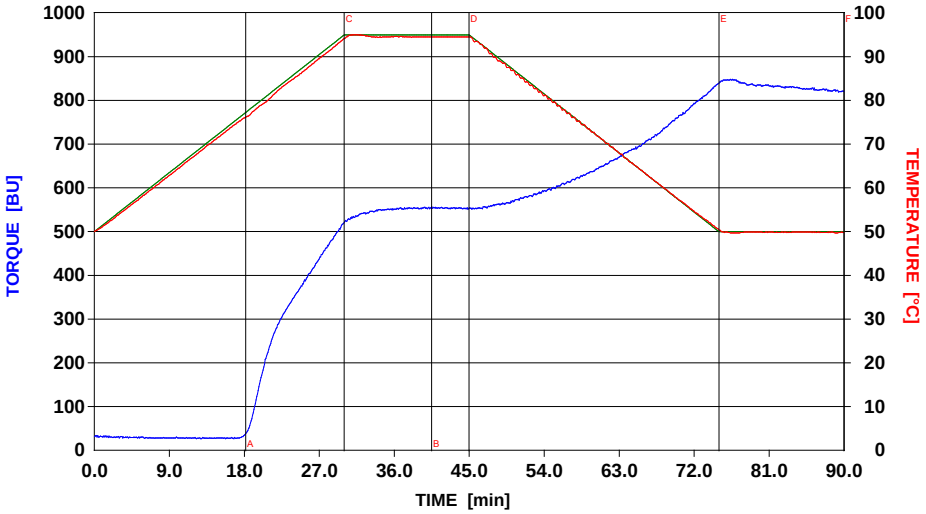
APPENDIX 6B: Cogelation at 30P:70S using 2.50kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	MR NAJAH		Date	:	7/30/2008	
Sample	:	2.5KGY 30P :70S		Method	:		
Moisture	:	10.51	[%]	Correction	:	11.06	[%]
Sample weight	:	40	[g]	Corr. to 11.06%	:	39.7	[g]
Water	:	420	[ml]	Corr. to 11.06%	:	420.2	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:18:10	39	76.2
B	Maximum viscosity	00:40:30	557	94.6
C	Start of holding period	00:30:00	522	94.2
D	Start of cooling period	00:45:00	554	94.6
E	End of cooling period	01:15:00	840	50.5
F	End of final holding period	01:30:00	821	49.9
B-D	Breakdown		4	
E-D	Setback		286	

File : Measurement V: 2.3.16

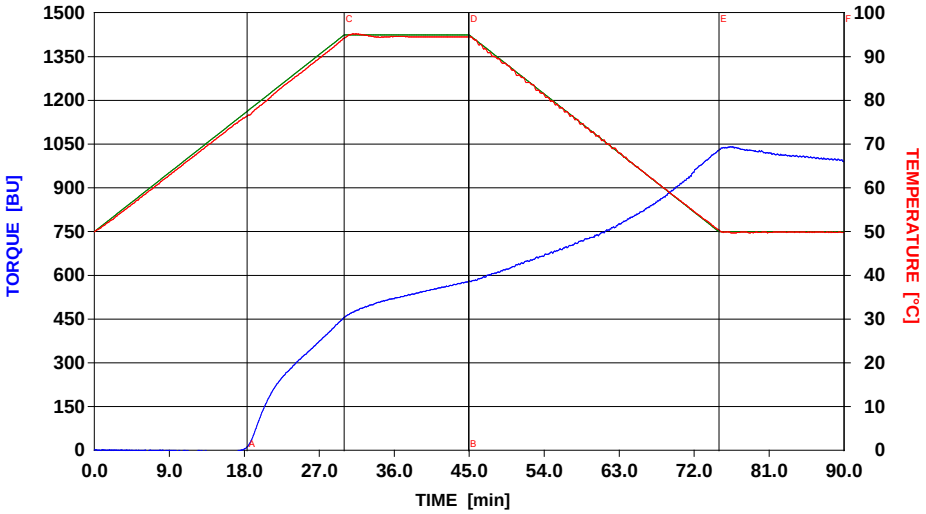
APPENDIX 6C: Cogelation at 30P:70S using 5.00kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	MR NAJAH		Date	:	8/4/2008	
Sample	:	5.0KGY 30P :70S		Method	:	METHOD 1	
Moisture	:	11.14	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	38.7	[g]
Water	:	420	[ml]	Corr. to 14%	:	421.2	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:18:20	13	76.5
B	Maximum viscosity	00:44:55	580	94.6
C	Start of holding period	00:30:00	457	94.3
D	Start of cooling period	00:45:00	581	94.6
E	End of cooling period	01:15:00	1031	50.5
F	End of final holding period	01:30:00	994	49.9
B-D	Breakdown		0	
E-D	Setback		448	

File : Measurement V: 2.3.16

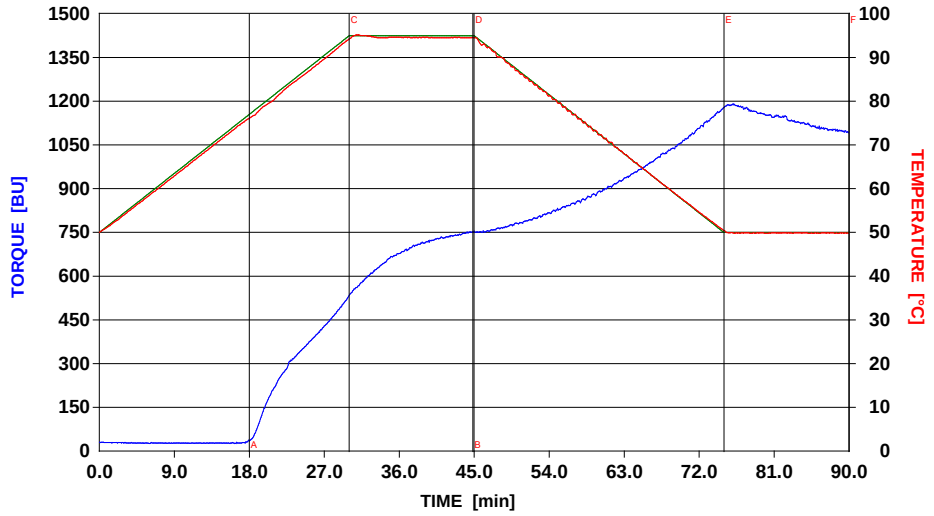
APPENDIX 6D: Cogelation at 30P:70S using 7.50kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	MR NAJAH		Date	:	7/31/2008	
Sample	:	7.5KGY 30P :70S		Method	:	METHOD 1	
Moisture	:	10.96	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	38.6	[g]
Water	:	420	[ml]	Corr. to 14%	:	421.3	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:18:00	36	76.2
B	Maximum viscosity	00:44:50	754	94.6
C	Start of holding period	00:30:00	535	94.3
D	Start of cooling period	00:45:00	755	94.6
E	End of cooling period	01:15:00	1180	50.5
F	End of final holding period	01:30:00	1092	49.9
B-D	Breakdown		0	
E-D	Setback		424	

File : Measurement V: 2.3.16

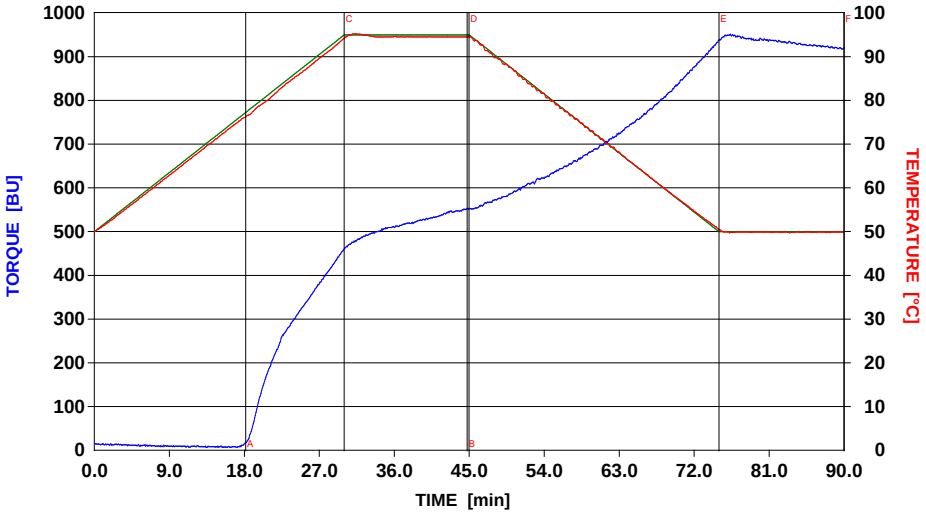
APPENDIX 6E: Cogelation at 30P:70S using 10.00kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	MR NAJAH		Date	:	8/4/2008	
Sample	:	10KGY 30P :70S		Method	:	METHOD 1	
Moisture	:	10.51	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	38.4	[g]
Water	:	420	[ml]	Corr. to 14%	:	421.6	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:18:10	18	76.3
B	Maximum viscosity	00:44:45	554	94.6
C	Start of holding period	00:30:00	461	94.2
D	Start of cooling period	00:45:00	553	94.6
E	End of cooling period	01:15:00	938	50.6
F	End of final holding period	01:30:00	921	49.9
B-D	Breakdown		2	
E-D	Setback		386	

File : Measurement V: 2.3.16

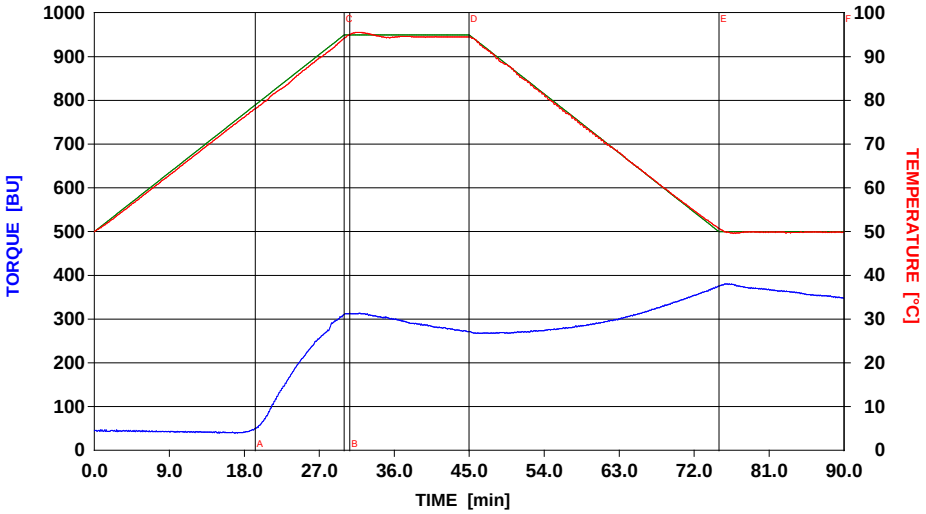
APPENDIX 6F: Cogelation at 50P:50S using 0.00kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	NII NORTEY		Date	:	7/29/2008	
Sample	:	0.00 KGY 50p:50s		Method	:	METHOD 1	
Moisture	:	10.51	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	38.4	[g]
Water	:	420	[ml]	Corr. to 14%	:	421.6	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:19:20	51	78.1
B	Maximum viscosity	00:30:40	314	95.1
C	Start of holding period	00:30:00	311	94.3
D	Start of cooling period	00:45:00	272	94.6
E	End of cooling period	01:15:00	376	50.8
F	End of final holding period	01:30:00	349	50.0
B-D	Breakdown		42	
E-D	Setback		104	

File : Measurement V: 2.3.16

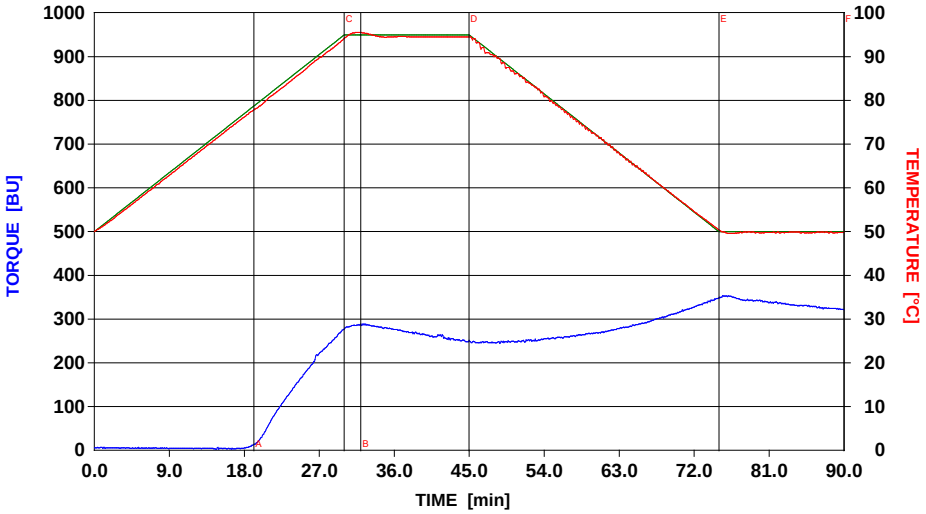
APPENDIX 6G: Cogelation at 50P:50S using 2.50kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	MR NAJAH		Date	:	8/6/2008	
Sample	:	2.5KGY 50P:50S		Method	:	METHOD 1	
Moisture	:	9.97	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	38.2	[g]
Water	:	420	[ml]	Corr. to 14%	:	421.7	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:19:10	14	77.9
B	Maximum viscosity	00:32:00	289	95.6
C	Start of holding period	00:30:00	278	94.2
D	Start of cooling period	00:45:00	252	94.6
E	End of cooling period	01:15:00	349	50.5
F	End of final holding period	01:30:00	322	50.0
B-D	Breakdown		40	
E-D	Setback		100	

File : Measurement V: 2.3.16

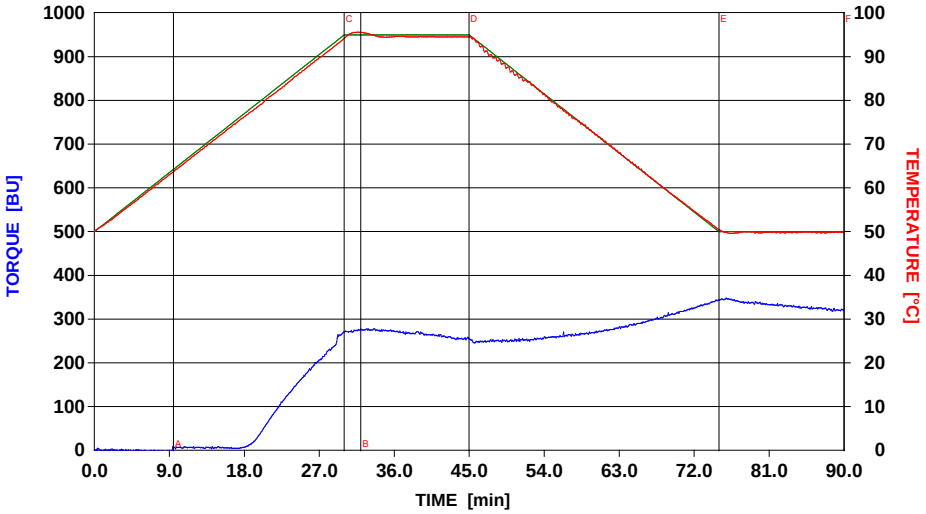
APPENDIX 6H: Cogelation at 50P:50S using 5.00kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	MR NAJAH		Date	:	7/31/2008	
Sample	:	5.00KGY 50P:50S		Method	:	METHOD 1	
Moisture	:	9.86	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	38.1	[g]
Water	:	420	[ml]	Corr. to 14%	:	421.8	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:09:30	8	63.8
B	Maximum viscosity	00:32:00	278	95.6
C	Start of holding period	00:30:00	271	94.3
D	Start of cooling period	00:45:00	255	94.6
E	End of cooling period	01:15:00	344	50.6
F	End of final holding period	01:30:00	323	49.9
B-D	Breakdown		21	
E-D	Setback		88	

File : Measurement V: 2.3.16

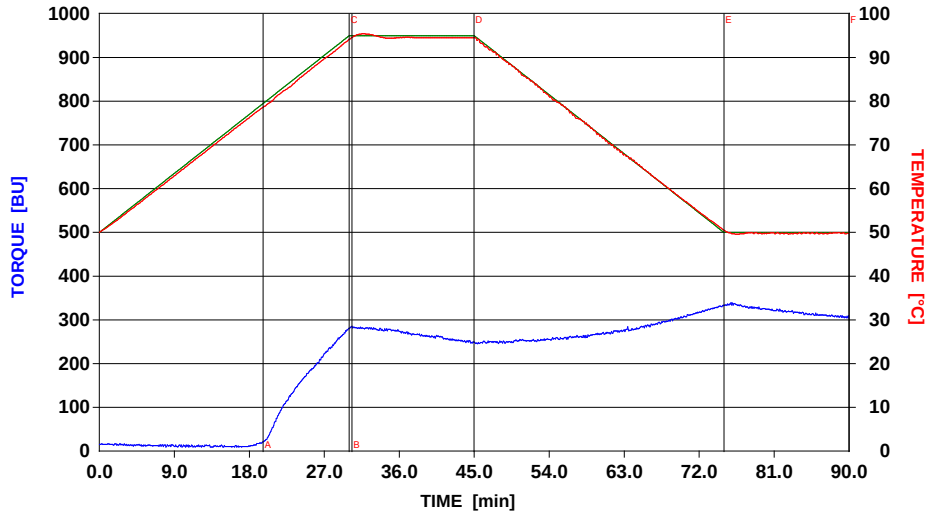
APPENDIX 6I: Cogelation at 50P:50S using 7.50kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	NII NORTEY		Date	:	7/28/2008	
Sample	:	7.50 KGY 50p:50s		Method	:	METHOD 1	
Moisture	:	10.18	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	38.2	[g]
Water	:	420	[ml]	Corr. to 14%	:	421.7	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:19:40	22	78.7
B	Maximum viscosity	00:30:20	286	94.6
C	Start of holding period	00:30:00	281	94.2
D	Start of cooling period	00:45:00	250	94.6
E	End of cooling period	01:15:00	335	50.6
F	End of final holding period	01:30:00	305	49.8
B-D	Breakdown		36	
E-D	Setback		83	

File : Measurement V: 2.3.16

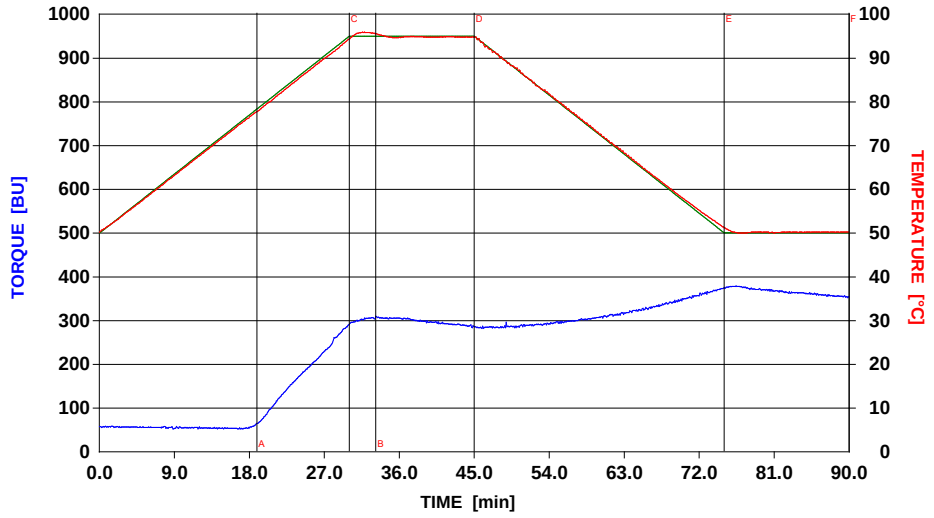
APPENDIX 6J: Cogelation at 50P:50S using 10.00kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	MR NAJAH		Date	:	8/6/2008	
Sample	:	10.00KGY 50P :50S		Method	:	METHOD 1	
Moisture	:	10.48	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	38.4	[g]
Water	:	420	[ml]	Corr. to 14%	:	421.6	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:18:55	64	77.6
B	Maximum viscosity	00:33:10	309	95.4
C	Start of holding period	00:30:00	291	94.2
D	Start of cooling period	00:45:00	285	94.6
E	End of cooling period	01:15:00	373	51.0
F	End of final holding period	01:30:00	353	50.0
B-D	Breakdown		23	
E-D	Setback		87	

File : Measurement V: 2.3.16

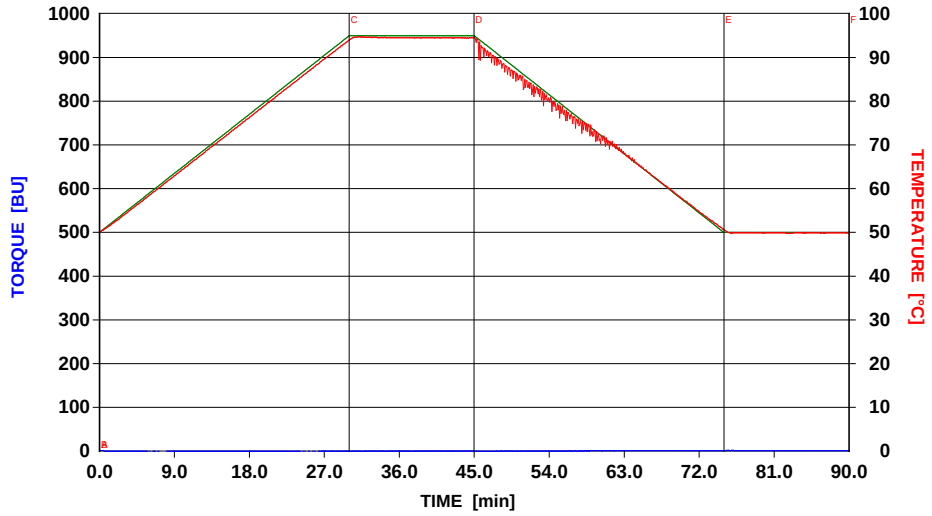
APPENDIX 6K: Cogelation at 70P:30S using 0.00kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	MR NAJAH		Date	:	7/17/2008	
Sample	:	0.00KGY 70P:30S		Method	:		
Moisture	:	10.07	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	38.2	[g]
Water	:	420	[ml]	Corr. to 14%	:	421.7	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	1000	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 1000 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:00:05	2	50.2
B	Maximum viscosity	00:00:00	2	50.0
C	Start of holding period	00:30:00	0	94.0
D	Start of cooling period	00:45:00	1	94.6
E	End of cooling period	01:15:00	2	50.7
F	End of final holding period	01:30:00	2	50.0
B-D	Breakdown		1	
E-D	Setback		1	

File : Measurement V: 2.3.16

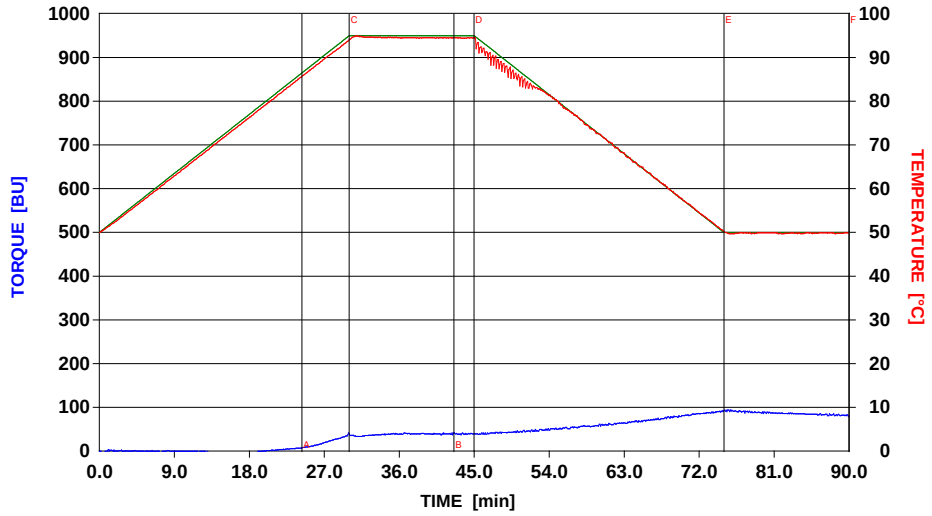
APPENDIX 6L: Cogelation at 70P:30S using 2.50kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	NII NORTEY		Date	:	7/28/2008	
Sample	:	2.5kgy 70p:30s		Method	:	METHOD 1	
Moisture	:	8.95	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	37.7	[g]
Water	:	420	[ml]	Corr. to 14%	:	422.2	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:24:20	8	85.8
B	Maximum viscosity	00:42:35	43	94.6
C	Start of holding period	00:30:00	40	94.1
D	Start of cooling period	00:45:00	40	94.5
E	End of cooling period	01:15:00	94	50.3
F	End of final holding period	01:30:00	82	50.0
B-D	Breakdown		3	
E-D	Setback		51	

File : Measurement V: 2.3.16

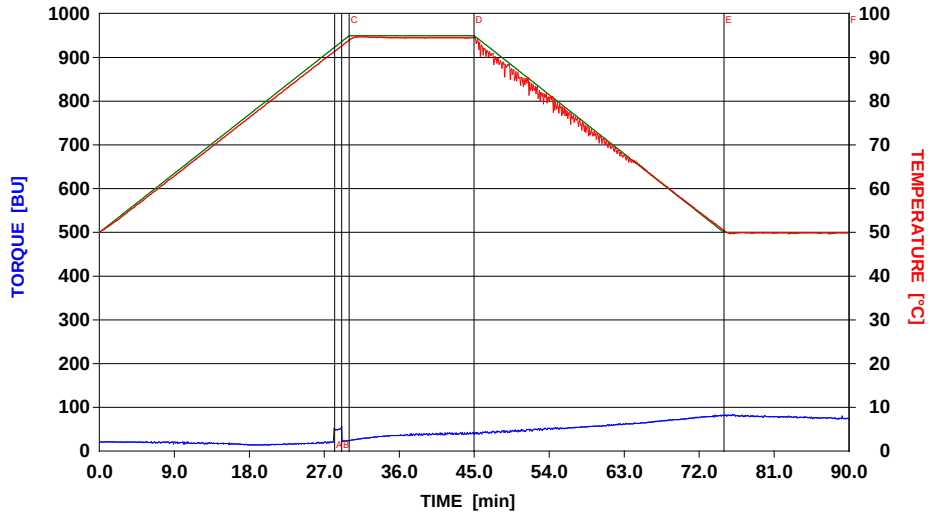
APPENDIX 6M: Cogelation at 70P:30S using 5.00kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	MR NAJAH		Date	:	7/21/2008	
Sample	:	5.00KGY 70P :30S		Method	:		
Moisture	:	8.90	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	37.7	[g]
Water	:	420	[ml]	Corr. to 14%	:	422.2	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:28:15	51	91.5
B	Maximum viscosity	00:29:05	57	92.7
C	Start of holding period	00:30:00	24	94.0
D	Start of cooling period	00:45:00	39	94.6
E	End of cooling period	01:15:00	82	50.6
F	End of final holding period	01:30:00	75	50.0
B-D	Breakdown		17	
E-D	Setback		42	

File : Measurement V: 2.3.16

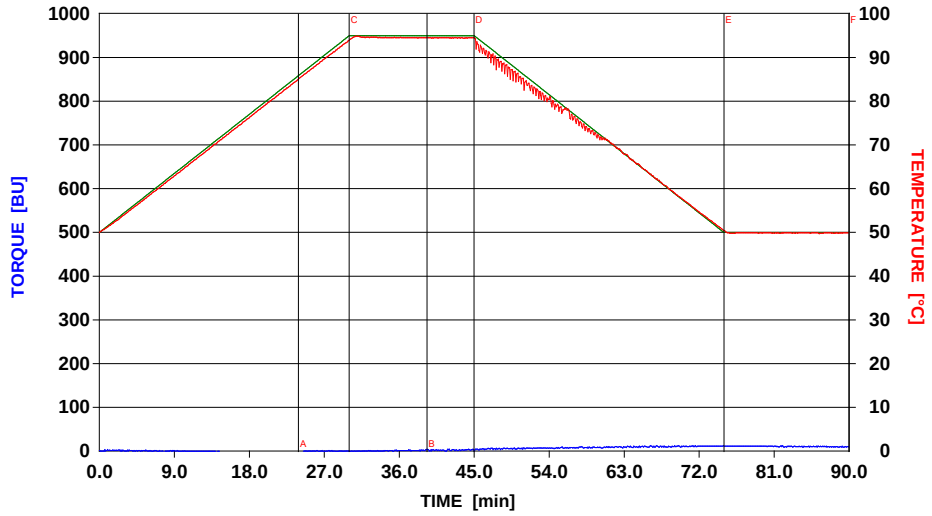
APPENDIX 6N: Cogelation at 70P:30S using 7.50kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	NII NORTEY		Date	:	7/29/2008	
Sample	:	7.5 KGY 70p:30s		Method	:	METHOD 1	
Moisture	:	8.90	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	37.7	[g]
Water	:	420	[ml]	Corr. to 14%	:	422.2	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:23:55	-1	85.1
B	Maximum viscosity	00:39:20	5	94.6
C	Start of holding period	00:30:00	0	94.0
D	Start of cooling period	00:45:00	2	94.6
E	End of cooling period	01:15:00	12	50.5
F	End of final holding period	01:30:00	10	50.0
B-D	Breakdown		1	
E-D	Setback		9	

File : Measurement V: 2.3.16

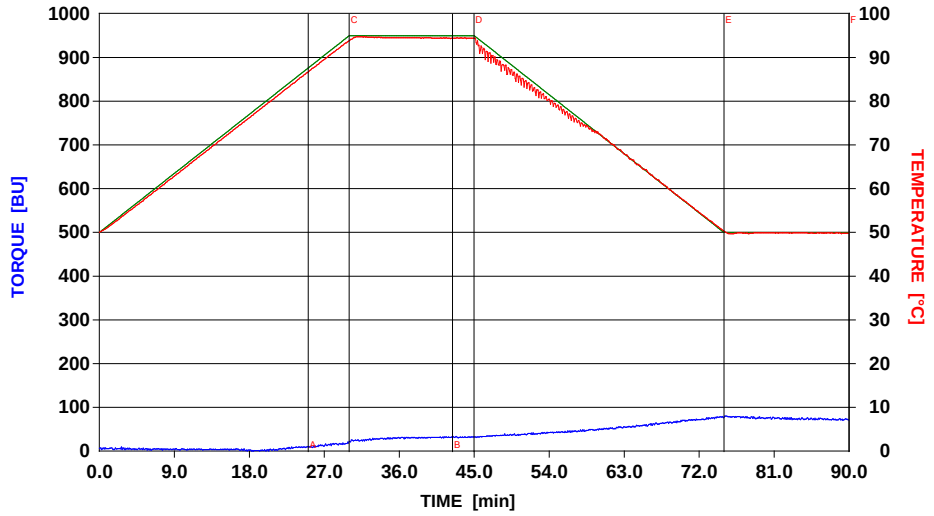
APPENDIX 6O: Cogelation at 70P:30S using 10.00kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	NII NORTEY		Date	:	7/29/2008	
Sample	:	10.00 KGY 70p:30s		Method	:	METHOD 1	
Moisture	:	9.44	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	37.9	[g]
Water	:	420	[ml]	Corr. to 14%	:	422.1	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:25:05	10	86.8
B	Maximum viscosity	00:42:25	34	94.5
C	Start of holding period	00:30:00	23	93.9
D	Start of cooling period	00:45:00	33	94.5
E	End of cooling period	01:15:00	78	50.4
F	End of final holding period	01:30:00	72	50.0
B-D	Breakdown		1	
E-D	Setback		44	

File : Measurement V: 2.3.16

APPENDIX 7 – ANALYSIS OF VARIANCE FOR PASTING PROPERTIES

APPENDIX 7A: Gelatinization temperatures at 30P:70S across irradiation doses

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	0.070	0.018	0.01	1.000
Residual	5	8.175	1.635		
Total	9	8.245			

APPENDIX 7B: Gelatinization temperature at 50P:50S across irradiation doses

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	460.49	115.12	1.52	0.325
Residual	5	379.33	75.87		
Total	9	839.82			

APPENDIX 7C: Gelatinization temperature at 70P:30S across irradiation doses

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	46.904	11.726	5.35	0.047
Residual	5	10.960	2.192		
Total	9	57.864			

APPENDIX 7D: Gelatinization temperatures within blends (30P:70S, 50P:50S, and 70P:30S) across irradiation doses

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments 1	4	114.16	28.54	1.07	0.405
Treatments 2	2	1240.27	620.13	23.26	<.001
TRTS 1. TRTS 2	8	393.31	49.16	1.84	0.146
Residual	15	399.91	26.66		
Total	29	2147.65			

APPENDIX 7E: Maximum viscosity within blends (30P:70S, 50P:50S, and 70P:30S) across irradiation doses

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments 1	4	1.12133	0.28033	6.95	0.002
Treatments 2	2	4.77800	2.38900	59.23	<.001
TRTS 1. TRTS 2	8	5.77867	0.72233	17.91	<.001
Residual	15	0.60500	0.04033		
Total	29	12.28300			

APPENDIX 7F: Maximum viscosity for 30P:70S blend

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	8598	2150	0.30	0.869
Residual	5	36306	7261		
Total	9	44905			

APPENDIX 7G: Maximum viscosity for 50P:50S blend

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	1697.6	424.4	3.13	0.121
Residual	5	678.0	135.6		
Total	9	2375.6			

APPENDIX 7H: Maximum viscosity for 70P:30S blend

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	8746.4	2186.6	12.37	0.008
Residual	5	884.0	176.8		
Total	9	9630.4			

APPENDIX 7I: Gel strength at the end of cooling period for 30P:70S blend

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	38152.	9538.	0.62	0.667
Residual	5	76638.	15328		
Total	9	114791			

APPENDIX 7J: Gel strength at the end of cooling period for 50P:50S blend

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	1556.6	389.1	1.77	0.271
Residual	5	1097.5	219.5		
Total	9	2654.1			

APPENDIX 7K: Gel strength at the end of cooling period for 70P:30S blend

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	15158.40	3789.60	41.92	<.001
Residual	5	452.00	90.40		
Total	9	15610.40			

APPENDIX 7L: Setback viscosity at 30P:70S

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	20777	5194	2.24	0.200
Residual	5	11610	2322		
Total	9	32386			

APPENDIX 7M: Setback viscosity at 50P:50S

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	788.00	197.00	23.18	0.002
Residual	5	42.500	8.50		
Total	9	830.50			

APPENDIX 7N: Setback viscosity at 70P:30S

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	2598.00	649.50	51.96	<.001
Residual	5	62.50	12.50		
Total	9	2660.50			

APPENDIX 8 – DATA ON THE PASTING CHARACTERISTICS OF PROTEIN-STARCH BLENDS

APPENDIX 8A: Gelatinization temperatures

BEGINNING OF GELATINIZATION TEMPERATURE (ºC)			
Irradiation dose (kGy)	PROTEIN-STARCH ADMIXTURES (%)		
	30P:70S	**50P:50S**	**70P:30S**
0.00	76.40(2.83)	78.10(0.00)	88.20(3.11)
2.50	76.20(0.00)	64.15(19.45)	86.60(1.13)
5.00	76.35(0.21)	63.80(0.00)	91.50(0.00)
7.50	76.45(0.35)	77.95(1.06)	85.10(0.00)
10.00	76.35(0.07)	77.40(0.28)	86.80(0.00)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 8B: Maximum viscosity temperatures

MAXIMUM VISCOSITY TEMPERATURE (ºC)			
Irradiation dose (kGy)	PROTEIN-STARCH ADMIXTURES (%)		
	30P:70S	**50P:50S**	**70P:30S**
0.00	94.40(0.00)	95.10(0.00)	94.50(0.42)
2.50	94.60(0.00)	95.35(0.35)	94.60(0.00)
5.00	94.60(0.00)	95.60(0.00)	92.70(0.00)
7.50	94.60(0.00)	94.60(0.00)	94.60(0.00)
10.00	94.60(0.00)	94.95(0.64)	94.50(0.00)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 8C: Maximum viscosity

MAXIMUM VISCOSITY (BU)			
Irradiation dose (kGy)	PROTEIN-STARCH ADMIXTURES (%)		
	30P:70S	**50P:50S**	**70P:30S**
0.00	541.00(5.66)	314.00(0.00)	67.50(34.65)
2.50	557.00(0.00)	288.00(1.41)	85.40(0.57)
5.00	575.00(7.07)	278.00(0.00)	57.00(0.00)
7.50	620.00(189.50)	279.00(9.90)	5.00(0.00)
10.00	541.50(17.68)	292.00(24.04)	34.00(0.00)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 8D: Gel strength at the start of holding period

GEL STRENGTH AT START OF HOLDING PERIOD			
Irradiation dose (kGy)	PROTEIN-STARCH ADMIXTURES (%)		
	30P:70S	**50P:50S**	**70P:30S**
0.00	618.50(9.19)	311.00(0.00)	105.50(0.00)
2.50	522.00(0.00)	277.00(1.41)	54.00(19.80)
5.00	479.00(31.11)	271.00(0.00)	24.00(0.00)
7.50	471.00(9.51)	270.50(14.85)	26.75(0.35)
10.00	461.00(0.00)	274.50(23.00)	23.00(0.00)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 8E: Gel strength at the start of cooling period

GEL STRENGTH AT START OF COOLING PERIOD			
Irradiation dose (kGy)	PROTEIN-STARCH ADMIXTURES (%)		
	30P:70S	**50P:50S**	**70P:30S**
0.00	532.50(0.00)	272.00(0.00)	70.50(0.71)
2.50	554.00(0.00)	253.00(1.41)	61.50(30.41)
5.00	575.50(7.78)	255.00(0.00)	39.00(0.00)
7.50	620.50(19.21)	254.50(6.36)	2.00(0.00)
10.00	541.00(16.97)	269.50(21.92)	33.00(0.00)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 8F: Gel strength at the end of cooling period

GEL STRENGTH at END OF COOLING PERIOD (BU)			
Irradiation dose (kGy)	PROTEIN-STARCH ADMIXTURES (%)		
	30P:70S	**50P:50S**	**70P:30S**
0.00	862.00(0.00	376.00(0.00)	126.00(1.41)
2.50	840.00(0.00)	356.50(10.61)	109.00(21.21)
5.00	974.00(80.61)	344.00(0.00)	82.00(0.00)
7.50	997.50(258.09)	340.50(7.78)	12.00(0.00)
10.00	896.00(59.40)	351.50(30.41)	78.00(0.00)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 8G: Gel strength at the end of final holding period

GEL STRENGTH AT END OF FINAL HOLDING PERIOD			
Irradiation dose (kGy)	PROTEIN-STARCH ADMIXTURES (%)		
	30P:70S	**50P:50S**	**70P:30S**
0.00	877.00(1.77)	349.00(0.00)	118.50(3.54)
2.50	821.00(0.00)	328.00(8.49)	99.00(24.04)
5.00	949.00(63.64)	323.00(0.00)	75.00(0.00)
7.50	943.50(21.01)	315.00(14.14)	10.00(0.00)
10.00	882.00(55.15)	331.50(30.41)	72.00(0.00)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 8H: Breakdown viscosity

BREAKDOWN VISCOSITY			
Irradiation dose (kGy)	PROTEIN-STARCH ADMIXTURES (%)		
	30P:70S	**50P:50S**	**70P:30S**
0.00	4.00(0.00)	42.00(0.00)	1.00(0.00)
2.50	4.00(0.00)	37.00(4.24)	2.00(1.41)
5.00	1.00(1.41)	21.00(0.00)	17.00(0.00)
7.50	0.00(0.00)	24.00(16.97)	1.00(0.00)
10.00	2.50(0.75)	21.50(2.12)	1.00(0.00)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 8I: Setback viscosities

SETBACK VISCOSITY (BU)			
Irradiation dose (kGy)	PROTEIN-STARCH ADMIXTURES (%)		
	30P:70S	**50P:50S**	**70P:30S**
0.00	288.50(2.12)	104.00(0.00)	57.00(1.41)
2.50	286.00(0.00)	104.50(6.36)	45.50(7.78)
5.00	397.50(71.42)	88.00(0.00)	42.00(0.00)
7.50	375.50(68.59)	84.00(1.41)	9.00(0.00)
10.00	356.00(42.43)	87.00(0.00)	44.00(0.00)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 9 – CORRELATION STUDIES ON FUNCTIONAL PROPERTIES OF PROTEINS, AND PASTING CHARACTERISTICS OF PROTEIN-STARCH BLENDS

APPENDIX 9A: Correlation Coefficient *(r)* Of Gel Strength Beginning of Gelatinization with Decreasing Starch and Increasing Proteins across Irradiation Doses

Gelatinization temperature (°C)					
Irradiated proteins within blends (kGy)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
	Correlation coefficient (***r***)				
Decreasing Starch % 70→50→30	-0.92*	-0.46	-0.55	-0.94*	-0.91*
Increasing Protein % 30→50→70	0.92*	0.46	0.55	0.94*	0.91*

Values with asterisks (*) are significantly correlated (p<0.05)

APPENDIX 9B: Correlation Coefficient *(r)* of Maximum Viscosity with Decreasing Starch and Increasing Proteins across Irradiation Doses

Maximum Viscosity					
Irradiated proteins within blends (kGy)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
	Correlation coefficient (***r***)				
Decreasing Starch % 70→50→30	1.00	1.00	1.00	1.00	1.00
Increasing Protein % 30→50→70	-1.00	-1.00	-1.00	-1.00	-1.00

APPENDIX 9C: Correlation Coefficient *(r)* of Gel Strength Start of holding Period with Decreasing Starch and Increasing Proteins Across Irradiation Doses

Gel Strength at start of holding period					
Irradiated proteins within blends (kGy)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
	Correlation coefficient (***r***)				
Decreasing Starch % 70→50→30	0.99	1.00	1.00	1.00	1.00
Increasing Protein % 30→50→70	-0.99	-1.00	-1.00	-1.00	-1.00

APPENDIX 9D: Correlation Coefficient *(r)* of Gel Strength start of cooling period with Decreasing Starch and Increasing Proteins across Irradiation Doses

Gel Strength at start of cooling period					
Irradiated proteins within blends (kGy)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
	Correlation coefficient (***r***)				
Decreasing Starch % 70→50→30	1.00	0.99	0.99	0.99	1.00
Increasing Protein % 30→50→70	-1.00	-0.99	-0.99	-0.99	-1.00

APPENDIX 9E: Correlation Coefficient *(r)* of Gel Strength at end of cooling Period with Decreasing Starch and Increasing Proteins across Irradiation Doses

Gel Strength at End Of Cooling Period					
Irradiated proteins within blends (kGy)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
	Correlation coefficient (***r***)				
Decreasing Starch % 70→50→30	0.98	0.98	0.97	0.98	0.98
Increasing Protein % 30→50→70	-0.98	-0.98	-0.97	-0.98	-0.98

APPENDIX 9F: Correlation Coefficient *(R)* of Gel Strength End of Final Holding Period With Decreasing Starch and Increasing Proteins across Irradiation Doses

Gel Strength at end of final holding period					
Irradiated proteins within blends (kGy)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
	Correlation coefficient (***r***)				
Decreasing Starch % 70→50→30	0.98	0.98	0.97	0.98	0.98
Increasing Protein % 30→50→70	-0.98	-0.98	-0.97	-0.98	-0.98

APPENDIX 9G: Correlation Coefficient *(r)* of Breakdown Viscosity with Decreasing Starch and Increasing Proteins across Irradiation Doses

Breakdown Viscosity					
Irradiated proteins within blends (kGy)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
	Correlation coefficient (***r***)				
Decreasing Starch % 70→50→30	0.07	0.05	-0.76*	-0.04	0.07
Increasing Protein % 30→50→70	-0.07	-0.05	0.76*	0.04	-0.07

Values with asterisks (*) are significantly correlated ($p<0.05$)

APPENDIX 9H: Correlation Coefficient *(r)* of Setback Viscosity with Decreasing Starch and Increasing Proteins across Irradiation Doses

Setback Viscosity					
Irradiated proteins within blends (kGy)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
	Correlation coefficient (***r***)				
Decreasing Starch % 70→50→30	0.96	0.96	0.92	0.95	0.92
Increasing Protein % 30→50→70	-0.96	-0.96	-0.92	-0.95	-0.92

CERTIFIED AS THE ORIGINAL WORK OF THE AUTHOR
MISS PAMELA AKU NUNOO

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CHAPTER ONE

1.0 Introduction

Legume seeds are of prime importance in human and animal nutrition due to their high protein content (20–50%) which is twice the level found in cereal grains and significantly more than the level found in their root crop counterparts (*Ustimenko-Bakumovsky, 1983*). Dry legumes are important ingredients of diet in many parts of the world and have been considered as the most significant food sources for people of low incomes (*Bressani and Elias, 1979*).

Legumes have historically been utilized mainly as whole seeds but in recent years, interest has grown in their utilization in other forms like flours, protein concentrates and protein isolates (*Saio, 1993; Doxastakis, 2000*). Plant proteins have been widely used in foods as functional ingredients to improve their stability and texture as well as their nutritional quality. Nevertheless, these applications in the food trade are almost limited to protein from soybean seeds, whereas other vegetable proteins are less used (*Makri et al., 2005*). Among these underutilized legumes are the lupins, peas and the beans of which the Bambara groundnut is part (*De Miguel Gordillo, 1991*). In spite of their underutilization, their high protein and their well-balanced amino-acid composition make these neglected and underutilized legumes important sources of protein, with potential to add in various products as novel food ingredients (*Alamanou et al., 1996*).

Various procedures or methods, including irradiation (*Abu et al., 2005*), physicochemical hydrolysis (*Fontana et al., 2004*; *Martinez et al., 2006),* and heat-moisture treatment (*Singh et al., 2005*) have been used to enhance some surface functional properties and pasting properties of flours and pastes obtained from various legume. However,

procedures which have been employed for the purposes of modifying plant proteins with the aim of enhancing and tailoring their food functional applications in their respective multi-component food systems may possibly be faced with some limitations of accuracy, sensitivity, specificity, toxicity, and in some cases equipment availability and efficiency, time constraint and cost, among others.

Food is submitted to gamma irradiation for various purposes. Among the several benefits, a food or an ingredient is irradiated to assure a physical or chemical change in such a way that a specific characteristic could be improved or its processability is facilitated. Gamma irradiation like other ionizing radiations, through the production of free radicals, can affect proteins by promoting covalent inter- and intra-molecular cross-links which may result in protein-protein association, deamination, scission of polypeptide chains and disulphide bonds, exposure of polar and non-polar protein sites or by association of aromatic and heterocyclic residues (*Simic, 1978*; *Urbain, 1986 and Cho et al., 1999*).

Gamma irradiation has been used to cross-link biodegradable films from whey, casein and soy proteins (*Lacroix et al., 2002*) and aided the digestibility of some legume flours (*Dario and Salgado, 1994*). Similarly, emulsion, foam, water and oil absorption capacities were affected by gamma irradiation in peanut flour (*Rahma and Mostafa, 1988)*. Protein solubility in soy (*Byun and Kang, 1995; Hafez et al., 1995*) and in red kidney beans (*Dogbevi et al., 1999*) have also been affected by gamma irradiation while decreases in swelling properties following gamma irradiation of cowpea starch (*Abu et al., 2004*) have been documented. Reports suggest that irradiation of cowpea flours and pastes at medium to high doses has resulted in significant changes in protein-related

functional properties (*Abu et al., 2004*), and studies conducted by Lee *et al*. (2004) also showed the disruption of the ordered structure of the soy protein molecules following irradiation of the protein isolates.

Various attempts have been made however to cross-link protein and starches to form protein-starch networks with improved synergistic food functionality. An example is a research conducted by Köber *et al*. (2004) to modify the water absorption capacity of a plastic based on bean protein using gamma irradiated starches as additives. Many more researchers have also used chemical and enzymatic modifications to improve the functionality of food proteins. Particularly, Ohtsuka *et al*. (2003) induced gelation of soy proteins using recombinant microbial transglutaminase, while Arntfield (2007) improved gelation of canola proteins through limited proteolysis with trypsin, ficin and bromelin either alone or in combination with the cross-linking enzyme, transglutaminase. Reports from Malhortra and Coupland (2004) have also shown the effect of surfactants on the solubility, zeta potential, and viscosity of soy proteins for food application.

1.1 Statement of the Problem

Perusal of literature reveals that although the effects of various modification processes on functionality have been studied extensively for soybean proteins, little work has been done on the less popular or neglected and underutilized legumes such as the Bambara groundnut. It will thus be proper to understand the surface functional properties of such less popular proteins when they are subjected to similar treatment as the soybean protein isolates. It will also be possible to understand the synergistic properties of the irradiated proteins-native starch admixtures of the Bambara groundnut.

1.2 Justification of Work

Due to the fact that crude proteins from the Bambara groundnut show reduced solubility leading to poor emulsion and foaming properties when used for food application as reported by Kato and Nakai (1980), it is unable to compete with others like the soy proteins for specific food applications. There is therefore the need for modification of the Bambara groundnut proteins to enhance functionality and refocus their use. This work would provide adequate information about the effects of irradiation on the functional performance of these protein isolates and their synergistic effect with the native starch on the pasting characteristics when used in food formulations.

1.3 Objectives

The main aim was to physically modify proteins extracted from the Bambara groundnut using gamma irradiation and study their respective food functional properties.

The specific objectives were;

- To evaluate the surface functional properties of gamma-irradiated Bambara groundnut proteins.
- To evaluate the performance of the irradiated Bambara groundnut proteins in a modeled starch system using the Brabender viscoamylograph.

CHAPTER TWO

2.0 Literature Review

2.1 An Overview of World Legumes

Legumes are second only to the grasses in their importance to humans and their domestic animals (*Seigler, 2005*). Legumes come in three varieties, namely, grain legumes (the Bambara groundnuts, Peas, Faba beans, Lupins, Chickpeas, and Lentils), perennial herbaceous legumes (Clover and Alfalfa) and legume trees (Robinia, Leucaena, and *Calliandra sp*.).

Globally, both the grain and forage legumes are grown on some 180 million hectares of land, or some 12% to 15% of the Earth's arable land (*Graham and Vance, 2003*). Grain legumes, commonly referred to as beans, have pods with seeds inside them and they account for 27% of the world's primary crop production, with grain legumes (e.g. Soybeans, Cowpea, Jack beans, Lima beans etc.) alone contributing 33% of the dietary protein needs of humans. They are an important component of the agricultural and food systems throughout the world, in that they complement cereal crops in dietary terms, as sources of proteins and minerals, showing two or more times the levels found in most cereals (*Seigler, 2005*).

2.2 Nutraceutical Benefits of Legumes

Legumes are generally an inexpensive source of proteins. Seeds of grain legumes contain at least 20% to 40% protein. They may also possess many desirable characteristics, including an abundance of carbohydrates, low fat (generally containing 5% of energy as fat except oilseeds, examples being chickpeas and soybeans), and high concentration of polyunsaturated fatty acids with α-linolenic acid making up about 7–8% of the total fat (*Human Nutrition Information Service, 1998*). In addition to B complex vitamins and minerals like iron, legumes are also a major source of fibre. The legume seeds which are green in the pod also contain a lot of vitamins A and C (*Rockland and Nishi, 1979*) and they generally have a long shelf life. Others, like the soy bean, are also known to contain certain bioactive compounds whose beneficial effects, such as the ability to lower the serum cholesterol *(Sridhar and Seena, 2005*), need to be explored for medicinal purposes. Recent research has revealed that some 'anti-aging' agents and antioxidants can be found in the bean seed coat. Research continues to reveal new attributes of beans; it has also been shown that beans have a perfect nutrient base for people interested in weight loss, while they also aid in reducing cholesterol, improving digestion and, as already mentioned, aid in cancer prevention (*Eborn, 2001*).

2.3 The Downside of Legumes

Typically, legumes have been associated with flatulence. They cause flatulence when they are digested by leaving some oligosaccharides that the human intestinal microflora cannot digest (*Sridhar and Seena, 2005*). These oligosaccharides then serve as food for the bacteria, resulting in an increase in their metabolic activities, and methane and

hydrogen sulfide gas production which causes the flatulence. However, boiling in water and sprouting of legume seeds decreases oligosaccharide content, and thus reduces flatulence significantly (*Sridhar and Seena, 2005*). Reports from Bösterling and Quast (1981) have also shown slowed digestion of the Bambara groundnut as a result of the presence of trypsin inhibitors while Kato and Nakai (1980) have also reported reduced solubility of its crude proteins which leads to poor emulsion and foaming properties when used for food application.

2.4 The Bambara groundnut

The Bambara groundnut, *Vigna subterranea*, commonly referred to as the Bambara beans is a herbaceous, intermediate, annual plant, with creeping stems at ground level. The pods usually develop underground, and may reach up to 3.7 cm, depending on the number of seeds they contain. Mature pods are indehiscent, often wrinkled, ranging from a yellowish to a reddish dark brown colour. Seed colour also varies, from white to cream, yellow, brown, purple, red or black (*Karikari, 1971*).

2.5 A Complete Nutritional Profile of the Bambara groundnut

The Bambara groundnuts have a carbohydrate content of approximately 54.5-69.3%, a proteins-content of 17.0-24.6% with levels of the essential sulphur-containing amino acid, methionine, higher than that found in most other legumes (*Linnemann, 1990; Brough and Azam-Ali, 1993*), a fats-content of 5.3-7.8%, and calories of about 36-414 Kcal per 100g. The beans are also a good source of fibre, calcium, iron and potassium and have the potential for providing a balanced diet in areas where animal protein is

expensive and the cultivation of other legumes is economically risky due to unfavourable environmental conditions. The red-coloured type of the beans could be useful in areas where iron deficiency is a problem as they contain almost twice as much iron as the cream-coloured (*de Kock, 2004*).

2.6 Traditional Uses of the Bambara groundnuts

The Bambara groundnut is eaten either boiled fresh or grilled while still immature. In Ghana, for instance, the fresh beans are boiled with a little pepper and salt, and sugar to taste sometimes, and served with fried ripe plantain, usually as lunch. The beans used to be canned in gravy by the GIHOC cannery-Nsawam, Ghana (*Karikari, 1971; Begemann, 1986*).

In Côte d'Ivoire, the beans are milled into flour with the aim of enhancing digestibility and used to bake small flat cakes or biscuits; while in some parts of East Africa, they are roasted, pulverized, and used to make soup (*Heller et al., 1995*), with or without other condiments. Bambara groundnut flour bread has been reported in Zambia as well (*Linnemann, 1987)* and many local foods, such as 'Akara', 'moin-moin' and 'okpa' which are common Nigerian varieties are made from ground Bambara groundnut (*Obizoba, 1983*).

A trial of Bambara groundnut milk was carried out which compared its flavour and composition with milks prepared from the "superior legumes" - soybean, cowpea, and pigeon pea (*Brough et al., 1993*). The Bambara groundnut milk was ranked first, but the other milks were more familiar as they were already on the market and therefore more

readily acceptable. However, the lighter colour of the Bambara groundnut milk made it somewhat more preferable during the trial (*Brough et al., 1993*).

2.7 Enhancement of Protein Functionality – A Current Trend in Food Technology

2.7.1 Novel food products

Perusal of literature seem to suggest that many past developments of fabricated foods may have been as a result of inspired creativity and trial and error manipulation of ingredients with little understanding of the underlying science. Current developments within the Food Science world however, have enabled the formulation of novel food products which have served their purposes in the consumer world. One such excellent endeavor which has been very beneficial to vegetarians and most health-conscious consumers is the simulation of the texture of meat using other sources of proteins, particularly soy proteins (*Kinsella, 1982*). Another is the production of a new biodegradable plastic material made of a protein matrix and starch which have been extracted from a bean variety (*Köber et al., 2007*).

2.7.2 Enzymatic modification

More specific modification methods to facilitate food protein use in nutritional, medical, and cosmetic applications include proteolytic treatments to produce protein hydrolysates with a degree of hydrolysis of less than 10% (limited proteolysis). Such biological modification methods may result in improved functional properties (*Mannheim and Cheryan, 1992; Sule, Tomoskozi, and Hajos, 1998*). Pedroche *et al.* (2004) enhanced some functional properties of protein isolates from *Brassica carinata* seeds using this

type of protein hydrolysis. The protein hydrolysate they obtained using 0.72AU of alcalase/g protein had a very good solubility in a wide range of pH, from 2 to 12, and high fat absorption capacities due to exposure of hydrophobic groups during the proteolytic process.

Similarly, Ruiz-Henestrosa *et al.* (2008) studied the effect of limited enzymatic hydrolysis on the interfacial and foaming characteristics of a soy globulin (glycinin, fraction 11S). Varying the degree of hydrolysis and soy protein concentrations, results revealed that hydrolysates with the low degree of hydrolysis had improved foaming capacities and stabilities, especially at pH close to the isoelectric point (pI).

The water insoluble gluten of wheat is one of the major limitations for its more extensive use in food processing. However, in a research conducted by Kong (2007), wheat gluten was enzymatically hydrolyzed by several commercially available proteases and results showed a remarkable increase in both emulsifying and foaming properties compared to the original gluten. On a whole, this biological method of modification, although specific and accurate, may be tedious and expensive.

2.7.3 Chemical modification

Krause (2001) also exhaustively modified protein isolates from rapeseed using three chemical modification procedures; acetylation, succinylation and phosphorylation and consequently studied the protein solubility, adsorption kinetics, surface pressure and surface potential of monolayer, wetting and foaming properties. Results from his study suggested that the chemical modifications distinctly increased the solubility and the foam capacity and foam expansion of the rapeseed protein within alkaline pH range.

Acetylation and phosphorylation specifically increased the reduction of the surface tension and increased foam stability whereas an overall improved pressure transformation and increase in surface potential in monolayer was observed after all three chemical modification procedures had been administered. The greatest changes were however achieved after succinylation.

Chemical modification is a very effective tool to investigate the structure-function relationship of proteins, but only useful in producing tailored proteins for non-food applications (*Kinsella and Shetty, 1979*).

2.7.4 Physical modification

Various studies using physical methods such as gamma irradiation as a preservation and functional modification tool in many food systems have been done. Examples of such food systems include cowpea flours and pastes (*Abu et al., 2005*), soy protein isolate films (*Lee et al., 2004*) and starch extrudates of maize (*Sokhey and Chinnaswamy, 1992*). Specifically, cowpea flours and pastes were irradiated at 2, 10 and 50 kGy and analyzed for their functional properties by Abu *et al.* (2005). Results showed that most of the protein-related functional properties of cowpea flours and pastes were not affected at low dose irradiation (2 kGy). At 10 and 50 kGy, however, all protein-related functional properties, except for water absorption capacity, were significantly ($p<0.05$) affected. Nitrogen solubility index decreased significantly ($p<0.05$), probably due to protein denaturation and/or aggregation, whereas oil absorption capacity increased significantly ($p<0.05$), possibly due to exposure of previously buried non-polar protein sites. Starch-

related functional properties, such as swelling and pasting properties, also decreased significantly ($p<0.05$) in a dose-dependent manner, most likely due to starch degradation.

A similar work done by Al-Kaisey *et al.* (2003) to determine the effect of cobalt-60 gamma irradiation on the antinutritional factors in broad bean showed that trypsin inhibitor activity was reduced by 4.5%, 6.7%, 8.5% and 9.2% at 2.5, 5.0, 7.5 and 10.0 kGy, respectively. Meanwhile, irradiation at 10.2, 12.3, 15.4 and 18.2 kGy reduced the phytic acid content. The flatulence-causing oligosaccharides were also decreased significantly ($p<0.05$) as the radiation dose increased.

Some researchers (*Bhattachary and Jena, 2007*) have used microwave treatment to alter gelling behaviours of defatted soybean flour dispersions. Defatted soybean flour dispersions of different solid concentrations (15–30%) were subjected to varying times of heating between15–75 seconds in a microwave and gels were characterized by texture measurement (penetration), dynamic testing (oscillation) and by sensory assessment. Results from this work showed that the gel strength, storage modulus, and complex viscosity of the legume flour were enhanced with increasing solid concentration and/or heating time. Others (*Hua et al., 2005*) have also used heat up to 95ºC for 15 minutes to form thermal-induced soy protein gels with improved functionality.

2.7.5 Effects of extraction techniques and conditions

Various studies have also been carried out with the aim of determining the effects of various extraction techniques and extraction conditions on the functional properties of protein isolates. The functional properties of pigeon pea and cowpea protein isolates were determined as a function of extraction technique and pH conditions of the extraction

medium by Mwasaru *et al.* (1996). The isolates extracted using the micellization technique showed significantly ($p<0.05$) higher solubility than those extracted using the isoelectric point precipitation technique and, for the latter, solubility was negatively correlated with the extraction pH. Results again indicated that the micellization technique of protein extraction gave higher oil absorption capacities and emulsifying activities than the isoelectric point precipitation technique for both cowpea and pigeon pea protein isolates. However, the micellization technique gave a lower foam expansion for pigeon pea isolates as against the higher foam expansion of the cowpea isolates, both compared to isolates obtained from the alternative technique of extraction.

Other works by Mwasaru *et al.* (2000) again showed the influence of altered solvent environment on the functionality of pigeon pea (*Cajanus cajan*) and cowpea (*Vigna unguiculata*) protein isolates. Functionality was determined as a function of pH and NaCl concentrations in this study. At low pH, nitrogen solubility decreased with increasing NaCl concentration but at high pH, it increased. However, addition of NaCl to the solvent medium resulted in a marginal improvement and a significant improvement in the emulsifying activity and emulsion stability of the pigeon pea isolate, respectively, but the same treatment decreased these properties for the cowpea isolates. It was clear that varying both the pH and NaCl concentrations resulted in significant improvements in the emulsifying and foaming properties as well as the least gelation concentration of the isolates relative to the control treatment. Most food preparations involve solvent environments that contain salt and pH in the range 4 to 8, therefore such a study is particularly relevant especially within the domain of food product development. Some potential applications may include the protein fortification of fruit juices, fermented milk

products, and fermented vegetables, in which the natural acidic pH will enhance protein solubility.

2.8 Co-gelation

Reports from Lin (1977) have indicated that some types of proteins can form gels when heated together with other proteins or with polysaccharide gelling agents, such as starch, pectin, carrageenan, and alginates. For example, the non-specific ionic interactions between positively charged gelatins and the negatively charged alginates or pectates produce gels with relatively high melting point of about 80ºC. In much the same way, specific ionic interactions are known to take place between the positively charged site of K-casein and polysulphated K-carrageenan at the pH of natural milk, making casein micelles entrapped in K-carrageenan gels.

Protein-polysaccharide interactions particularly at air/water interfaces have also been studied by Martinez *et al.* (2006). The aim of this study was to gain knowledge on the interaction between the two biopolymers at the air/water interface under dynamic conditions of neutral pH, where limited incompatibility between macromolecules can occur. Having evaluated the rheological properties of soy protein-polysaccharide films with a drop tensiometer, it was clear that the presence of hydroxypropylmethylcellulose and lambda carrageenan used as the surface active and non-surface active polysaccharide systems respectively, greatly increased the surface pressure, surface dilatational elasticity and relative viscoelasticity of the films.

In other studies conducted by Makri *et al.* (2005), native lupin, pea, and broad bean protein isolates were used as emulsifiers and stabilizers, in admixture with

polysaccharides (xanthan gum), in order to create a rigid and viscoelastic film around the oil or air droplets and/or for creation of network structure, with desirable texture for the benefits of the consumer. Results indicated that the addition of incompatible xanthan gum enhanced protein adsorption at air/water and oil/water interfaces, increasing their emulsion and foam stabilities. The reason for this observation may be due to xanthan gum's ability to increase the viscosity of the continuous phase, and also to its effect on protein adsorption at the interfaces.

The extent and type of change on a food or food ingredient is largely dependent on the type of modification process administered to it. Ultimately the choice of a modified food protein or any other food biopolymer as an ingredient in any food system would be largely dictated by its functional properties. Hopefully proteins from cheap vegetable sources such as the Bambara groundnut could be suitably modified in much the same way to mimic the egg proteins in many food products.

2.9 The Use of Unconventional Food Materials in New Product Development

Technology has always allowed us to obtain new products or develop new applications for existing agricultural raw materials. Trends in research have shown clearly that it was not until the last quarter of the century that food stuffs of exclusively industrial origin made their appearance, and the indications are that more of such products may appear on the market in the not too distant future. Good examples of such developments currently are the appearance of margarine from plant other than animal sources, and confectionery sugar derived from cereals other than the usual sugar cane and beetroot. The exotic single and double tomato concentrate, commonly referred to as tomato ketchup, is also an

example. Others include: the highly nutritious pasta which has been developed with added protein isolates and concentrate as their functional ingredients; the high protein fruit pie; and the addition of proteins to effervescent drinks. Such innovations are gradually gaining acceptance as young consumers continue to adapt to and embrace new kinds of foods and drinks.

Since some of these products involve new applications of existing and commonly used proteins, it is worth investigating the possible utilization of unconventional proteins in much the same way. It is possible that the new food products obtained from these unconventional proteins can compete favourably with their already existing counterparts on the world food market, judging from the advances in research in the area of sourcing for new biomaterials for food applications. This would help address the current global food shortages, augment the global protein resource base to meet the world's current high demand for more protein-based foods, and ultimately alleviate poverty, especially, in Africa. The relative price levels, dietetic qualities, physical and functional properties, taste attributes, and the ease of use should be the main focus for the use of these unconventional protein sources. Their future market will probably be found in a whole range of products yet unknown.

CHAPTER THREE

3.0 Materials and Methods

3.1 Source of Bambara groundnuts

Two 50 kg bags each of one variety of Bambara groundnuts (*Vigna subterranea* (L.) Verdc.) were obtained from the Crop Research Institute of the Plant Generic Resource Unit, Bunso, Ghana. The beans were sorted and solar-dried (to 12% moisture content) for a minimum of three days, milled and stored in plastic bags for further analysis.

3.2 Milling and Defatting of dried Bambara groundnuts into flour

The dried Bambara groundnuts were milled into fine powder comprising 8.42% moisture, 8.97% fat, 22.10% protein, 3.80% ash, 3.84% fibre and 52.87% carbohydrates. Defatting of the seed meal was done using petroleum spirit in a ratio of seed meal-to-solvent, 1:10 w/v, in a large scale Soxhlet's extractor. The defatted meal was spread on trays and solar-dried for about two to three hours to expel the volatile extraction solvent and stored in plastic bags for further analysis.

3.3 Protein Extraction from Defatted Bambara groundnut meal

A total of 1.477 kg (15.98%) of isolated proteins on dry weight basis was extracted from, 9.2 kg of dried defatted Bambara groundnut flour. Alkaline extraction of protein from the dried de-fatted meal was done using 0.01M NaOH with a meal-to-solvent ratio of 1:10 w/v, agitated at 150 rpm at room temperature for two hours, using G24 Environmental incubator shaker. Proteins and oligosaccharides entered into solution while the insoluble polysaccharides and residues were removed by centrifuging at 2500rpm for 20 minutes.

Supernatant obtained after centrifugation was acidified to a pH within the range of 4.5-5.0 to allow protein precipitation. The resulting mixture was then centrifuged at 3000rpm for 20 minutes to separate the proteins from the soluble polysaccharides. The centrifugation process was repeated thrice for any suspended precipitated proteins to dissolve in distilled water, after which the recovered proteins were washed and freeze-dried using the HETO POWER DRY LL300 freeze dryer and kjeldahl analysis run (%N × 6.25) to obtain 94% protein.

3.4 Starch Extraction from Dried Defatted Bambara groundnut Flour

Starch was extracted from the solid fibrous portion obtained after the very first round of centrifugation. This portion constituted the insoluble polysaccharide including cellulose and starch. Pure starch was then obtained by repeated washing. Pure starch particles settled after the filtrate was allowed to stand and the recovered starch was solar-dried for about four to five hours to a moisture content of, approximately, 10.440%.

3.5 Irradiation of Isolated Proteins

The initial total amount of 1.477 kg of freeze dried proteins was divided into five portions (samples), each weighing 295.41 g were tightly sealed in transparent polyethylene bags and labeled according to radiation doses. Each of the five samples was then placed in the inner region of the irradiation chamber which was calibrated with the Fricke Dosimetry System. With the Fricke Dosimetry, irradiated Fricke solution’s optical density was measured in a quartz cuvette (1 cm path length) with a UV-VIS (150-800 nm) Beckman Coulter DU 640 spectrophotometer (EUA), and the temperature was measured with

Eutechnics digital thermometer Moodel 4400 (EUA). The Fricke solution itself was synthesized with 0.392 g of hexahydrated ferrous-ammonium sulfate ammonium P.A. (10^{-3} MERCK), 0.060 g of sodium chloride P.A. (10^{-3} MERCK), and 22 mL of concentrated sulfuric acid P.A. (MERCK) diluted in Milli-Q (18.2 MΩ.cm) water in a 1000 mL volumetric balloon (corresponding to a total mass of 1022.70 g). Once the solution was ready, 3.5 mL aliquots were transferred to several 6-mL sterilized vacutainer plastic vials and placed within the inner region of the ionization chamber. The irradiation chamber was positioned on a Plexiglas simulator with the centre of the entrance window perpendicular to the radiation beam's central axis. Finally the measuring system was irradiated with standard radiation qualities recommended by the DIN 6809. The dose absorbed in the Fricke solution in grays (Gy) was obtained from the average of five readings, and an appropriate formula used to calibrate the radiation chamber. Samples were then exposed to 2.5, 5.0, 7.5 and 10.0 kGy of ^{60}Co gamma radiation at 1.63 $kGyh^{-1}$ at 20.00 °C and 101.33 kPa, in a one factor design. The non-irradiated sample (0.00 kGy) was set as control in the subsequent tests.

3.6. Determination of Moisture Content of Samples

The hydration state of the sample may affect the final changes that might occur in food sample after the irradiation process, with respect to its functionality. Therefore the moisture content of samples was therefore determined by the AOAC (1990) approved method to establish the extent to which the samples were hydrated. Five grams of each protein sample, as well as the isolated starch, were measured into separate crucibles, and their respective gross weights taken. These were then placed in the oven at 150°C for 24

hours. The moisture content was expressed as percentage loss in weight of sample. The process was repeated for proteins after irradiation (*Simic, 1978*).

3.7 Solubility Profile of Samples

The solubility of each irradiated sample was determined as a function of pH. Such factors as buffer pH, weight of sample, slurry concentration of sample and vortex time, were the variables used to generate a composite design for optimum conditions for the solubility of the irradiated protein samples from the face-centered option of central composite design of the response surface methodology (*Design Expert, 2007*). Protein solutions were then centrifuged at 3000xg for 20 minutes, after vortexing for appropriate time under the specific conditions of buffer pH and slurry concentrations. The filtrates containing soluble proteins were then decanted into separate centrifuge tubes, labeled accordingly and stored.

The optimum conditions with their corresponding maximum solubility for each irradiated Bambara groundnut protein sample were determined using Bradford's reagent. This was done by reading the absorbance of the various protein-Bradfords reagent solutions at 595.0 nm with the Helios Gamma Spectrophotometer, and an appropriate formula used to calculate for protein solubility in g/ml.

3.8 Water Absorption Capacity (WAC)

Water absorption capacity was estimated by the method described by Wang and Kinsella (1976) with modification. One gram each of both the irradiated and the non-irradiated samples was suspended in 10ml distilled water in 15ml graduated centrifuge tubes and

the weights taken before and after addition of distilled water. Samples were then shaken for 30 minutes and centrifuged at 2500rpm for 25 minutes at room temperature. The freed water was carefully decanted and the weight of test tube plus the content was taken. The density of water was taken as 1.0 g/cm^3. Water absorbed was calculated as the difference between the initial volume of water added to the sample and the volume of the supernatant and WAC was expressed as the volume of water retained by one gram of the sample.

3.9 Oil Absorption Capacity (OAC)

The oil absorption capacity was measured by the method described by Wang and Kinsella (1976) and also with modification. One gram each of both the irradiated and the non-irradiated samples was suspended in 10ml vegetable oil in 15ml graduated centrifuge tubes and the weights taken before and after addition of oil. Samples were then shaken for 30 minutes and centrifuged at 2500 rpm for 25 minutes at room temperature. The freed oil was carefully decanted and the weight of test tube plus the content was taken. Oil absorbed was calculated as the difference between the initial volume of oil added to the sample and the volume of the supernatant. Oil absorption capacity was expressed as the volume of oil retained by one gram of the sample with the density of oil 0.890 g/cm^3.

3.10 Emulsifying Activity (EA) and Emulsifying Stability (ES)

The procedure described by Volker and Kelin (1979) was used for both emulsifying activity and emulsifying stability. Emulsions were prepared with one gram of each protein sample, 50ml distilled water and 50ml vegetable oil. The mixtures were

homogenized thoroughly at room temperature for 30 minutes. Each emulsified sample was divided equally into 50ml centrifuge tubes. Content of one 50ml tube was centrifuged directly at 3000xg for 30minutes while the other centrifuged under the same conditions after heating in a water bath at 80°C for 30 minutes and cooling to 15°C. The heights of the emulsified layers, as a percentage of the total height of the material in the unheated tubes was used to calculate the emulsifying activity and emulsifying stability using appropriate formulae.

3.11 Foaming Capacity (FC)

The foaming capacity was determined by the method of Lawhon *et al*. (1972) with modifications. 50ml of 0.01M NaOH was added to five grams of protein samples. The mixtures were thoroughly homogenized for 10 minutes using a laboratory homogenizer, (L4R model) set at high speed (approximately 10,000rpm) at room temperature. Homogenized samples were poured into 100ml measuring cylinders and the volume of foam after 30 seconds taken. The increase in volume of content of cylinder was expressed as a percent foam capacity. Procedure was repeated for a five gram egg white sample of the same moisture content.

3.12 Foaming Stability (FS)

Foam stability was determined by measuring the decrease in volume of foam as a function of time up to a period of 120 minutes (*Suliman et al., 2006*). The stable foam volumes were recorded at time intervals of 10, 30, 60 and 120 minutes.

3.13 Viscographic Analysis

The method of Demiate *et al.* (2001) was used with only slight modifications. Specific weights of starch-protein combinations depending on the moisture content was suspended in a specific volume of solvent and was analyzed with a Brabender viscoamylograph at constant heating and cooling rates. The equipment was able to increase the temperature and to rotate the vessel at a fixed rate, 1.5ºC/minute and 75 rpm, respectively. Total procedure included an initial heating phase, from 50 to 95°C, in order to observe the viscosity features as: beginning of gelatinization, maximum viscosity, start of holding period, start of cooling period, end of cooling period, end of final holding period, breakdown viscosity, and setback viscosity. The whole system was maintained at 95°C for 30 minutes to observe the resistance of the paste to mechanical stirring and finally, the cooling phase was kept at 50°C to observe retrogradation. The influence of the different combinations of protein-starch blends on the pasting properties was also studied. Results were recorded directly from the equipment as digitized viscoamylograph.

3.14 Statistical Analysis

Functional properties were determined at least in duplicates. For all surface functional properties, sample variations effects were analyzed by one-way ANOVA (no blocking), while sample and treatment effects were analyzed by a two-way ANOVA (no blocking), using the GenStat statistical tool (*GenStat, 2007*). Significant differences ($p<0.05$) between means of sample variations and between variations and treatments were determined using variance ratio (*v.r*). Correlations coefficients (*r*) of functional properties were obtained. The level of significance used was 95%.

CHAPTER FOUR

4.0 Results and Discussions

4.1 Defatted Bambara groundnut Flour

A total of 10.50kg of the Bambara groundnut flour was defatted using a meal to solvent ratio of 1.5 kg:18 L while running the large scale Soxhlet's extraction procedure for approximately three hours per batch. An average weight of about 9.20 kg of Bambara groundnut meal was obtained after Defatting.

4.2 Extracted Proteins and Starches

A total of 1.48 kg of isolated proteins on dry weight basis was extracted from the 9.20 kg of dried defatted Bambara groundnut flour. The initial total amount of 1.48 kg freeze dried proteins was divided into five, each weighing 295.41 g. similarly, 2.54 kg of native starch was obtained from the 9.20 kg of dried defatted Bambara groundnut flour after repeated washing.

4.3 Moisture Content of Protein Sample before and after Irradiation

The moisture content of various irradiated protein samples ranged from 5.770%-5.810% (Figure 4.1). There were no significant differences ($p<0.05$) among samples both before and after irradiation. This however is an indication that the irradiation procedure had no effect on the moisture content of the samples, evidenced by the correlation obtained (r = -0.12) between irradiation dosage and moisture content of the Bambara groundnut samples.

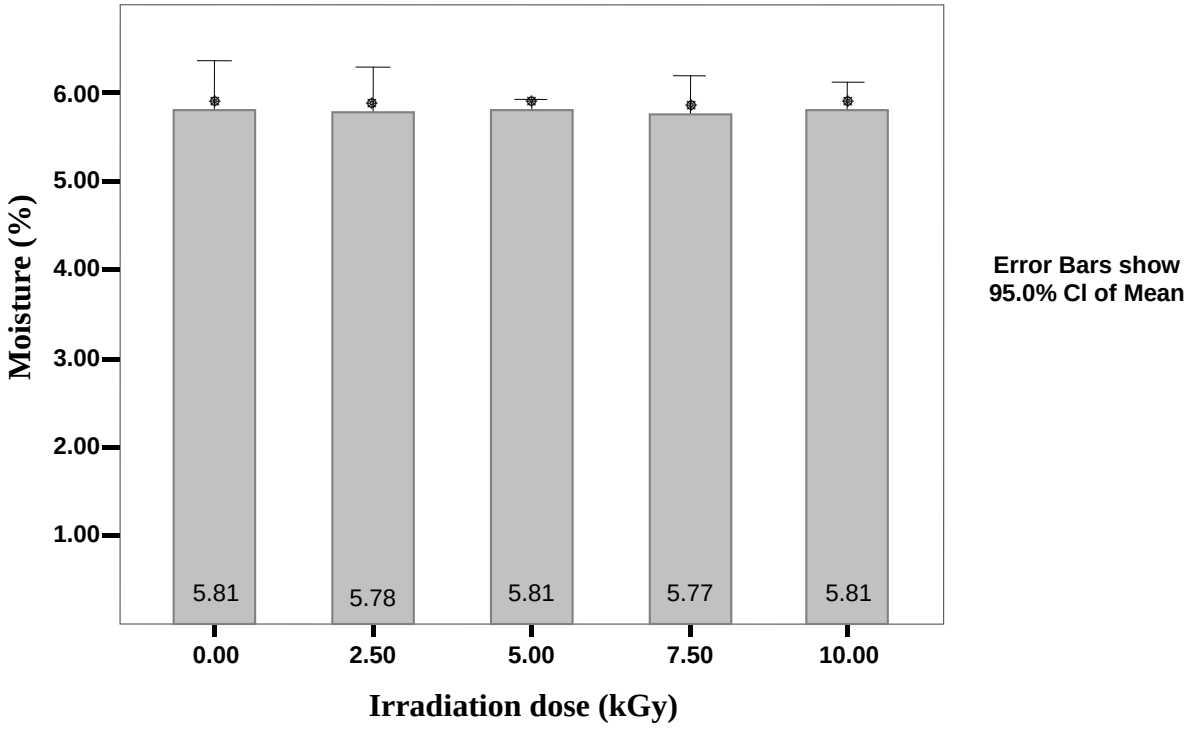

FIGURE 4.1: MOISTURE CONTENT OF BAMBARA GROUNDNUT

PROTEIN SAMPLES

4.4 Solubility Profile of Gamma Irradiated Proteins

Protein-related functional properties such as protein solubility are in general influenced by various factors such as protein denaturation, size, structure and conformation, charge, amino acid composition and amino acid sequence of the protein molecules (*Zayas, 1997*). Under the given conditions for optimum solubility, which are pH, protein slurry concentration and vortexing time, the control sample (non-irradiated sample) recorded the highest value (0.172688). A decrease in solubility then occurred at the 2.50kGy sample, followed by small increases up to the 7.50kGy irradiated sample and then a decrease at a 10.00kGy dose (Table 4.2). There was generally a decrease in solubility following irradiation probably due to some degree of protein denaturation even though samples did not show any significant differences ($p<0.05$) with respect to their solubility profile. However, all five samples of irradiated Bambara groundnut proteins readily solubilized at pH 8.00 when their respective slurries were vortexed for one hour each. This is a good property for product development since most food preparations involve solvent that contain salt with pH within the range of 4.0 to 8.0 (*Mwasaru et al., 2000*).

Table 4.1: Optimum Conditions for Maximum Solubility of the Irradiated Bambara groundnut Protein Isolates

Irradiation doses (kGy)	Optimum conditions for maximum solubility			
	pH of buffer solution	Protein slurry concentration (g/20ml)	Vortex time (hrs)	Protein solubility (g/ml)
0.00	8.00	3.26	1.00	0.172688
2.50	8.00	3.66	1.00	0.171666
5.00	8.00	3.91	1.00	0.171802
7.50	8.00	2.50	1.00	0.172450
10.00	8.00	2.00	1.00	0.171892

4.5 Water and Oil Absorption Capacities (WAC and OAC)

Water and oil absorption generally increased across irradiation doses with the 10.00kGy irradiated sample recording the highest for both Water Absorption Capacity (WAC) and Oil Absorption Capacity (OAC) - 18.45% and 10.09% respectively (Figures 4.2 and 4.3). The increase in the WAC may be as a result of the coupled effect of water adsorption via existing polar binding sites distributed over the protein surface, and molecular rearrangement leading to the exposure of more polar binding sites, following irradiation (*Privalov, 1979*). Increases in OAC following irradiation have been recorded for cowpea flours and pastes (*Abu et al., 2005*). Lipophilic tendencies of samples increased with increasing irradiation probably due to more exposed hydrophobic sites as scission and rearrangement of polypeptides occurred following a progressive increase in irradiation doses, compared to the non-irradiated proteins. Figure 4.2 shows higher values for WAC than OAC in Figure 4.3, estimating a hydrophilic to hydrophobic ratio of, approximately,

64.64%:35.36%. However, OAC values, unlike WAC, values did not vary significantly ($p<0.05$) between any two successive irradiation doses.

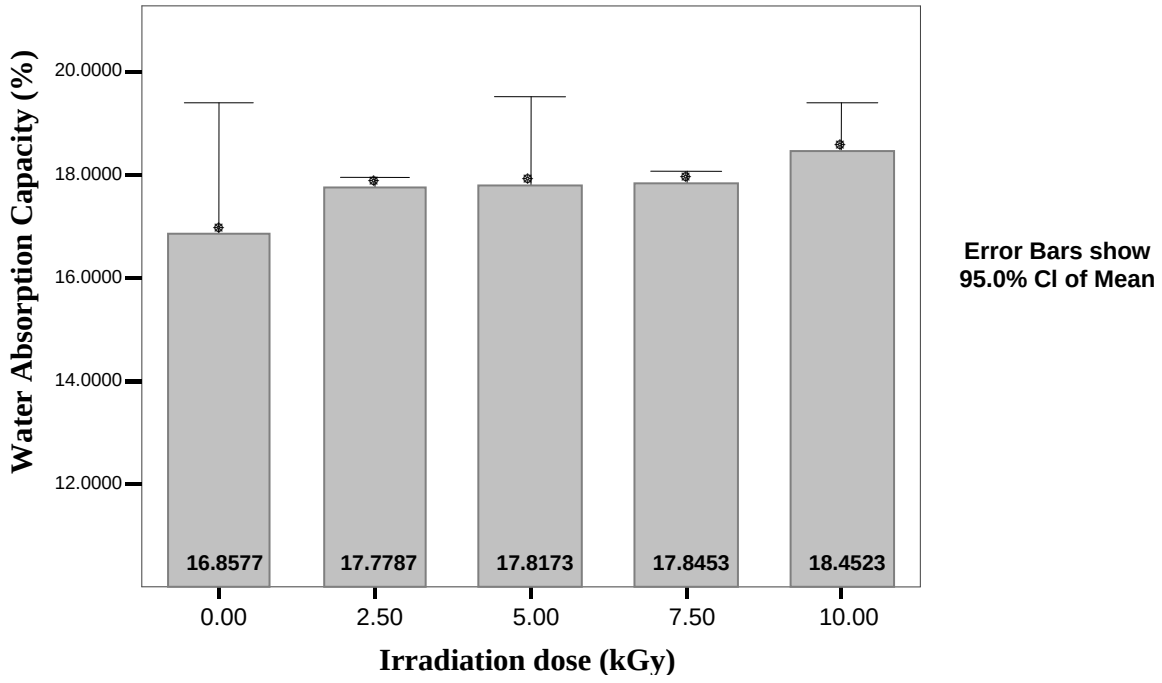

FIGURE 4.2: WATER ABSORPTION CAPACITY OF BAMBARA GROUNDNUT PROTEIN SAMPLES

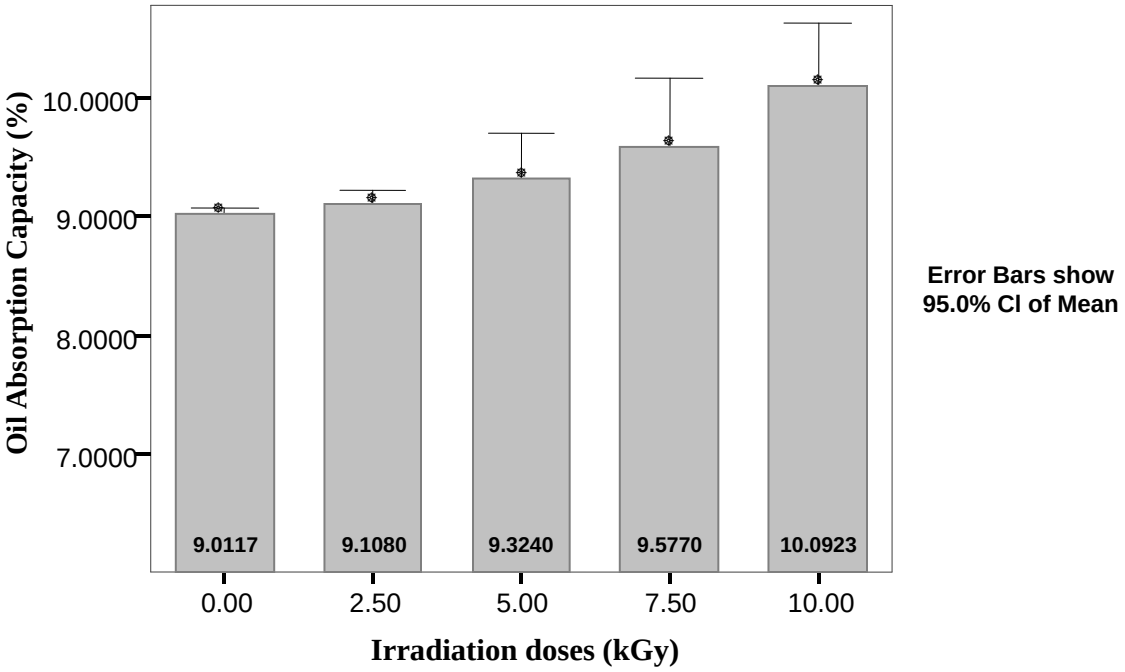

FIGURE 4.3: OIL ABSORPTION CAPACITY OF BAMBARA GROUNDNUT PROTEIN SAMPLES

4.6 Foaming Capacity (FC) and Foam Stability (FS)

Irradiation caused a progressive increase in Foaming Capacity (FC) for all protein samples (Figure 4.4). This observation may be due, in part, to increased diffusion of the dose-dependent unfolded and fragmented proteins towards the air/water interface. Increased unfolding and fragmentation of protein following irradiation, may have enabled the formation of more continuous phases of thin liquid layers which trapped air bubbles, hence the progressive increases in foaming capacity of irradiated protein samples. The egg white which has excellent foaming properties and therefore often used as the standard, recorded the highest FC (95%) and Foaming Stability (FS) values.

The 10.00kGy irradiated sample which recorded 80.00% FC exhibited a fairly high ability to foam, therefore could serve as much a foaming agent as the egg white in confectionery products, such as cakes and breads. FS values for each sample decreased progressively from 30 seconds to 120 minutes after whipping (Figure 4.5). Breakdown of foams in a dose-dependent manner, resulting from drainage of lamella liquid, may have been due to the effect of gravitational force on the protein masses obtained from various degrees of unfolding and fragmentation after irradiation. However, foams of 2.50kGy protein were more stable than those of 5.00kGy over the two-hour period for reasons that could not be explained.

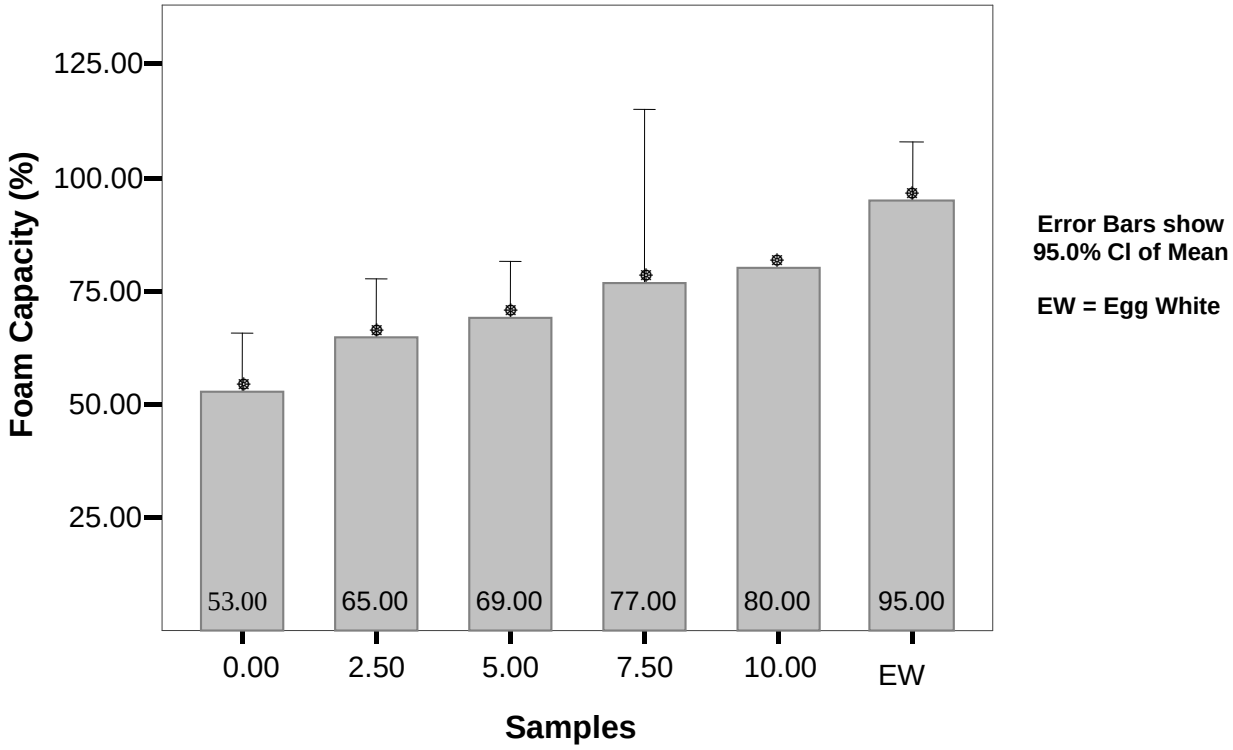

FIGURE 4.4: FOAMING CAPACITIES OF BAMBARA GROUNDNUT PROTEINS SAMPLES COMPARED WITH EGG WHITE (EW)

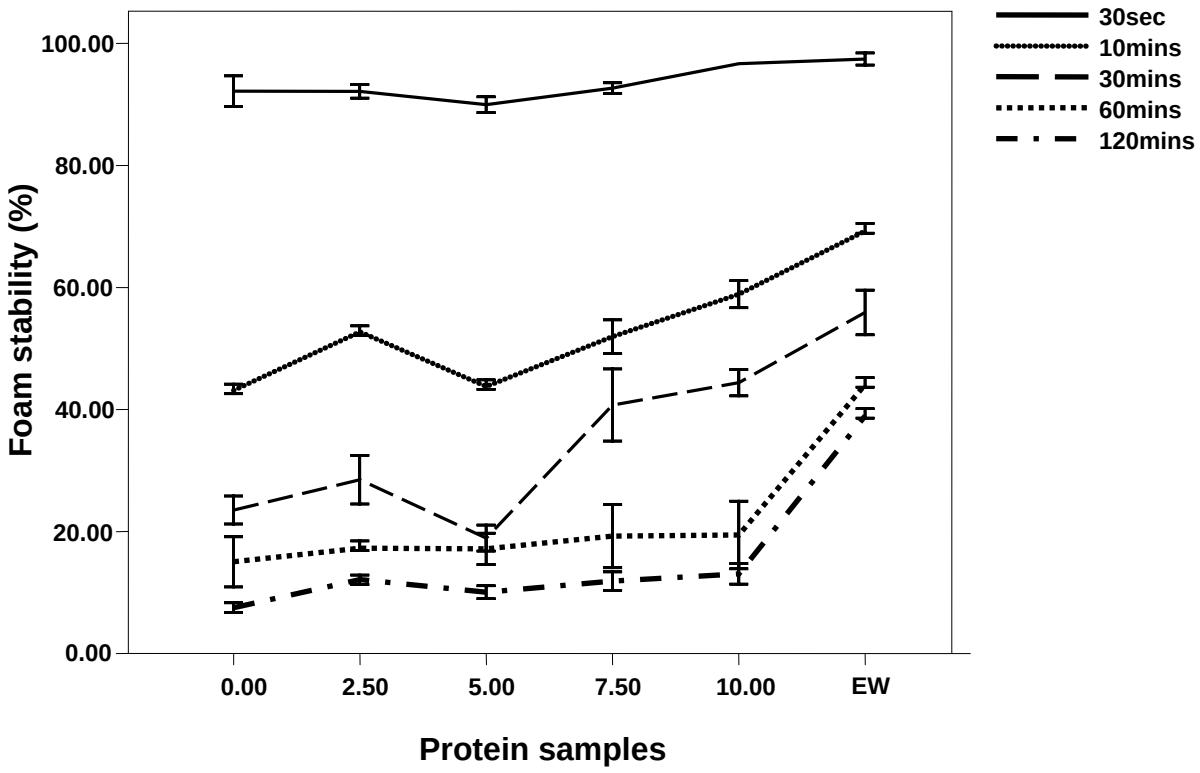

FIGURE 4.5: FOAM STABILITY OF WHIPPED BAMBARA GROUNDNUT PROTEINS COMPARED WITH EW OVER A TWO-HOUR PERIOD

4.7 Emulsifying Activity (EA) and Emulsion Stability (ES)

Superior emulsifying properties are desired to make milk-like beverages and meat analogues (*Friberg, 1976*). Irradiation caused a progressive decrease in EA and ES for Bambara groundnut protein samples (Figures 4.6 and 4.7).

The non-irradiated samples (0.00 kGy) recorded lower EA and ES values than the 2.5 kGy irradiated samples for reasons that could not be explained. However as irradiation increased from 2.50 kGy to 10.00 kGy, the various degrees of protein unfolding and fragmentation resulting from the breakages of weak bonds might have caused the exposure of previously hidden polar sites on protein surfaces, making them more hydrophilic, thereby adsorbing more within the aqueous phase, thus reducing their emulsifying properties in a dose-dependent manner.

A negative correlation (r = -0.14) was recorded between WAC and EA. Similarly, Abu *et al.* (2005) obtained a negative correlation coefficient (r = -0.02 and r = -0.17) between WAC and EC for irradiated cowpea flours and pastes respectively, for the same reason of irradiation-induced increases in protein surface hydrophilicity.

As already stated, once a reasonable portion of the proteins comes into contact with the interface, the non-polar amino acids residues orient toward the non-aqueous phase (oil), and with a corresponding decrease in free energy of the system, the remainder of the proteins spontaneously adsorb at the interface. But according to Kato and Nakai (1980), the more hydrophilic the protein, (as in the case of the irradiated Bambara groundnut proteins), the lesser the concentration of proteins at the interface, hence the higher the interfacial tension and the less stable the emulsions.

Many theoretical and experimental studies have been carried out on the behaviour of proteins at water/oil or oil/water interfaces. However, much uncertainty still remains as to the conformation that proteins adopt at these interfaces and the relationship between initial conformation and at the interface, versus emulsifying or foaming properties (*Phillips, 1981*). Even though many similarities exist between emulsions and foam formation, there is no strict correlation between the emulsifying and foaming abilities of proteins. This perhaps can be attributed to the fact that foam stability has a greater requirement for residual protein structure than emulsion stability (*Halling, 1981*).

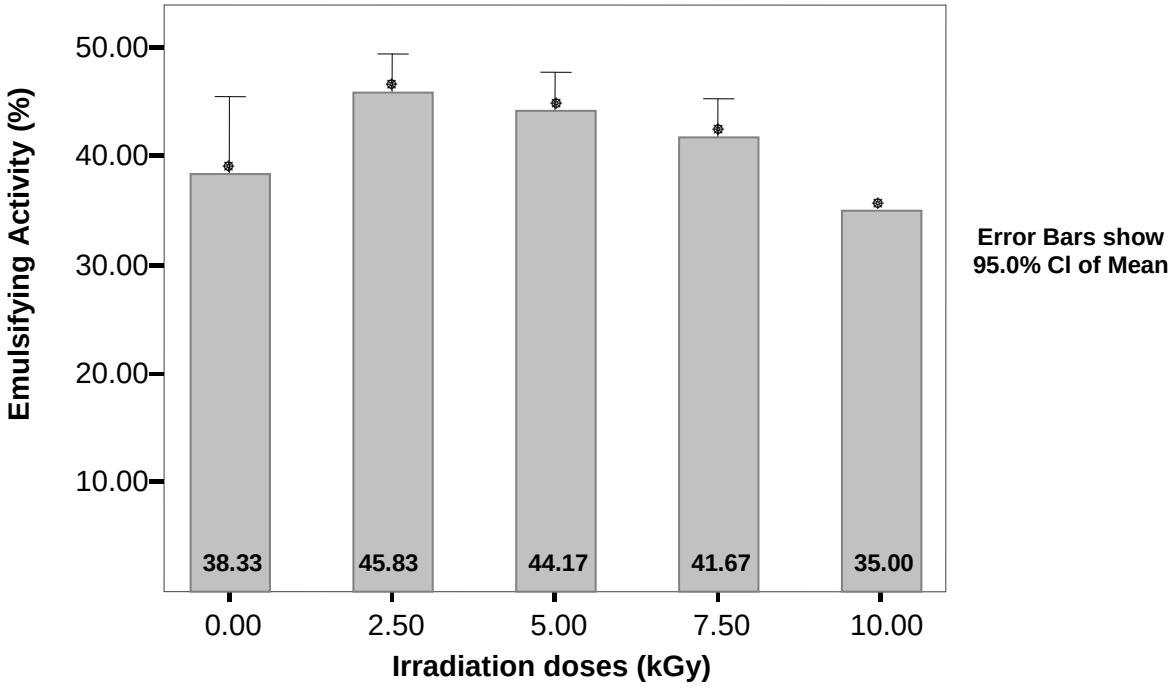

FIGURE 4.6: EMULSIFYING ACTIVITY OF WHIPPED BAMBARA GROUNDNUT PROTEIN SAMPLES

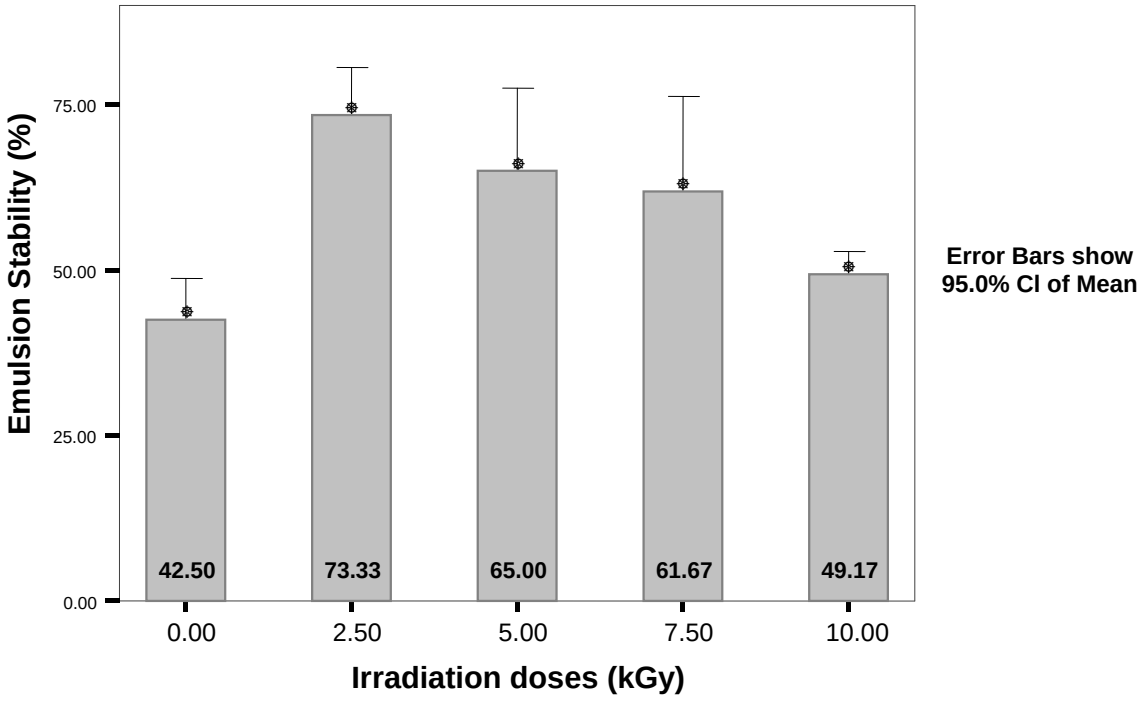

FIGURE 4.7: EMULSION STABILITY OF WHIPPED BAMBARA GROUNDNUT PROTEIN SAMPLES

Table 4.2: Correlation Coefficient *(r)* of the Functional Properties of Bambara groundnut Gamma Irradiated Proteins

	Functional Properties						
	SOL	**WAC**	**OAC**	**FC**	**FS**	**EA**	**ES**
	Correlation coefficients (*r*)						
SOL		-0.67	-0.21	-0.38	-0.04	-0.40	-0.67
WAC	-0.67		0.84*	0.92*	0.73	-0.14	0.31
OAC	-0.38	0.92*		0.87*	0.92*	-0.62	-0.21

Values with asterisks (*) are significantly correlated with each other (p<0.05)

4.8 Viscographic analysis

Gelation of protein is very important for the preparation of puddings, jams and sauces that require thickening and jelling. Some kinds of proteins form gels through interactions with polysaccharide gelling agents such as starch and gelatin (*Lin, 1977*). Pasting temperatures provide an indication of the minimum temperatures required to cook the admixtures. Clearly cooking temperatures (T°C) for each pasting characteristic did not differ significantly ($p<0.05$) from one another across all five levels of protein irradiation within each of the three levels of the protein-starch blends (Appendices 10A, 10B and 10C).

Pasting temperature and peak time: However, of the three admixtures, the 70P:30S blends recorded the highest gelatinization temperatures of 88.20ºC, 86.60ºC, 91.50ºC, 85.10ºC and 86.80ºC, approximately, within 30 minutes for 0.00, 2.50, 5.00, 7.50 and 10.00kGy levels of irradiated proteins. The 50P:50S blends recorded intermediate

temperatures while the 30P: 70S blends had the least gelatinization temperatures. However, at irradiation levels 2.50 and 5.00kGy, gelatinization temperatures for 30P:70S (76.20ºC and 76.35ºC) were higher than 50P:50S (64.15ºC and 63.80ºC), for reasons that could not be explained (Figure 4.8).

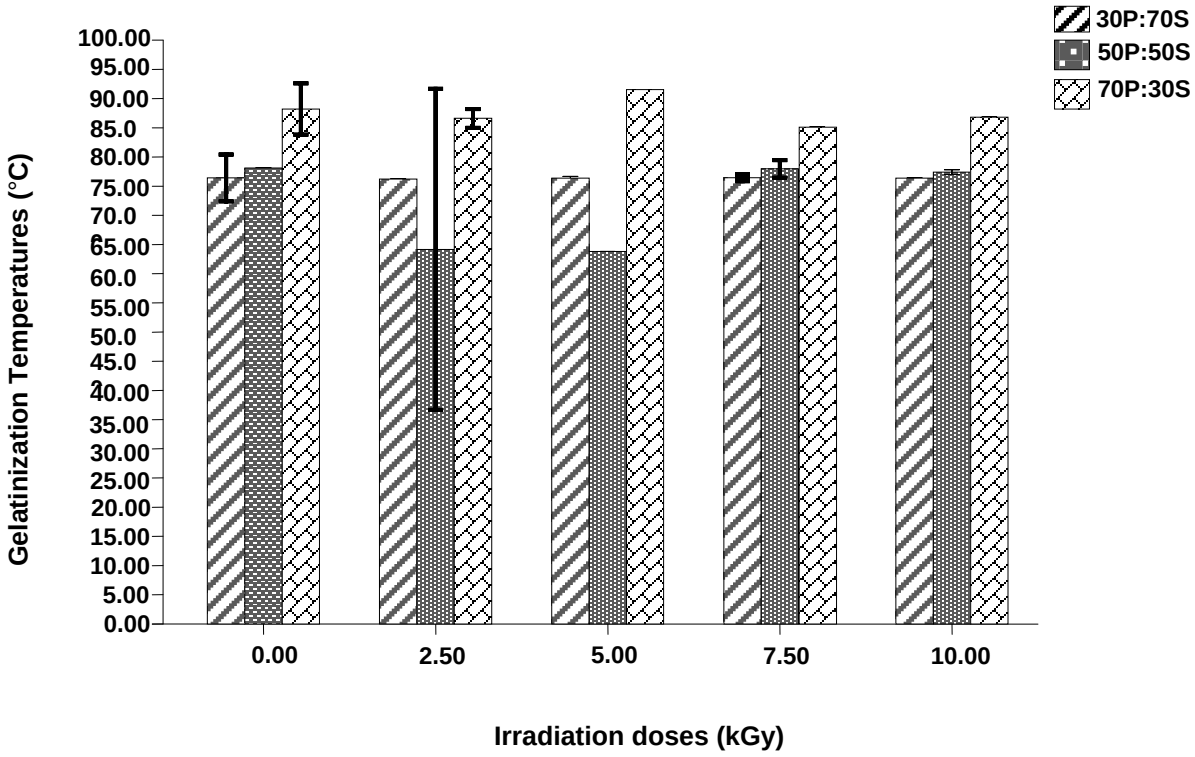

FIGURE 4.8: GELATINIZATION TEMPERATURES OF PROTEIN-STARCH ADMIXTURES

Peak viscosity: Maximum viscosity also decreased with decreases in starch and increases in protein concentrations (Figure 4.9). This is evidenced by the strong negative correlation (r = -1.00) between increased protein-starch ratio and maximum viscosity.

Peak viscosity ranged between 620.00 and 5.00BU. Blends constituted of 30% Proteins and 70% Starch attained the highest values for peak viscosity at all levels of irradiation (541.00, 557.00, 575.00, 620.00 and 541.00 BU for 0.00, 2.50, 5.00, 7.50 and 10.00kGy doses respectively). The least viscosity values were observed in the 70P:30S blends while the 50P:50S recorded intermediate values. For instance, at 2.50kGy level protein irradiation the viscosity values for the three blends, 30P:70S, 50P:50S, 70P:30S were 557.00, 288.00 and 64.00 BU, respectively. However, across the levels of irradiation peak viscosity did not differ significantly ($p<0.05$). A significant negative correlation of protein with peak viscosity (r = -0.863, $p<0.01$) of corn flour had also been reported earlier by Sandhu and Singh (2007). Proteins did not gel when cooked and therefore exhibited very low to almost negligible viscosities. Possibly, the high maximum viscosity observed within the 30P:70S blend may be attributed to the high starch concentration and not to the proteins or the interaction between the two biopolymers. This observation also implies that the less starch within the blend, the less viscous the paste upon cooking at optimum temperatures.

However, temperatures at which the different blends, each constituted of different modified proteins with corresponding attained maximum viscosities, did not differ significantly ($p<0.05$). The temperature values ranged between 92.70 and 95.60°C. Again, all protein-starch admixtures exhibited increased viscosity during cooling at 50°C (Appendices 10A, 10B and 10C).

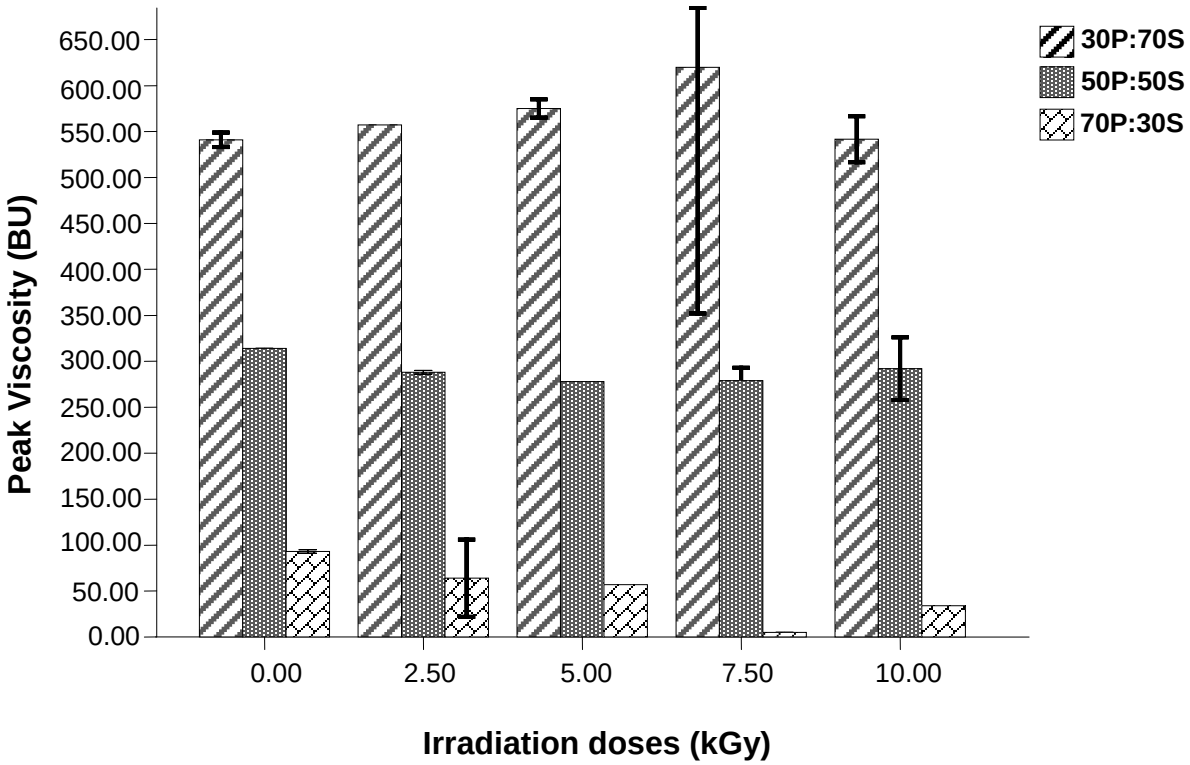

FIGURE 4.9: PEAK VISCOSITY OF PROTEIN-STARCH ADMIXTURES

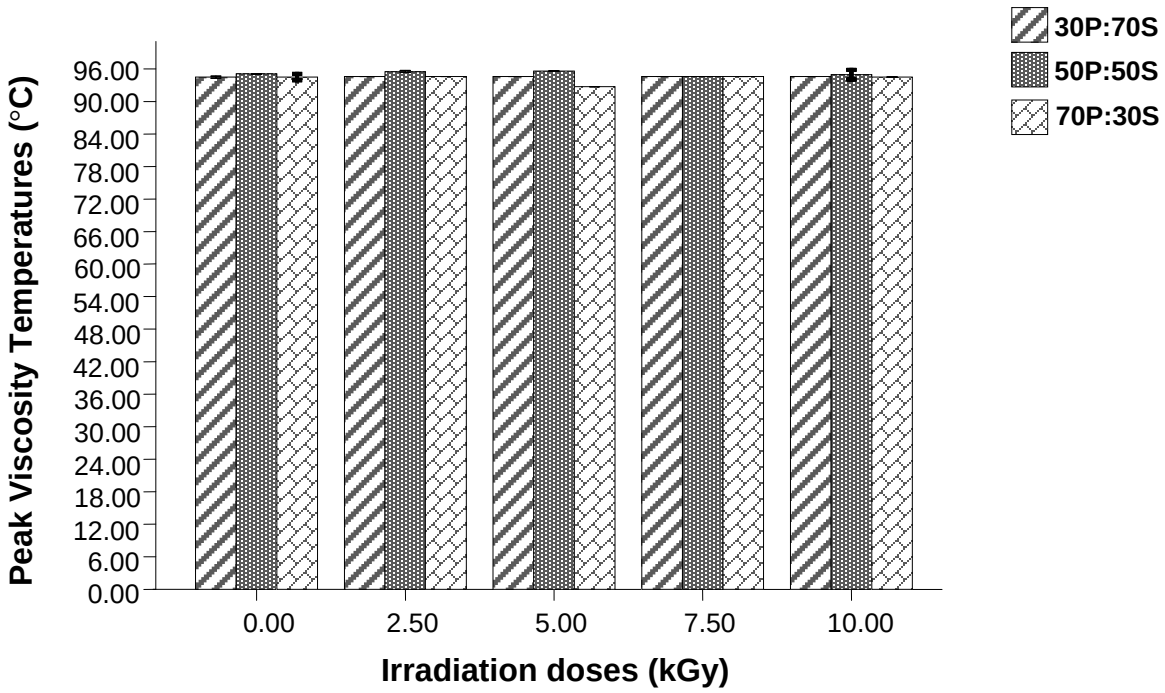

FIGURE 4.10: PEAK VISCOSITY TEMPERATURES OF PROTEIN-STARCH ADMIXTURES

Gel strength was a measure of the torque (BU) applied to the gels during pasting. In more basic terms, torque measures how hard something is rotated. Results showed that gel strengths decreased with decreasing starch concentrations within blends. The reason advanced for this observation may be as a result of the direct consequence of the decreasing peak viscosities with decreased starch concentrations. Gel strength for all blends generally increased from start of holding period, through the cooling period, to the end of the final holding period. For instance, the gel strength of the 30P:70S blends constituted of 10.00kGy proteins increased from 541.00BU at start of holding period to 896.00BU at the end of the final holding time (Figures 4.11 and 4.12). Similar observations were made for the other two blends with proteins at the various levels of irradiation. There were, however, no dose-dependent significant differences ($p<0.05$) in gel strengths with respect to the irradiated proteins used to constitute the three different protein-starch admixtures.

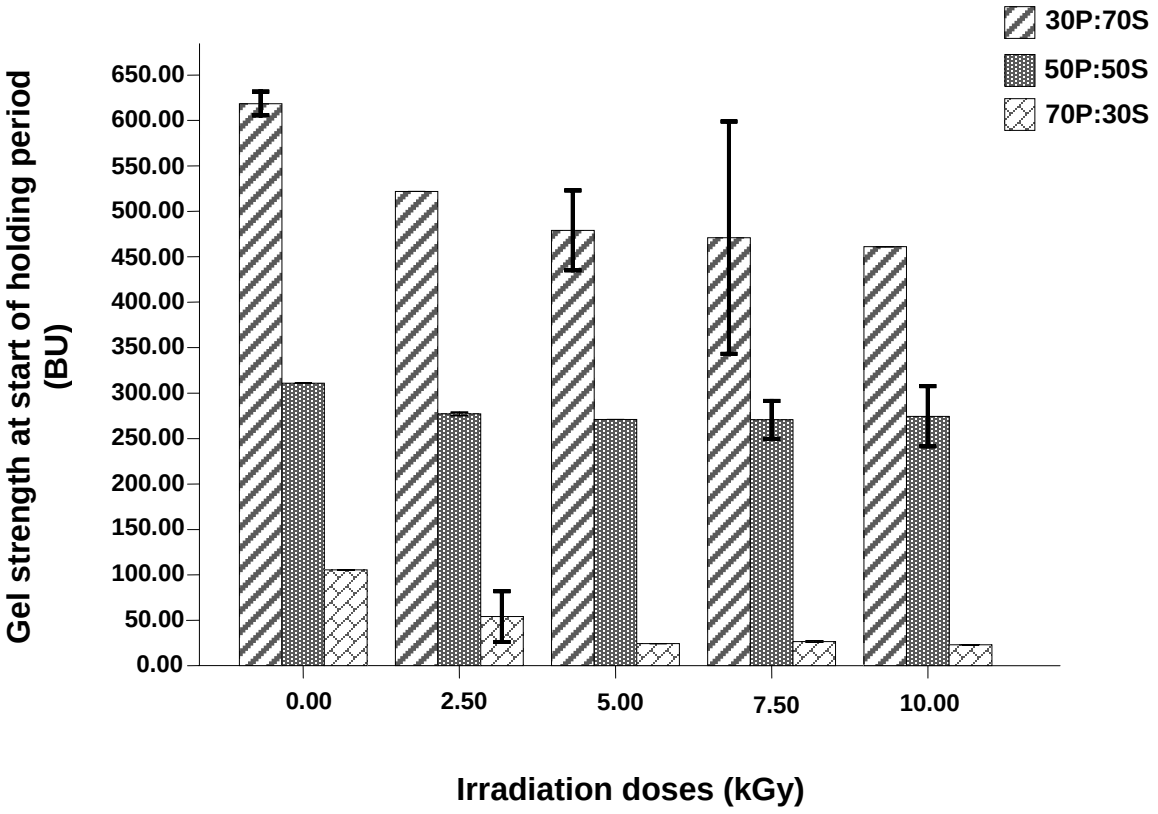

FIGURE 4.11: GEL STRENGTH OF PROTEIN-STARCH ADMIXTURES

AT START OF HOLDING PERIOD

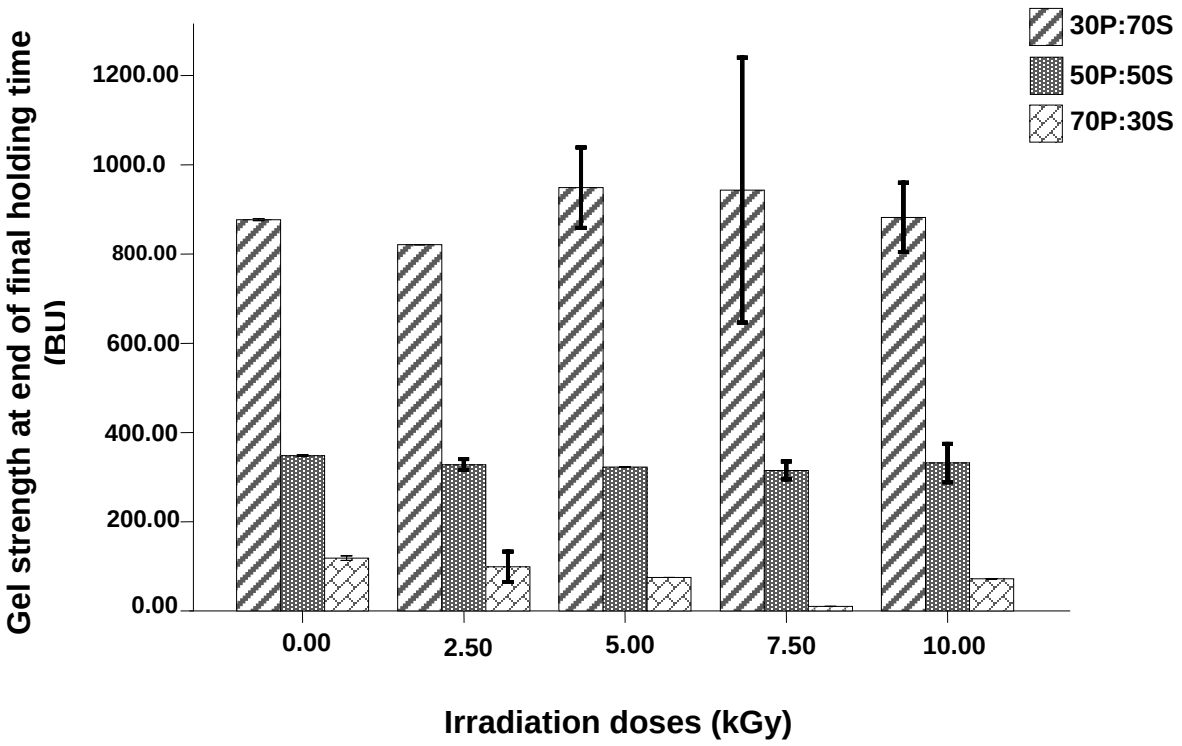

FIGURE 4.12: GEL STRENGTH OF PROTEIN-STARCH ADMIXTURES

AT END OF FINAL HOLDING PERIOD

Breakdown viscosity: Breakdown viscosity values were between 0.00 and 42.00BU, and were higher in the 50P:50S blends with all five levels of irradiated proteins compared with the 30P:70S and 70P:30S blends. There were dose-dependent significant differences ($p<0.05$) in breakdown viscosity with respect to the proteins used in constituting the 50P:70S and 70P:30S blends (Figure 4.13). However, no viscosity breakdown was observed in the 30P:70S blend constituted of 7.50kGy irradiated protein. These observations could not also be explained.

Setback and final viscosity: setback viscosity is a measure of retrogradation. Retrogradation is a general term for the behaviour of recrystallization of gelatinized starches on cooling and storage, and is accompanied by gel hardening and the leakage of water (syneresis) from the starch gel (*Ishiguro et al., 2000*). Setback viscosity is an important factor for starch used as a food ingredient in processing and preservation, because the quality of the food's texture and physical properties deteriorate due to retrogradation as time passes. Setback viscosity ranged between 397.50 and 9.00 BU, and had an increasing order of 70P:30S < 50P:50S < 30P:70S (i.e. least viscous to highest). The reason for this observation was that higher starch concentrations encouraged the formation of a more ordered structure, which in turn trapped enough water, forming stronger gels with higher viscosities. This phenomenon may have increased the tendencies for retrogradation accompanied by syneresis, hence the increasing setback viscosity order of 70P:30S < 50P:50S < 30P:70S. However, no dose dependent significant differences ($p<0.05$) were recorded for the setback viscosities with respect to the type of irradiated protein used in constituting the admixtures (Figure 4.14).

Results from the pasting characteristics of the protein-starch admixture models suggest the sole dependence of the pasting properties on the starch concentration within the blends, meaning that the contribution of the Bambara starch to the pasting properties of the blends was greater than that of the irradiated Bambara proteins. This seem to suggest that the modified proteins were simply unable to form strong gel matrices or gel networks with starches, perhaps, due to poor ionic interactions between the individual molecules and the improper balance between protein-protein and protein-solvent interactions.

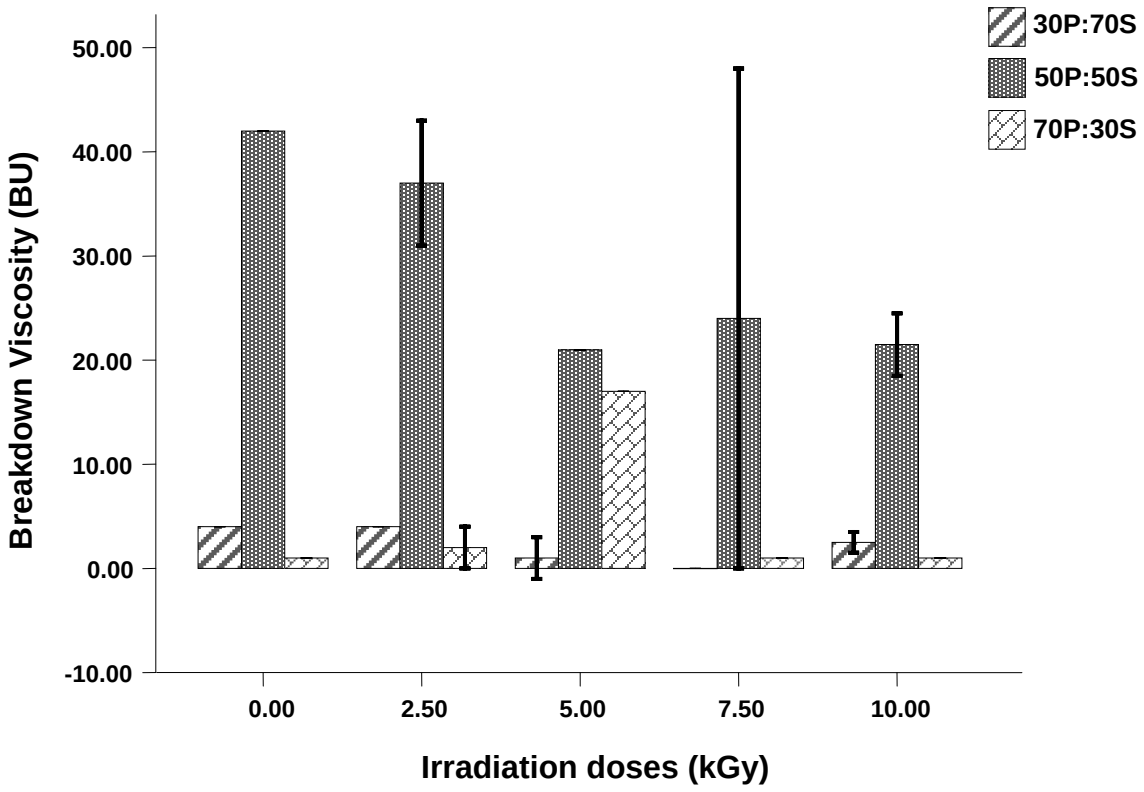

FIGURE 4.13: BREAKDOWN VISCOSITY OF PROTEIN-STARCH ADMIXTURES

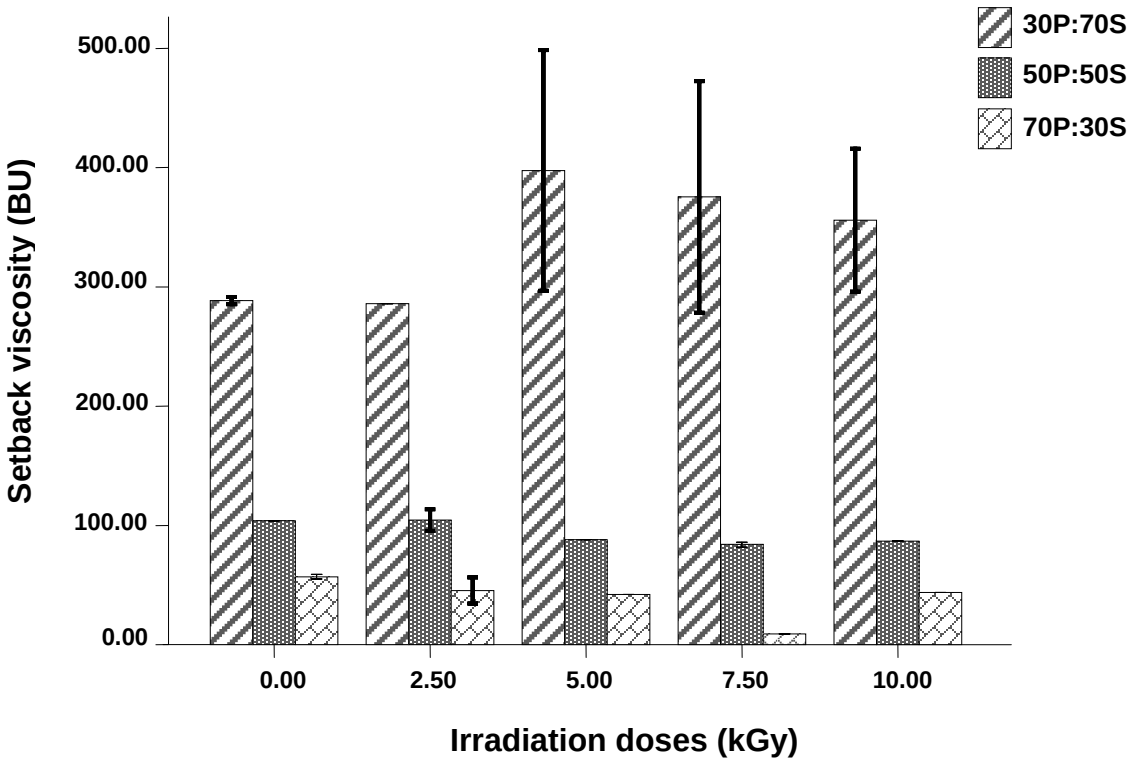

FIGURE 4.15: SETBACK VISCOSITY OF PROTEIN-STARCH ADMIXTURES

5.0 CONCLUSION

Results from this research showed significant ($p<0.05$) effects of increasing irradiation doses on some protein related functional properties, while pasting characteristics of admixtures showed no dose-dependent significant ($p<0.05$) changes. Protein solubility decreased following irradiation, even though there were no significant differences ($p<0.05$) among samples. All samples readily solubilized at pH 8.00 with the non-irradiated (0.00kGy) showing the highest solubility value of 0.173g/ml. Increases in Water and Oil Absorption Capacities (WAC and OAC) were dose-dependent, with samples showing significant differences ($p<0.05$). The 10.00kGy samples recorded the highest values of 18.45% and 10.09% for WAC and OAC respectively. Foaming Properties increased across irradiation doses with some significant differences ($p<0.05$) among samples. However the 10.00kGy irradiated samples compared to Egg White, recorded lower values for foaming properties. Significant decreases ($p<0.05$) in Emulsifying Properties were also recorded after irradiation, with the 2.50kGy sample recording the highest values of 45.83% and 73.33% for Emulsifying Activity and Emulsion Stability respectively. Pasting characteristics again increased significantly ($p<0.05$) with increasing starch:protein ratios. Of the three admixtures, the 70P:30S blends recorded the highest Gelatinization Temperatures of 88.20ºC, 86.60ºC, 91.50ºC, 85.10ºC and 86.80ºC, approximately, within 30 minutes for 0.00, 2.50, 5.00, 7.50 and 10.00kGy levels of irradiated proteins. Blends constituted of 30% Proteins and 70% Starch attained the highest values for Peak Viscosity at all levels of irradiation (541.00, 557.00, 575.00, 620.00 and 541.00 BU for 0.00, 2.50, 5.00, 7.50 and 10.00kGy doses respectively). Gel strengths decreased with decreasing starch concentrations within

blends, whereas Breakdown Viscosity values which ranged between 0.00 and 42.00BU, were higher in the 50P:50S blends with all five levels of irradiated proteins compared with the 30P:70S and 70P:30S blends. Finally, Setback Viscosity, which ranged between 397.50 and 9.00 BU, had an increasing order of 70P:30S < 50P:50S < 30P:70S. Conclusively, correlation studies showed that the pasting properties were solely dependent on the starch concentration within the blends, indicating the insignificant contribution of modified Bambara groundnut proteins to the pasting properties of the blends. This observation may have been due to poor ionic interactions between the individual molecules and the improper balance between protein-protein and protein-solvent interactions

6.0 RECOMMENDATIONS

1. Gamma irradiated Bambara groundnut protein isolates could be potential flavour retention, palatability improvement and shelf life extension agents due to their moderately low water and oil absorption capacities.

2. Since foam contributes to smoothness, lightness, flavour dispersions, and palatability, the moderately high foaming properties of the modified proteins could make them potential replacements of egg white in foods like cakes, breads, and desserts.

3. The 2.50kGy irradiated Bambara protein sample makes it a potential ingredient in food formulations such as comminuted meats due to its moderately high emulsifying properties.

4. The starch-protein admixture models may also serve as a potential protein based thickening agents for foods that require various degrees of viscosity modifying effects.

5. Further studies should be done to investigate the effect of low dose irradiation on the surface functional properties on Bambara groundnut protein pastes with varying moisture content.

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REFERENCES

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CHAPTER ONE

1.0 Introduction

Legume seeds are of prime importance in human and animal nutrition due to their high protein content (20–50%).Their protein content is twice the level found in cereal grains and significantly more than the level found in their root crop counterparts (*Ustimenko-Bakumovsky, 1983*). Dry legumes are important ingredients of diet in many parts of the world and have been considered as the most significant food sources for people of low incomes (*Bressani and Elias, 1979*). Legumes have historically been utilized mainly as whole seeds but in recent years, interest has grown in the utilization of legumes in other forms like flours, protein concentrates and protein isolates (*Doxastakis, 2000; Saio, 1993*). The use of plant protein products in food as functional ingredients to improve the stability and texture as well as the nutritional quality of the product or for economic reasons is much extended. Nevertheless, these applications in the food trade are almost limited to proteins from soybean seeds, whereas other vegetable proteins are less used (*Makri et al., 2005*). Among these underutilized legumes are the lupins, peas and the beans of which the Bambara groundnut is part (*De Miguel Gordillo, 1991*). In spite of their underutilization, their high protein and their well-balanced amino-acid composition make these neglected and underutilized legumes important sources of protein, with potential to add in various products as novel food ingredients (*Alamanou et al., 1996*). In this light, various procedures or methods, including irradiation (*Abu et al., 2006*), hydrolysis (*Martinez et al., 2006*), proteolysis (*Fontana et al., 2004*), and heat-moisture treatment (*Singh et al., 2005*) have been used to enhance some surface functional properties and pasting properties of flours and pastes obtained from various legume. Food is submitted to gamma irradiation process for different purposes. Among the several benefits, a food or an ingredient is irradiated to assure a chemical change in such a way that a specific characteristic could be improved or its processability is facilitated. Gamma irradiation like other ionizing radiations,

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through the production of free radicals, can affect proteins by promoting covalent inter- and intra-molecular cross-links which may result in protein-protein association, deamination, scission of polypeptide chains and disulphide bonds, or by association of aromatic and heterocyclic residues (*Cho et al., 1999; Simic, 1978* and *Urbain, 1986*).

Gamma irradiation has been used to cross-link biodegradable films from whey, casein and soy proteins (*Lacroix et al., 2002*) and aided the digestibility of some legume flours (*Dario and Salgado, 1994*). Similarly, emulsion, foam, water and oil absorption capacities were affected by gamma irradiation in peanut flour (*Rahma and Mostafa, 1988).* Protein solubility , in soy (*Byun and Kang, 1995 and Hafez et al., 1995*) and in red kidney beans (*Dogbevi et al., 1999*) have also been affected by gamma irradiation while decreases in swelling properties following gamma irradiation of cowpea starch (*Abu et al, 2004*) have been documented. Reports suggest that irradiation of cowpea flours and pastes at medium to high doses has resulted in significant changes in protein-related functional properties (*Abu et al, 2004*), and studies conducted by Lee et al., (2004) also showed the disruption of the ordered structure of the soy protein molecules following irradiation the protein isolates. Various reasons such as protein denaturation, dissociation, exposure of polar and non-polar protein sites, deamination and hydrophobicity were advanced for these irradiation-induced changes.

Due to the high amylose content (*Whistler and Daniel, 1985*) that tends to confer poor functional properties in many food applications, starch is often overlooked by-product after protein isolation from legume. However with the current advances made so far by food technology, it is possible to cross-link or cogel starches with proteins in order to create a rigid and viscoelastic films around the oil or air droplets and/or for creation of network structure, with desirable texture for the benefits of the consumer. Such a modification may enhance the use of legume starches such as the Bambara

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groundnut starch with a great reduction of the over-dependence of the conventional root crop and cereal starches.

Various attempts have been made however to cross-link protein and starches to form protein-starch networks with improved synergistic food functionality. An example is a research conducted by Köber et al., (2004) to modify the water absorption capacities of a plastic based on bean protein using gamma irradiated starches as additives. Many more researchers have also used chemical and enzymatic modifications to improve the functionality of food proteins. Particularly, Ohtsuka *et al* (2003) induced gelation of soy proteins using recombinant microbial transglutaminase, while Arntfield (2007) improved gelation of canola proteins through limited proteolysis with trypsin, ficin and bromelin either alone or in combination with the cross-linking enzyme, transglutaminase. Reports from Malhorta and Coupland (2004) have also shown the effect of surfactants on the solubility, zeta potential, and viscosity of soy proteins for food application.

1.2 Statement of the Problem

Perusal of literature reveals that although the effects of various modification processes on functionality have been studied extensively for soybean proteins, little work has been done on others legume such as the Bambara groundnut protein. However, procedures which have been employed for the purposes of modifying plant proteins with the aim of enhancing and tailoring their food functional applications in their respective multi-component food systems have been faced with some limitations of accuracy, sensitivity, specificity, toxicity, and in some cases equipment availability and efficiency, time constraint and cost, among others.

1.3 Justification of work

Due to the fact that crude proteins from the Bambara groundnut have shown some level of impairment in their functional properties (*Kato, et al., 1987*) is it therefore unable to compete with

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others like the soy proteins, it is therefore necessary is the need for such modification procedures to enhance their functionality and refocus their use in food applications. To do this, the use of a less expensive and less tedious ^{60}Co gamma irradiation up to 10kGy dose to cause protein conformational changes and enhance their functionality would be appropriate. This work would then provide adequate information about the effects of irradiation on the functional performance of these protein isolates and their synergistic effect with the Bambara native starch on the pasting characteristics when used in food formulations.

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1.4 Objectives

The main aim was to physically modify proteins extracted from the Bambara groundnut using γ-irradiation, combine same with extracted native starches at various ratios, and study their respective food functional properties.

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The specific objectives are;

- To evaluate the surface functional properties of gamma irradiated Bambara groundnut proteins.
- To evaluate the pasting properties of modified proteins native starch admixtures using the Brabender viscoamylograph

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CHAPTER TWO

2.0 Literature Review

2.1 An overview of world legumes

Legumes are second only to the grasses in their importance to humans and their domestic animals (*Seigler, 2005*). Legumes come in three varieties, namely, grains legumes (the Bambara groundnuts, Peas, Faba beans, Lupins, Chickpeas, and Lentils), perennial herbaceous legumes (Clover and Alfalfa) and legume trees (Robinia, Leucaena, and *Calliandra sp.*).

Globally, both the grain and forage legumes are grown on some 180 million hectares of land, or some 12% to 15% of the Earth's arable land (*Graham and Vance, 2003*). Grain legumes, commonly referred to as beans, have pods with seeds inside them and they account for 27% of the world's primary crop production, with grain legumes (e.g. Soybeans, Cowpea, Jack beans, Lima beans etc.) alone contributing 33% of the dietary protein needs of humans. They are an important component of the agricultural and food systems throughout the world, in that they complement cereal crops in dietary terms, as sources of proteins and minerals, showing two or more times the levels found in most cereals (*Seigler, 2005*).

2.1.1 Nutraceutical benefits of Legumes

Legumes are generally an inexpensive source of proteins. Seeds of grain legumes contain at least 20% to 40% of protein. They may also possess many desirable characteristics, including an abundance of carbohydrates, low fat (generally containing, approximately, 5% of energy as fat except oilseeds, examples being chickpeas and soybeans), high concentration of polyunsaturated fatty acids with α-linolenic acid making up about 7–8% of the total fat (*Human Nutrition Information Service, 1988*). In addition to B complex vitamins and minerals, like iron, legumes are

also a major source of fiber. The legume seeds, green in the pod, also contain a lot of vitamins A and C (*Rockland and Nishi, 1979*) and they generally have a long shelf life. Others, like the soy bean, are also known to contain certain bioactive compounds whose beneficial effects, such as the ability to lower the serum cholesterol *(Sridhar and Seena, 2005*), need to be explored for medicinal purposes. Recent research has revealed that some 'anti-aging' agents or antioxidants can be found in the bean seed coat. Research continues to reveal new attributes of beans; it has also been shown that beans have a perfect nutrient base for people interested in weight loss. They also aid in reducing cholesterol, improve digestion and, as already mentioned, are an aid in cancer prevention (*Eborn, 2001*).

2.1.2 The downside of legumes

Typically, legumes have been associated with flatulence. They cause flatulence when they are digested; by leaving some oligosaccharides that the human intestinal microflora cannot digest (*Sridhar and Seena, 2005*). These oligosaccharides then serve as food for the bacteria, resulting in an increase in their metabolic activities, and methane and hydrogen sulfide gas production which causes flatulence. However, boiling in water and sprouting of legume seeds decreases oligosaccharide content, and thus reduces flatulence significantly (*Sridhar and Seena, 2005*). Reports from Bösterling and Quast, (1981) have also shown slowed digestion of the Bambara groundnut as a result of the presence of trypsin inhibitors while Kato, *et al.*, (1987) have also reported reduced solubility of its crude proteins which leads to poor emulsion and foaming properties when used for food application.

2.2 The Bambara groundnut

The Bambara groundnut also referred to as the Bambara beans is a herbaceous, intermediate, annual plant, with creeping stems at ground level. The pods usually develop underground, and may reach up to 3.7 cm, depending on the number of seeds they contain. Mature pods are indehiscent, often wrinkled, ranging from a yellowish to a reddish dark brown colour. Seed colour also varies, from white to creamy, yellow, brown, purple, red or black (*Karikari, 1971*).

2.2.1 A complete nutritional profile of the Bambara groundnut

The Bambara groundnuts have a carbohydrates content of approximately 54.5-69.3%, a proteins-content of 17-24.6% with levels of the essential sulphur-containing amino acid, methionine, higher than that found in most other legumes (*Linnemann, 1990; Brough and Azam-Ali, 1992*), a fats-content of 5.3-7.8%, and calories of about 36 - 414 Kcal per 100g. The beans are also a good source of fibre, calcium, iron and potassium and have the potential for providing a balanced diet in areas where animal protein is expensive and the cultivation of other legumes is economically risky due to unfavourable environmental conditions. The red-coloured type of the beans could be useful in areas where iron deficiency is a problem as they contain almost twice as much iron as the cream-coloured (*de Kock, 2004*).

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2.2.2 Traditional uses of the Bambara groundnuts

The Bambara is eaten either boiled fresh or grilled while still immature. In Ghana, for instance, the fresh beans are boiled with a little pepper and salt, and sugar to taste sometimes, and served with fried ripe plantain, usually as lunch. The beans used to be canned in gravy by the GIHOC cannery-Nsawam, Ghana (*Karikari, 1971; Begemann, 1986*).

In Côte d'Ivoire, the beans are milled into flour with the aim of enhancing digestibility and used to bake small flat cakes or biscuits; while in some parts of East Africa, they are roasted, pulverized, and used to make soup (*Heller et al., 1995*), with or without other condiments. Bambara flour bread has been reported in Zambia as well (*Linnemann, 1987) and*. many local foods, such as 'Akara', 'moin-moin' and 'okpa' which are common Nigerian varieties are made from ground Bambara beans (*Obizoba, 1983*).

A trial of Bambara groundnut milk was carried out which compared its flavour and composition with milks prepared from the "superior legumes" - soybean, cowpea, and pigeon pea (*Brough et al., 1993*). The Bambara groundnut milk was ranked first, but the other milks were more familiar as they were already on the market and therefore more readily acceptable. However, the lighter colour of the Bambara groundnut milk made it somewhat more preferable during the trial.

2.3 Proteins

Cantor and Schimmel (*1980*) have described proteins as complex macromolecules that may constitute 50% or more of the dry weight of living cells. Proteins play a fundamental role in the structure and function of cells. These biopolymers are usually made up of carbon, hydrogen, oxygen, nitrogen and sulphur while others may contain iron, copper, phosphorus or zinc. Complete hydrolysis (acid, alkaline or enzymatic) of proteins produces α-amino acids of L-configuration, which differ from one another by the nature of their side chains (*Cantor and Schimmel, 1980*). For most proteins, the component amino acids belong to the 20 different kinds which are linked together by peptide bonds, forming polypeptide chains containing up to several hundreds of units of amino acid residues.

The primary structure of proteins have been described by Neurath and Hill (*1979*) as the sequential order of amino acids within those proteins, the secondary and tertiary structures however related to the three-dimensional organization of the polypeptide chain while the quaternary structure refers to the geometric arrangement among various polypeptide chains and these chains linked together by bonds that in most cases are not covalent. Proteins according to Neurath and Hill (*1979*) possess an extraordinary diversity of functions which allows them to be classified into three main categories, namely, structural proteins, proteins with biological activity, and food protein.

2.3.1 Food proteins

According to Neurath and Hill (*1979*), food proteins in particular, do not represent any unique group because many of the structural or biologically active proteins are food proteins. They are simply those that are edible, palatable, digestible, nontoxic and available economically for humans. Food proteins are believed to be largely responsible for functional properties, such as foaming, emulsification, nitrogen solubility, oil and water absorption (*Kinsella and Shetty, 1979*). However, the production of food proteins in sufficient amounts has for long remained a global problem, especially since they are more expensive to produce than carbohydrates or lipids. In order to satisfy this steadily growing demand for proteins, a school o f thought suggests that there is the need to find and exploit to the fullest potential, unconventional protein sources, such as those from the Bambara groundnut, and ultimately develop new methods for their technological applications.

2.4 Food protein functionality

Food functionality in general has been defined by Kinsella (1977), as any property of a food or a food ingredient that affects its application, except its nutritional properties. Most functional properties affect the sensory characteristics of foods, especially, the textural characteristics, but can

also play a major role in the physical behaviour during preparation, processing or storage of the foods. Specifically, the functional properties of food proteins are those physicochemical properties that enable those proteins to impart their desirable characteristics to the whole multi-component food system of which they are part (*Kinsella, 1977*). Studies have indicated that protein functionality basically revolves around the structural features (amino acid composition, primary sequence and conformation), molecular properties (size, shape and charge distribution) and particularly the secondary interactions between and among molecules (intra- and intermolecular bonding respectively). Therefore detailed knowledge of these intrinsic properties as related to the structure and function will enable the Food Scientist to manipulate food proteins to perform specific and targeted functions in food systems (*Kinsella, 1982*).

Listed below are some of the most important functional properties of proteins to consider during the development of some common food product.

Table 2.1: Functional Properties of Proteins in Foods (*Kinsella, 1982*)

Type of food	Functional properties of protein constituent
Beverages	Solubility at different pHs, heat stability, viscosity.
Soups and sauces	Viscosity, emulsification, water retention.
Dough products	Formation of a matrix and film with viscoelastic properties, cohesion.
Baked products	Heat denaturation, water absorption, emulsification, foaming, browning and gelation.
Dairy product	Emulsification, fat retention, viscosity, foaming, gelation and coagulation.
Egg substitutes	Foaming and adhesion.
Meat products	Emulsification, gelation, cohesion, water and fat absorption and retention.
Meat extenders	Water and fat absorption and retention, insolubility, hardness,

	chewiness, cohesion and heat denaturation.
Food coatings	Cohesion and adhesion.
Confectionary products	Dispersability and emulsification.

The ultimate aim of the Food Scientist is to provide adequate and concrete explanations to the functional behavior of food proteins, but the current limited knowledge coupled with the complex nature of various multi-component food systems undermines the clear understanding of how a particular protein will typically affect the final food functionality. One most challenging issue is the fact that in most cases, the initial protein structure in the food system is inevitably modified as the food is processed into the final consumer product (*New Notes, 1982*).

Notwithstanding these major challenges, the functional properties of food proteins have since been classified into three main groups.

Table 2.2: Classification of the Functional Properties of Food Proteins (*Fox, 1982*).

Functional Properties	Composition of functional properties
Hydration properties	Encompasses properties as water absorption and retention, swelling, adhesion, dispersability, solubility and viscosity.
Protein-protein interaction properties	Operative during occurrences as precipitation, gelation and formation of various structures as protein dough and fibre.
Surface active properties	These relate primary to surface tension, emulsification and foaming characteristics.

According to Fox (*1982*), these three groups are not totally independent of each other. For instance, gelation involves not only protein-protein interactions but also protein-water interactions; and, viscosity and solubility both depend on protein-water and protein-protein interactions. Also, protein manifests functionality by interacting with other components within the food system. These interactions may involve solvent molecules, solute molecules, other protein molecules or substances that are dispersed in the solvent such as oil or air (*Branden and Tooze, 1991*).

The Food Scientist once again is faced with the challenge whereby the proteins being used in food systems possess the tertiary structures and conformations that presumably have evolved to perform only specific biological functions but not necessarily designed for the needed specific functional application in food systems (*Kinsella, 1982*). This has inevitably made the physical properties of particular food proteins in several instances, dictate the manner and way in which they should be used (*Kinsella, 1982*). For instance, the peculiar nature of milk proteins but no other, under appropriate conditions allows the formation of a curd from whence the cheese products evolved. Similarly, in the case of soy protein, their ability to coagulate in the presence of calcium facilitated the manufacture of *tofu.* The particular composition and conformation of egg white proteins made it the premier and still the traditional whipping protein and led to the evolution of many foam-based foods. On the other hand, most vegetable crude proteins have been reported to have reduced solubility (*Kato, et al., 1987*) giving these proteins very poor abilities to foam and to serve as emulsifying agents therefore, unlike the egg and soy proteins, cannot be incorporated into foods that require such protein functionality.

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2.5 Enhancement of food protein functionality – A current trend in Food Technology

As pointed out by Mitchell and Ledward (1986), many past developments of fabricated foods have been as a result of inspired creativity and trial and error manipulation of ingredients with little understanding of the underlying science. Current developments within the Food Science world however, have enabled the formulation of novel food products which have served their purposes in the consumer world. One such excellent endeavor which has been very beneficial to vegetarians and most health-conscious consumers is the simulation of the texture of meat using other sources of proteins, particularly soy proteins (*Kinsella, 1982*) and a new biodegradable plastic material

made of a protein matrix and starch which have been extracted from a bean variety (*Kober et al., 2007*).

2.5.1 Biological modification

More specific food protein modification methods to facilitate their use in nutritional, medical, and cosmetic applications include proteolytic treatments to produce protein hydrolysates with a degree of hydrolysis of less than 10% (limited proteolysis). This method may result in improved functional properties (*Mannheim and Cheryan, 1992; Sule, Tomoskozi, and Hajos, 1998*). Pedroche *et al.*, (2004) enhanced some functional properties of protein isolates from *Brassica carinata* seeds using this type of protein hydrolysis. The protein hydrolysate they obtained using 0.72 UA of alcalase/g protein had a very good solubility in a wide range of pH, from 2 to 12, and a high fat absorption capacities due to exposure of hydrophobic groups during the proteolytic process. However, this improved solubility did not translate into an improvement of foaming and emulsifying properties probably because the peptides generated were too small to stabilize the air/water and oil/water interfaces.

Similarly, Ruiz-Henestrosa *et al.*, (2008) studied the effect of limited enzymatic hydrolysis on the interfacial (dynamics of adsorption and surface dilatational properties) and foaming (foam formation and stabilization) characteristics of a soy globulin (glycinin, fraction 11S). Varying the degree of hydrolysis and soy protein concentrations, results revealed that hydrolysates with the low degree of hydrolysis had improved foaming capacities and stabilities, especially at pH close to the isoelectric point (pI). The water insoluble gluten of wheat is one of the major limitations for its more extensive use in food processing. However, in a research conducted by Kong Xiang Zhen *et al.*, (*2007*), wheat gluten was enzymatically hydrolyzed by several commercially available proteases and results showed a remarkable increase in both emulsifying and foaming properties

compared to the original gluten. On a whole, this method of modification, although specific and accurate, may be tedious and expensive.

2.5.2 Chemical modification

Krause (2001) exhaustively modified protein isolates from rapeseed using three chemical procedures; acetylation, succinylation and phosphorylation and consequently studied the protein solubility, adsorption kinetics, surface pressure and surface potential of monolayer, wetting and foaming properties. Results from his study suggested that the chemical modifications distinctly increased the solubility and the foam capacity and foam expansion of the rapeseed protein within the alkaline pH range. Acetylation and phosphorylation however increased the reduction of the surface tension and increased foam stability whereas an overall improved pressure transformation and increase in surface potential in monolayer after all modifications was observed, while the highest changes were achieved after succinylation.

Chemical modification although a very effective tool to investigate the structure-function relationship of proteins is mostly useful in producing tailored proteins but for non-food applications (*Kinsella and Shetty, 1979*).

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2.5.3 Physical modification

Various studies using gamma irradiation as a preservation and functional modification tool in many food systems have been done. Examples of such food systems include starch extrudates of maize (*Sockey and Chinnaswamy, 1992*), cowpea flours and pastes (*Abu et al., 2004*), and soy protein isolate films (*Lee et al., 2004*), not to mention but a few. Specifically, cowpea flours and pastes were irradiated at 2, 10 and 50 kGy and analyzed for their functional properties by Abu *et*

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al., (2005). Results showed that most of the protein-related functional properties of cowpea flours and pastes were not affected at low dose irradiation (2 kGy). At 10 and 50 kGy, however, all protein-related functional properties, except for water absorption capacity, were significantly ($p<0.05$) affected. Nitrogen solubility index decreased significantly ($p<0.05$), probably due to protein denaturation and/or aggregation, whereas oil absorption capacity increased significantly ($p<0.05$), possibly due to exposure of previously buried non-polar protein sites. Starch-related functional properties, such as swelling and pasting properties, were also decreased significantly ($p<0.05$) in a dose-dependent manner, most likely due to starch degradation.

Similar work done by Al-Kaisey *et al.,* (2003) to determine the effect of cobolt-60 gamma irradiation on the antinutritional factors in broad bean showed that trypsin inhibitor activity was reduced by 4.5%, 6.7%, 8.5% and 9.2% at 2.5, 5, 7.5 and 10 kGy, respectively. Meanwhile, irradiation at 10.2, 12.3, 15.4 and 18.2 kGy reduced the phytic acid content. The flatulence causing oligosaccharides were also decreased significantly ($p<0.05$) as the radiation dose increased.

In their work, Kang *et al.,* (1999) modified corn starches using cobolt-60 gamma irradiation together with four different kind of inorganic peroxides. The addition of inorganic peroxides without gamma irradiation or gamma irradiation without the addition of inorganic peroxides effectively decreased initial viscosity, but did not sufficiently keep viscosity stable, meaning that a more functional modified starch with low to moderately high viscosity as well as with sufficient moderate viscosity stability can be produced by controlling the dose levels of the irradiation and the amount of added peroxide.

β-glucan with high viscosity and low solubility poses several impediments to being applied widely in food industries. However, results of a study conducted by Byun et al., (2008) on the effect of

^{60}Co gamma irradiation (10, 30 and 50kGy) on the physical and structural properties of β-glucan revealed that the irradiation process improved the solubility and decreased the viscosity of the homopolysaccharide by the radiolysis of the glycosidic bonds, and this effect was dependent upon the absorbed dose. Conclusively gamma irradiation could be a useful tool in commercial processes as an effective method to resolve the physical problems involved in the use of polymers with high viscosity and low solubility such as the β-glucan.

In much the same way, Rheological characteristics of some food hydrocolloids processed with ^{60}Co gamma irradiation have been studied extensively by Mahmut, Kayacier and Ic (2007). It was however concluded from the results of this study that irradiation significantly ($p<0.005$) affected the consistency indices and particularly decreased the apparent viscosities of the hydrocolloids, enhancing their functional performance for their specific food applications.

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Some researchers (*Bhattachary and Jena, 2007*) have used microwave treatment to alter gelling behaviours of defatted soybean flour dispersions. Defatted soybean flour dispersions of different solid concentrations (15–30%) were subjected to varying time of heating between15–75 seconds in a microwave and gels were characterized by texture measurement (penetration), dynamic testing (oscillation) and by sensory assessment. Results from this work showed that the gel strength, storage modulus, and complex viscosity of the legume flour were enhanced with increasing solid concentration and/or heating time. Other researchers (*Hua et al., 2005*) have used heat up to 95ºC for 15 minutes to form thermal-induced soy protein gels with improved functionality.

2.5.4 Alternative means of modification

Various studies have been carried out with the aim of determining the effects of various extraction techniques and conditions on the functional properties of protein isolates. The functional properties of pigeon pea and cowpea protein isolates were determined as a function of extraction technique

and pH conditions of the extraction medium by Mwasaru *et al.*, (1996). The isolates extracted using the micellization technique (MP) showed significantly ($P<0.05$) higher solubility than those extracted using the isoelectric point precipitation technique (IP) and, for the latter, solubility was negatively correlated with the extraction pH. Results from their study again indicated that MP technique of protein extraction gave higher oil absorption capacities and emulsifying activities than the PI technique for both cowpea and pigeon pea protein isolates. However, the MP technique gave a lower foam expansion for pigeon pea isolates as against the higher foam expansion of the cowpea isolates, both compared to isolates obtained from the alternative technique of extraction.

Other works by Mwasaru *et al.*, (2000) again showed the influence of altered solvent environment on the functionality of pigeon pea (*Cajanus cajan*) and cowpea (*Vigna unguiculata*) protein isolates. Functional properties of pigeon pea and cowpea isolates were determined as a function of pH and NaCl concentrations in this study. At low pH, nitrogen solubility decreased with increasing NaCl concentration but, at high pH, it increased. However, addition of NaCl to the solvent medium resulted in a marginal improvement and a significant improvement in the emulsifying activity and emulsion stability of the pigeon pea isolate, respectively, but the same treatment decreased these properties for the cowpea isolates. It was clear that varying both the pH and NaCl concentrations resulted in significant improvements in the emulsifying and foaming properties as well as the least gelation concentration of the isolates relative to the control treatment. Most food preparations involve solvent environments that contain salt and pH in the range 4 to 8. Such a study is particularly relevant to those situations and in further applications, especially in the domain of product development. Some potential applications include the protein fortification of fruit juices, in which the natural acidic pH will enhance protein solubility, fermented milk products, and fermented vegetables.

In the same light, the influence of preparation methods, that is, alkaline extraction followed by isoelectric precipitation or ultrafiltration, and extraction at a mildly acidic environment and ultrafiltration, on physicochemical and gelation properties of chickpea protein isolates have been reported by Papalamprou *et al.*, (2008) with results revealing that although gelling behaviour depended mainly on the method preparation rather than the protein composition, isolates obtained by ultrafiltration exhibited lower gelling concentrations and gel networks of higher elasticity at protein contents below 12% (w/v).

In another study conducted by Papalamprou and Doxastakis (2005), native lupin, pea, and broad bean protein isolates were used as emulsifiers and stabilizers, in admixture with polysaccharides (xanthan gum), in order to create a rigid and viscoelastic film around the oil or air droplets and/or for creation of network structure, with desirable texture for the benefits of the consumer. Results indicated that the addition of incompatible xanthan gum enhances protein adsorption at air/water and oil/water interfaces, increasing their emulsions and foams stabilities. This may be due to xanthan gum's ability to increase the viscosity of the continuous phase, but also to its effect on protein adsorption at the interface.

Another study to determine the synergistic effect of protein-polysaccharide combinations on enhancement of protein film functionality was conducted by Rhim *et al.*, (1998). In their work, the effect of dialdehyde starch (DAS) on Hunter color values, tensile strength, percentage elongation at break, water vapor permeability, moisture content after conditioning at 50% RH and 25°C for 48 h, and total soluble matter after immersion in water at 25°C for 24 h, of cast soy protein isolate films, was determined. The outcome however revealed that DAS addition increased film yellowness, suggesting occurrence of cross-linking between soy protein isolate and DAS. Further more DAS-containing films, compared to control films, had increased tensile strength, water vapour permeability and moisture content, probably due to water absorption by hydrophilic groups

along the DAS polymer chains. On the whole, DAS showed potential for increasing resistance of soy protein isolate films to breakdown in water, thus improving their functionality in prospective packaging and mulching applications.

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Similarly, soy protein-polysaccharide interactions at air/water interfaces have been studied by Martinez *et al.,* (2006). The aim of this study was to gain knowledge on the interaction between the two biopolymers at the air/water interface under dynamic conditions at neutral pH where a limited incompatibility between macromolecules can occur. Having evaluated the rheological properties of the films with a drop tensiometer, it was clear that the presence of hydroxypropylmethylcellulose and lambda carrageenan used as the surface active and non-surface active polysaccharide systems respectively, greatly increased the surface pressure, surface dilatational elasticity and relative viscoelasticity on the basis of different mechanisms.

2.6 Co-gelation

Reports from Lin (*1977*) have indicated that some types of proteins can form gels when heated together with other proteins, and also, with polysaccharide gelling agents, such as starch, pectin, carrageenan, alginates, and others. For example, the non-specific ionic interactions between positively charged gelatins and the negatively charged alginates or pectates produce gels with relatively high melting point of about 80ºC. In much the same way, specific ionic interactions are known to take place between the positively charged site of K-casein and polysulphated K-carrageenan at the pH of natural milk, making casein micelles entrapped in K-carrageenan gels.

The extent and type of change on a food or food ingredient is largely dependent on the type of modification process administered to it. Ultimately the choice of a modified food protein or any other food biopolymer as an ingredient in any food system would be largely dictated by its

functional properties. It is therefore hopeful that proteins from cheap vegetable sources such as the Bambara groundnut which is free of cholesterol could be suitably modified in much the same way to mimic egg proteins in many food products.

Comment [I W26]: are you suggesting that some plant proteins have intrinsic cholesterol?

2.7 The use of unconventional food materials in new product development

Technology has always allowed us to obtain new products or develop new utilization for existing agricultural raw materials. However, records have shown that it was not until the last quarter of the century that food stuffs of exclusively industrial origin made their appearance, and the indications are that more of such products may appear on the market in the not too distant future. Good examples of such developments, currently, are the appearance of margarine from plant sources other than butter and cheese (from animal sources), and confectionery sugar derived from cereals other than the usual sugar cane and beetroot, while not forgetting the exotic single and double tomato concentrates, commonly referred to as “tomato ketchup”. Also, some novel industrial food products, such as the highly nutritious “pasta”, have been developed with added protein isolates and concentrate as their functional ingredients. There is also a recipe for high protein fruit pie; and, the addition of proteins to effervescent drinks. Such innovations are gradually gaining acceptance as young consumers continue to adapt to and embrace new kinds of foods and drinks.

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Since some of these products involve new utilization of existing and commonly used proteins, it is worth investigating the possible new applications of unconventional proteins in much the same way. There are already signs that the new food products obtained from these unconventional proteins can compete favourably with their already existing counterparts on the (world) food market, and this would help, somewhat, to address the current global food shortages, augment the global protein resource base to meet the world’s current high demand for more protein-based foods

and, ultimately, alleviate poverty, especially, in Africa. However, relative price levels, dietetic qualities, physical and functional properties, taste attributes, and the ease of use should be the main focus for the use of these unconventional protein sources, and then their future market will probably be found in a whole range of products yet unknown.

CHAPTER THREE

3.0 Materials and Method

3.1 Source of Bambara groundnuts

Two 50kg bags each of one variety of Bambara groundnuts were obtained from the Crop Research Institute of the Plant Generic Resource Unit, Bunso, Ghana. The beans were sorted and solar-dried (to 12% moisture content) for a minimum of three days, milled and stored in plastic bags for further analysis.

3.2 Milling and de-fatting of dry beans into flour

The dried beans were milled into fine powder and de-fatting of the seed meal was done using petroleum spirit in a ratio of seed meal to solvent, 1:10w/v, in a large scale Soxhlet's extractor. The de-fatted meal was spread on trays and solar-dried for about two to three hours to expel the volatile extraction solvent and stored in plastic bags for further analysis.

3.3 Protein extraction from defatted Bambara meal

A total of 1.47703 kg (15.98% of isolated proteins) on dry weight basis was extracted from, approximately, 9.2 kilograms of dried defatted Bambara flour. Alkaline extraction of protein from the dried de-fatted meal was done using 0.01M NaOH with a meal-to-solvent ratio of 1:10 w/v, agitated at 150 rpm at room temperature for two hours, using G24 Environmental incubator shaker. Proteins and soluble polysaccharides (oligosaccharides) entered into solution while the insoluble polysaccharides and residues were removed by centrifuging at 2500rpm for 20 minutes. Supernatant obtained after centrifugation was then acidified to a pH range of 4.5-5.0 to allow protein precipitation. The resulting mixture was then centrifuged at 3000rpm for 20 minutes to

Comment [I W30]: remve

separate the proteins from the soluble polysaccharides. The centrifugation process was repeated thrice for any suspended precipitated proteins to dissolve in distilled water, after which the recovered proteins were washed and freeze-dried using the HETO POWER DRY LL300 freeze dryer.

3.4 Starch extraction from dried defatted Bambara flour

Starch was extracted from the solid fibrous portion obtained after the very first round of centrifugation. This portion constitutes the insoluble polysaccharide including cellulose and starch. Pure starch was then obtained by repeatedly washing. Pure starch particles settled after the filtrate was allowed to stand and the recovered starch was solar-dried for about four to five hours to a moisture content of, approximately, 10.440%.

3.5 Irradiation of isolated proteins

The initial total amount of 1.47703 kg of freeze dried proteins was divided into five portions (samples), each weighing 295.41 g. Each of the five samples of isolated proteins was then exposed to 0, 2.5, 5.0, 7.5 and 10.0 kGy of ^{60}Co gamma radiation at 1kGyh^{-1} in a one factor design. The non-irradiated sample was set as control in the subsequent tests.

3.6. Determination of moisture content of samples

The hydration state of the sample (*Simic, 1978*) may affect the final changes that might occur in food sample after the irradiation process, with respect to its functionality. The moisture content of samples was therefore determined by the AOAC (1990) approved method to establish to extent to which the samples were hydrated. Five grams of each protein sample, as well as the isolated starch, were measured into separate crucibles, and their respective gross weights taken. These were then

placed in the oven at 150°C for 24 hours. The moisture content was expressed as percentage loss in weight of sample. The process was repeated for proteins after irradiation.

3.7 Solubility profile of samples

The solubility of each irradiated sample was determined as a function of pH. Such factors as buffer pH, weight of sample, slurry concentration of sample and vortex time, were the variables used to generate a composite design for optimum conditions for the solubility of protein sample, from the Response Surface Methodology from the Design Expert software, version 7.13. After vortexing for appropriate time under the specific conditions of buffer pH and slurry concentrations, protein solutions were then centrifuged at 3000xg for 20 minutes. The filtrates containing soluble proteins were then decanted into separate centrifuge tubes, labeled accordingly and stored.

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A model solubility profile for proteins was obtained from a standard absorbance curve of Bovine Serum Albumin (BSA) with the Bradford's reagent an R^2 value of 0.968 and y = 6139x-41.80 for the equation of the straight line were generated from the regression graph of mass of Bovine Serum Albumin (BSA) against average absorbance were established. The X and Y values of the equation however, represent the average amount of gamma irradiated proteins in g/ml, and the average absorbance of the protein-Bradford's reagent read at 595.0nm, respectively.

3.8 Water absorption capacity (WAC)

Water absorption capacity was estimated by the method described by Wang and Kinsella (1976) with modification. One gram each of both the irradiated and the non-irradiated samples was suspended in 10ml distilled water in 15ml graduated centrifuge tubes and the weights taken before and after addition of distilled water. Samples were then shaken for 30 minutes and centrifuged at

2500rpm for 25 minutes at room temperature. The freed water was carefully decanted and the weight of test tube plus the content was taken. The density of water was taken as 1.0 g/cm^3. Water absorbed was calculated as the difference between the initial volume of water added to the sample and the volume of the supernatant and WAC was expressed as the volume of water retained by one gram of the sample.

3.9 Oil absorption capacity (OAC)

The oil absorption capacity was measured by the method described by Wang and Kinsella (1976) and also with modification. One gram each of both the irradiated and the non-irradiated samples was suspended in 10ml distilled water in 15ml graduated centrifuge tubes and the weights taken before and after addition of distilled water. Samples were then shaken for 30 minutes and centrifuged at 2500rpm for 25 minutes at room temperature. The freed water was carefully decanted and the weight of test tube plus the content was taken. The density of water was taken as 1.0 g/cm^3. Water absorbed was calculated as the difference between the initial volume of water added to the sample and the volume of the supernatant and WAC was expressed as the volume of water retained by one gram of the sample. Oil absorption capacity was expressed as the volume of oil retained by one gram of the sample with the density of oil 0.890g/cm^3.

3.10 Emulsifying activity (EA) and emulsifying stability (ES)

The procedure described by Volker and Kelin (*1979*) was used for both emulsifying activity and emulsifying stability. Emulsions were prepared with one gram of each protein sample, 50ml distilled water and 50ml vegetable oil. The mixtures were homogenized thoroughly at room temperature for 30 minutes. Each emulsified sample was divided equally into 50ml centrifuge tubes. Content of one 50ml tube was centrifuged directly at 3000xg for 30minutes while the other

centrifuged under the same conditions after heating in a water bath at 80°C for 30 minutes and cooling to 15°C. The heights of the emulsified layers, as a percentage of the total height of the material in the unheated tubes was used to calculate the emulsifying activity and emulsifying stability using appropriate formulae.

3.11 Foaming capacity (FC)

The foaming capacity was determined by the method of Lawhon *et al*. (*1972*) with modifications. 50ml of 0.01M NaOH was added to five grams of protein samples. The mixtures were thoroughly homogenized for 10 minutes using a laboratory homogenizer, L4R model, set at high speed (approximately 10,000rpm) at room temperature. Homogenized samples were then poured into 100ml measuring cylinders and the volume of foam at 30 seconds taken, and the increase in volume of content of cylinder expressed as a percent foam capacity. Procedure was repeated for a five gram egg white sample of the same moisture content.

3.12 Foaming stability (FS)

Foam stability was determined by measuring the decrease in volume of foam as a function of time up to a period of 120 minutes (*Suliman et al., 2006*). The stable foam volumes were recorded at time intervals of 10, 30, 60 and 120 minutes.

3.13 Viscographic analysis

The method of Wosiacki and Cereda (*1989*) was used with only slight modifications. Specific weights of starch-protein combinations depending on the moisture content was suspended in a specific volume of solvent and was analyzed with a Brabender viscoamylograph at constant heating and cooling rates. The equipment was able to increase the temperature and to rotate the

vessel at a fixed rate, 1.5ºC/minute and 75 rpm, respectively. Total procedure included an initial heating phase, from 50 to 95°C, in order to observe the viscosity features as: beginning of gelatinization, maximum viscosity, and start of holding period, start of cooling period, end of cooling period, end of final holding period, breakdown viscosity and setback. The whole system was maintained at 95°C for 30 minutes to observe the resistance of the paste to mechanical stirring; and finally, the cooling phase was kept at 50°C to observe retrogradation. The influence of the different combinations of protein-starch blends was also studied. Results were recorded directly from the equipment as digitized viscoamylograph.

3.14 Statistical analysis

Functional properties were determined at least in duplicates (n=2 and n=3). For all surface functional properties, sample variations effects were analyzed by one-way ANOVA (no blocking), while sample and treatment effects were analyzed by a two-way ANOVA (no blocking), using the GenStat statistical tool (Discovery Edition 3). Significant differences (P <0.05) between means of sample variations and between variations and treatments were determined using standard error of differences (s.e.d.) of means. Correlations of selected functional properties were obtained to estimate the degree of dependency of one functional property on another.

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CHAPTER FOUR

4.0 Results and Discussions

4.1 Defatted Bambara groundnut flour

A total of 10.50kg of the Bambara groundnut flour was defatted using a meal to solvent ratio of 1.5kg:18L and running the large scale Soxhlet's extraction procedure for approximately three hours per batch. An average weight of about 9.20kg (87.62%) of Bambara groundnut meal was obtained after defatting, indicating 12.40% oil extracted.

4.2 Extracted protein

About 1.48 kg (15.98%) of isolated proteins on dry weight basis was extracted from the 9.20 kg of dried defatted Bambara groundnut flour. The initial total amount of 1.48 kg freeze dried proteins was divided into five portions (samples), each weighing 295.41 g.

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4.3 Extracted starch

An amount of 2.54 kg (27.58%) of pure starch was obtained from approximately 9.20 kg of dried defatted Bambara groundnut flour after repeated washing.

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Table 4.1: Percentage proteins and starches on dry weight basis from dried defatted Bambara groundnut meal

Defatted meal (kilograms)	Protein content (kilograms)	Starch content (kilograms)	% proteins	% starch
9.20	**1.48**	**2.54**	**15.98**	**27.58**

4.4 Moisture content of protein sample before and after irradiation

The moisture content of various irradiated protein samples ranged from 5.770%-5.810% (Figure 4.1). Clearly, there were no significant differences (p<0.05) among samples both before and after irradiation, (APPENDIX 5A). This however is an indication that the irradiation procedure had no effect on the moisture content of the samples, evidenced by the negative correlation obtained (r = -0.12).

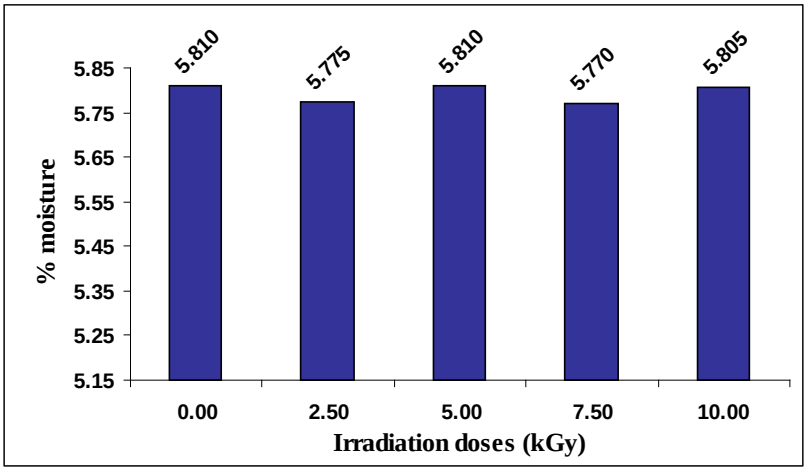

FIGURE 4.1: MOISTURE CONTENT OF MODIFIED PROTEIN SAMPLES

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4.5 Solubility profile of gamma irradiated proteins

Solubility of irradiated proteins generally increased with increasing irradiation doses under all conditions of optimum solubility. Protein-related functional properties, such as protein solubility are, in general influenced by various factors such as protein denaturation, size, structure and conformation, charge, amino acid composition and amino acid sequence of the protein molecules (*Zayas, 1997*). The control sample (0.00kGy) recorded high values for proteins solubility across all conditions for optimum solubility, which are pH, protein slurry concentration and vortexing time.

A sudden drop in solubility then occurred from the 0.00kGy, followed by a steady rise across the doses under most conditions optimum for optimum solubility (Figures 1-17). The high values for solubility of the control sample may be due to increased initial polar sites on protein surfaces which aided hydrogen bonding with water, coupled with the fact that proteins carried more negative and positive charges when placed in the various buffers, aiding once again, hydrogen bonding with water and hence a marked increase in solubility for 0.00kGy irradiated samples. The sudden drop in solubility following irradiation, as in the second observation, may be partly due to irradiation-induced partial protein denaturation or protein-protein aggregation which lead to molecular rearrangements causing the displacement of polar sites on protein surfaces and consequently leading to poor hydrogen bond formation with water. However, as the irradiation dosage increased progressively from 2.50kGy to 10.00kGy, previously hidden hydrophilic sites might have been exposed, leading to increased hydrophilicity and a subsequent increase in solubility (*Urbain, 1986*). The various irradiated protein samples however solubilized between the pH ranges of 5.0 to 9.02. Most food preparations involve solvent environments that contain salt and pH in the range 4.0 to 8.0 (*Mwasaru et al., 2000*).

Figure 4.2: Solubility of modified proteins at buffer pH 6.5, slurry concentration of 1.75g/20ml, and vortex time of 2.00hours

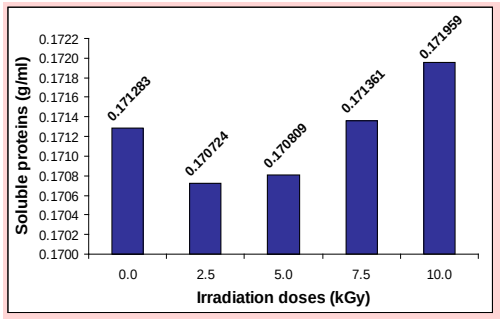

Figure 4.3: Solubility of modified proteins at buffer pH 3.98, slurry concentration of 1.75g/20ml, and vortex time of 2.00hours

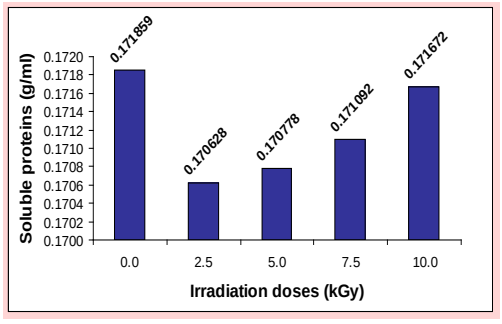

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Figure 4.4: Solubility of modified proteins at buffer pH 5, slurry concentration of 1.00g/20ml, and vortex time of 3.00hours

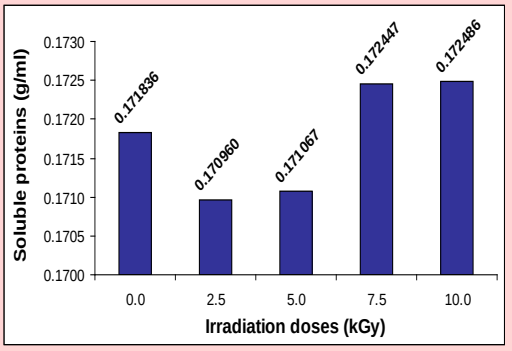


Figure 4.5: Solubility of modified proteins at buffer pH 8, slurry concentration of 2.50g/20ml, and vortex time of 1.00hour

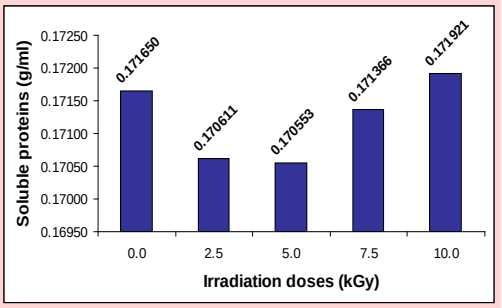


Figure 4.6: Solubility of modified proteins at buffer pH 6.5, slurry concentration of 1.75g/20ml, and vortex time of 4.00hours

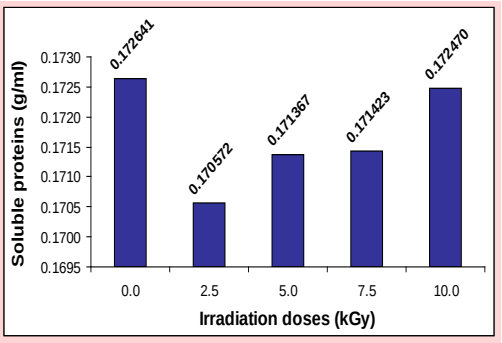


Figure 4.7: Solubility of modified proteins at buffer pH 6.5, slurry concentration of 3.01g/20ml, and vortex time of 2.00hours

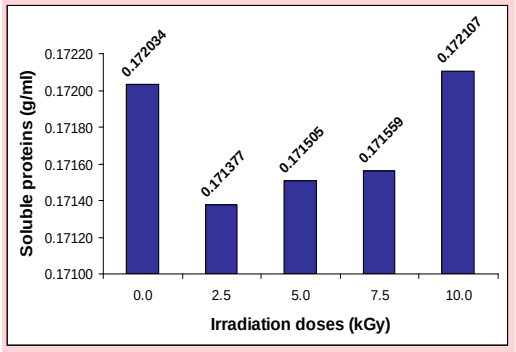


Figure 4.8: Solubility of modified proteins at buffer pH 5, slurry concentration of 2.50g/20ml, and vortex time of 3.00hours

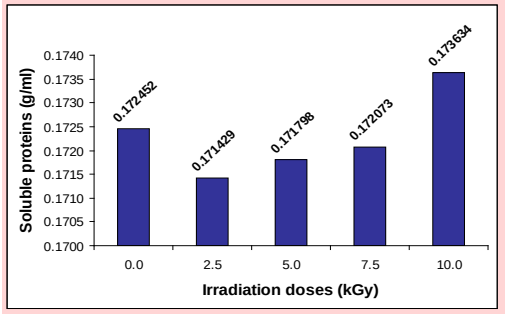


Figure 4.9: Solubility of modified proteins at buffer pH 6.5, slurry concentration of 0.50g/20ml, and vortex time of 2.00hours

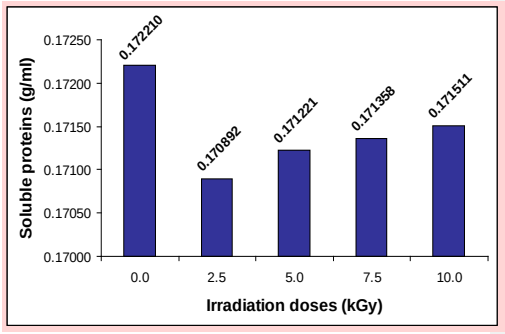


Figure 4.10: Solubility of modified proteins at buffer pH 8, slurry concentration of 1.00g/20ml, and vortex time of 3.00hours

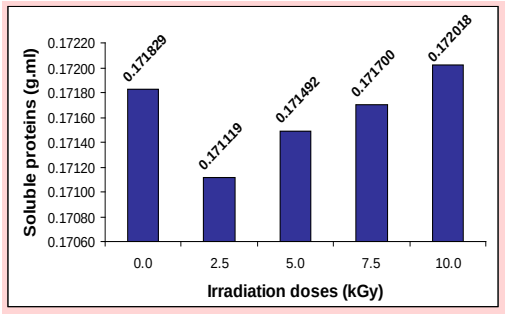


Figure 4.11: Solubility of modified proteins at buffer pH 5, slurry concentration of 1.00g/20ml, and vortex time of 1.00hour

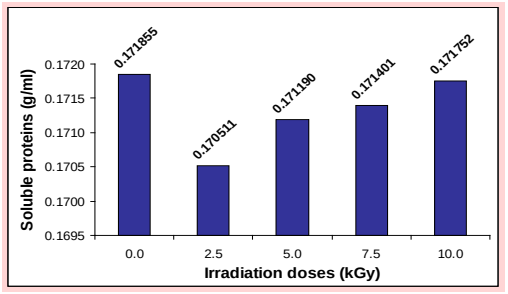


Figure 4.12: Solubility of modified proteins at buffer pH 9.02, slurry concentration of 1.75g/20ml, and vortex time of 2.00hours

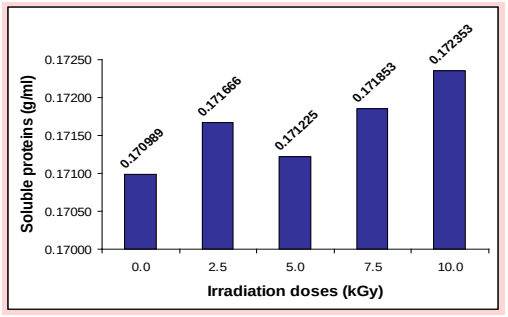


Figure 4.13: Solubility of modified proteins at buffer pH 8, slurry concentration of 2.50g/20ml, and vortex time of 3.00hours

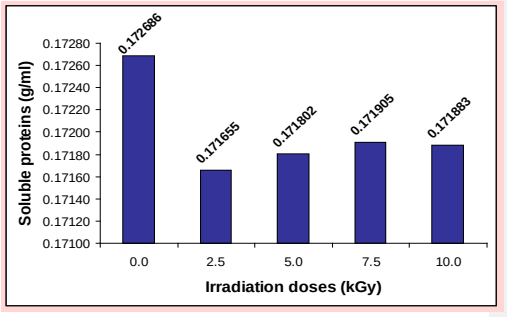


Figure 4.14: Solubility of modified proteins at buffer pH 5, slurry concentration of 2.50g/20ml, and vortex time of 1.00hour

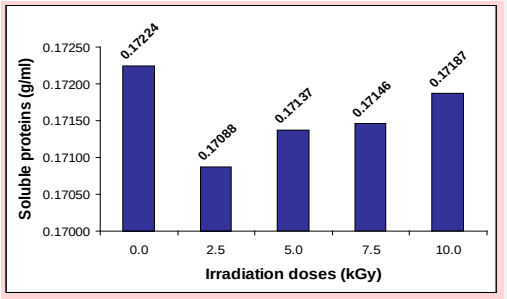


Figure 4.15: Solubility of modified proteins at buffer pH 8, slurry concentration of 1.00g/20ml, and vortex time of 1.00hour

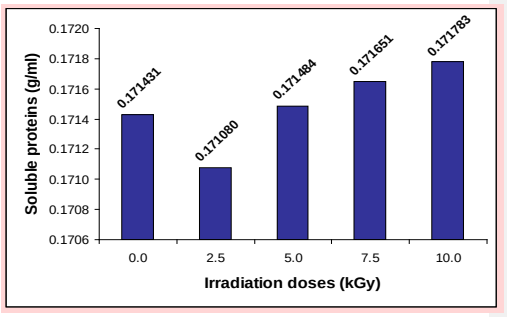


Figure 4.16: Solubility of modified proteins at buffer pH 6.50, slurry concentration of 1.75g/20ml, and vortex time of 30 minutes

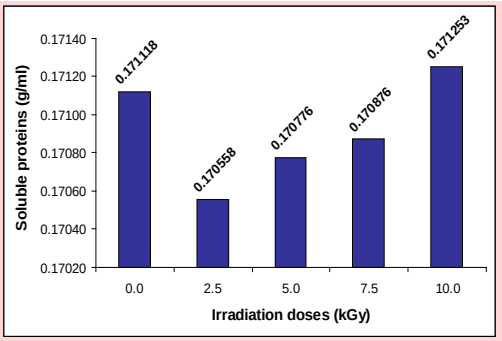
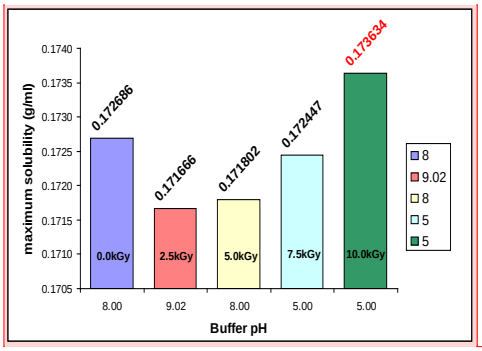


Figure 4.17: Maximum solubility of modified proteins optimum buffer pH



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4.6 Water and Oil absorption capacities

Water and oil absorption generally increased across irradiation doses with the 10.00kGy irradiated sample recording the highest for both WAC and OAC (Figures 4.19 and 4.20). The increase in the WAC may be as a result of the coupled effect of water adsorption via polar binding sites distributed over protein surface, and the denaturation of proteins following irradiation (*Privalov, 1979*). Increases in OAC following irradiation have been recorded elsewhere for cowpea flours and pastes (*Abu et al., 2005*). However, lipophilic tendencies of samples increased with increasing irradiation probably due to more exposed hydrophobic sites as scission and rearrangement of polypeptide occurred following a progressive increase in irradiation doses. Figures 4.19 and 4.20 indicate higher for WAC than OAC for both treated and untreated samples, estimating a *hydrophilic:hydrophobic* ratio of approximately *64.64%:35.36%*. However, OAC values did not vary significantly (p<0.05) between any two successive modifications probably due mainly to the narrow range of irradiation dosage.

Comment [I W42]: How does denaturation affect water absorption? Explain please.

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FIGURE 4.18: WATER ABSORPTION CAPACITY OF BAMBARA PROTEINS

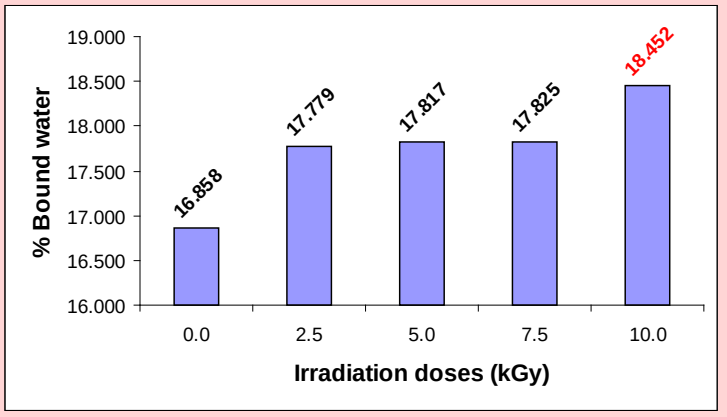
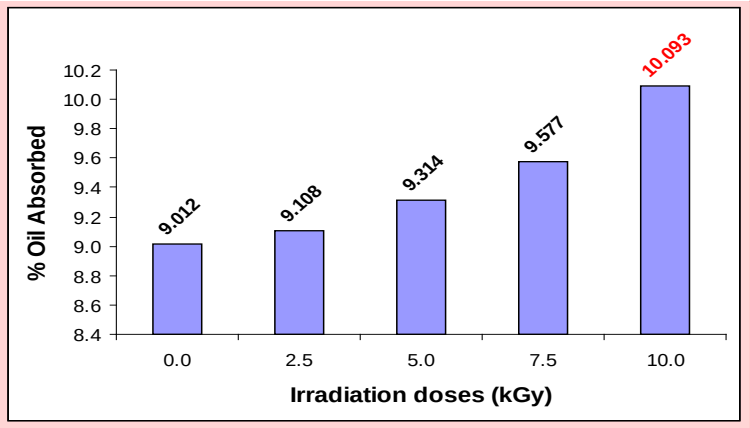


FIGURE 4.19: OIL ABSORPTION CAPACITY OF MODIFIED BAMBARA PROTEINS



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4.7 Foaming Capacity (FC) and Foam Stability (FS)

Irradiation caused a progressive increase in FC for all protein samples (Figure 4.21). This observation may be due, in part, to increased diffusion of fragmented proteins toward the air/water interface caused by increased solubility following irradiation. A positive correlations (r = 0.56) between foaming properties and protein solubility, such as was observed in Bambara proteins have been reported elsewhere for cowpea (*Okaka and Potter, 1979*) and winged bean flours (*Narayana and Rao, 1982*). However the egg white which has excellent foaming properties therefore often used as the standard, recorded the highest FC value. The 10.00kGy irradiated sample which recorded 80.00% FC exhibited a fairly high ability to foam, therefore could serve as much a foaming agent as the egg white in confectionery products such as cakes, meringues, marshmallows, bread, etc. Foams of irradiated proteins were less stable than the egg white foam which had a high viscosity of the liquid bulk phase, but generally, FS increased with increasing doses except that, foams of 2.50kGy protein recorded higher stability values compared to foams of 5.00kGy over the two-hour period.

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FIGURE 4.20: FOAMING CAPACITIES OF MODIFIED BAMBARA PROTEINS COMPARED WITH EGG WHITE

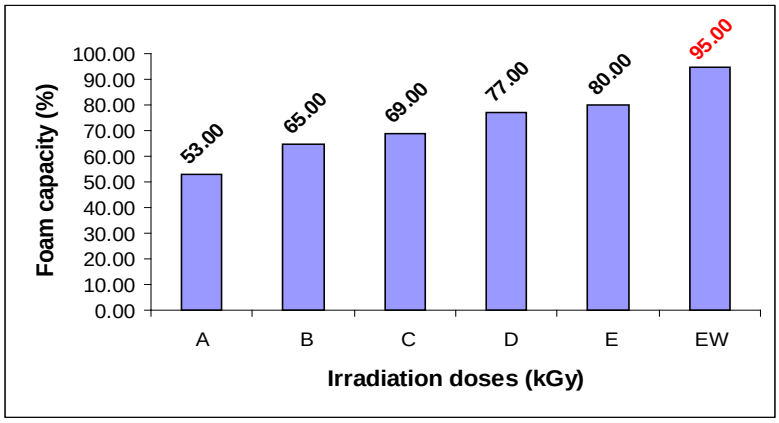
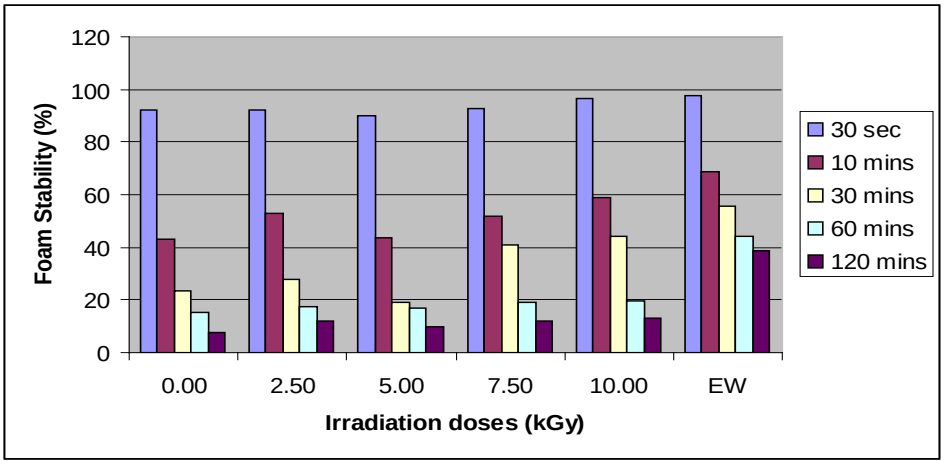


FIGURE 4.21: FOAM STABILITY OF WHIPPED MODIFIED BAMBARA PROTEINS AND EGG WHITE OVER A TWO-HOUR PERIOD



4.8 Emulsifying Activity (EA) and Emulsion Stability (ES)

Superior emulsifying properties are desired to make milk-like beverages and meat analogues (*Friberg, 1976*). Irradiation, at all doses studied, caused a progressive decreases in EA and ES for Bambara protein samples (Figures 4.28 and 4.29), presenting a significant negative correlation (r = -9.8 and r = -0.78) with protein solubility at all doses. With increasing protein solubility following irradiation treatment, increases in EA and ES would have been expected since these two functional properties are known in general to have high positive correlation (*Zayas, 1997*). The reason for the reverse observation may be attributed to the predominantly hydrophilic nature of the proteins resulting from polar active sites that reside on the protein surfaces coupled with additional irradiation-induced increases in the ratio of exposed hydrophilic:hydrophobic amino acid residues of the proteins samples, thereby causing the bulk of the adsorbed protein molecules to reside within the water side of the interface, hence the reduced ability to stabilize the emulsions produced (*Friberg, 1976*). For this same reason of irradiation-induced increases in proteins surface hydrophilicity, a significant negative correlation coefficient (r = -0.62 and r = -0.14) was recorded between OAC and EA and WAC and EA respectively at all levels of irradiation.

As already stated, once a reasonable portion of the proteins comes into contact with the interface, the non-polar amino acids residues orient toward the non-aqueous phase (oil), and with a corresponding decrease in free energy of the system, the remainder of the proteins spontaneously adsorb at the interface. But according to some investigators, the bigger the hydrophilic:hydrophobic ratio of the protein (as in the case of the Bambara proteins,

64.64%:35.36%), the lesser the concentration of proteins at the interface, the higher the interfacial tension and the less stable the emulsions (*Kato and Nakai, 1980*)..

Many theoretical and experimental studies have been carried out on the behaviour of proteins at water/oil or oil/water interfaces. However, much uncertainty remains as to the conformation that proteins adopt at these interfaces and the relationship between initial conformation and conformation at the interface, versus emulsifying or foaming properties (*Philips, 1981*). Again, many similarities exist between emulsions and foam formation, but there is no strict correlation between the emulsifying and foaming abilities of proteins. This perhaps can be attributed to the fact that foam stability has a greater requirement for residual proteins structure than emulsion stability (*Halling, 1981*).

FIGURE 4.22: EMULSIFYING ACTIVITY OF WHIPPED MODIFIED BAMBARA PROTEINS AND EGG WHITE AFTER 120 MINUTES

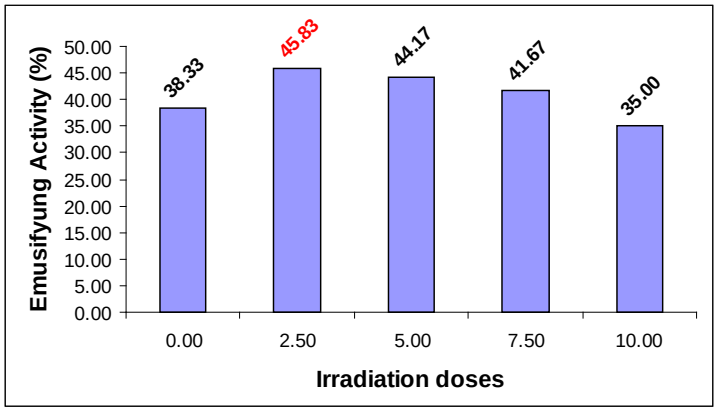


FIGURE 4.23: EMULSION STABILITY OF WHIPPED MODIFIED BAMBARA PROTEINS AND EGG WHITE AFTER 120 MINUTES

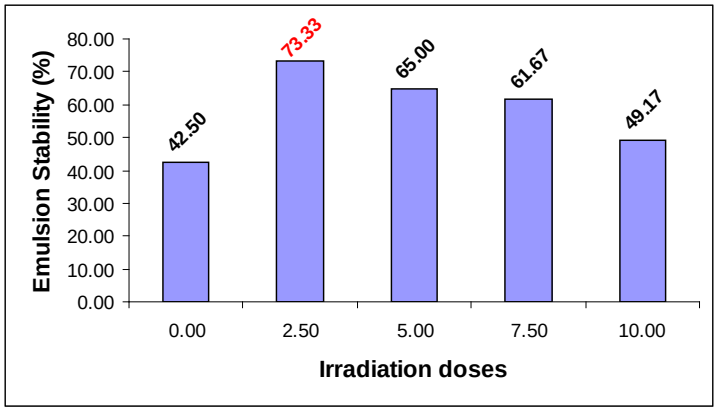


TABLE 4.2: CORRELATION COEFFICIENT *(r)* OF THE FUNCTIONAL PROPERTIES OF BAMBARA GAMMA IRRADIATED PROTEINS

	Functional Properties						
	Solubility	Water Absorption Capacity	Oil Absorption Capacity	Foaming Capacity	Foam Stability	Emulsifying Activity	Emulsion Stability
	Correlation coefficients (*r*)						
Solubility		**0.31**	**0.74**	**0.37**	**0.75**	**-0.98***	**-0.78**
WAC			**0.84***	**0.92***	**0.73**	**-0.14**	**0.31**
OAC				**0.87***	**0.92***	**-0.62**	**-0.21**

Values with asterisks (*) are significantly correlated with each other ($p<0.05$)

4.9 Pasting characteristics of protein-starch admixtures

Gelation of protein is very important in the preparation of puddings, jams and sauces that require thickening and jelling. Some kinds of proteins form gels through interactions with polysaccharides gelling agents such as starch and gelatin (*Lin, 1977*). The pasting temperatures provided an indication of the minimum temperatures required to cook the admixtures. Clearly cooking temperatures for each pasting characteristic did not differ significantly ($p<0.05$) from one another across the different levels of protein irradiation within all three levels of the protein-starch blends (Appendix 10).

However, results generally showed there no dose-dependent significant ($p<0.05$) increases in the gelatinization temperatures within the three levels of irradiated protein-native starch admixtures. However, of the three, the 70P:30S blend, regardless of which irradiated protein used, recorded the highest gelatinization temperatures (Table 4.3 and Figure 4.3). Correlation studies as shown in Tables 4.13 indicate that, a decrease in starch concentration caused a corresponding increase in the gelatinization temperature, while increase in protein concentration correlated positively with rises in gelatinization temperatures right across all three blends. It is worth knowing that a higher gelatinization temperature is just an indication that more energy is required to initiate gelatinization for the same starch.

Maximum viscosity also decreased with decreases and increases in starch and protein concentrations respectively (Table 4.6 and Figure 4.33), evidenced by the strong negative correlation (r = -1.00) between increased protein-starch ratio and maximum viscosity. A significant negative correlation of protein with peak viscosity (r = -0.863, $p<0.01$) of corn flour

have also been observed earlier by (*Sandhu and Singh, 2007*). However, temperatures at which the different blends, each constituted with the five modified proteins, attained maximum viscosities did not differ significantly ($p<0.05$) from each other (Table 4.5 and Figure 4.32). On the other hand, all protein-starch admixtures exhibited an increased viscosity during cooling to 50°C (Appendix 10)

Similarly the strength of the gels formed at start of holding period, start of cooling period, end of cooling and at the end of the final holding time, also recorded decreases with decreasing and increasing starch and protein concentrations respectively with 30P:70S blends recording the strongest gels values (Tables 4.7-4.10). The reason for the observation again could be attributed to the strong positive correlations between increased starch-protein ratios with the parameters mentioned above (Tables 4.15-4.18)

Breakdown viscosity values were higher in the 50P:50S blends compared with the other two blends. However no viscosity breakdown was observed in the 30P:70S blend constituted with 7.50kGy irradiated protein (Table 4.11). Setback viscosity, which is the measure of retrogadation accompanied by syneresis of starch upon cooling of the cooked starch pastes, had an increasing order of 70P:30S < 50P:50S < 30P:70S (44.00, 87.00 and 356.00 BU, respectively). The bulky protein in the 70P:30S blend could have prevented the formation of an ordered structure of the starch paste, thus retarding retrogradation, hence the low value of 44.00 BU.

Conclusively, correlation studies obviously proofs the sole dependence of the pasting properties on the starch concentration within the blends, meaning that the contribution of the Bambara starch to the pasting properties of the blends was greater than that of the irradiated Bambara proteins. This however seem to suggest that the modified proteins were simply unable to form strong gel matrices or gel networks with starches perhaps due to poor ionic interactions between the individual molecules and due also to the improper balance between protein-protein and protein-solvent interactions.

TABLE 4.3: BEGINNING OF GELATINIZATION TEMPERATURES OF BAMBARA PROTEIN-STARCH ADMIXTURES

Beginning of gelatinization Temperature (°C)					
	Irradiation Doses (kGy)				
Blends (%)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
30P:70S	76.40	76.20	76.35	76.45	76.35
50P:50S	78.10	64.15	63.80	77.95	77.40
70P:30S	88.20	86.60	91.50	85.10	86.80

FIGURE 4.240: BEGINNING OF GELATINIZATION TEMPERATURES ADMIXTURES

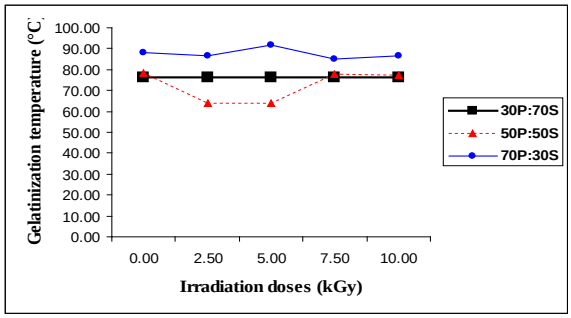


TABLE 4.4: GEL STRENGTH AT BEGINNING OF GELATINIZATION

Gel Strength at Beginning of gelatinization					
	Irradiation Doses (kGy)				
Blends (%)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
30P:70S	80.00	39.00	36.00	26.50	40.00
50P:50S	51.00	12.00	8.00	34.00	42.50
70P:30S	2.00	34.50	51.00	11.00	10.00

FIGURE 4.25: GEL STRENGTH AT BEGINNING OF GELATINIZATION

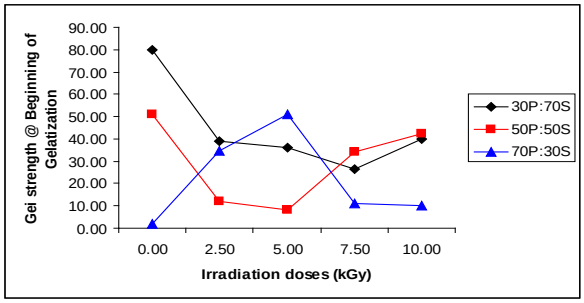


TABLE 4.5: MAXIMUM VISCOSITY TEMPERATURES OF BAMBARA PROTEIN-STARCH ADMIXTURES

Maximum Viscosity Temperature (ºC)					
	Irradiation Doses (kGy)				
Blends (%)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
30P:70S	94.40	94.60	94.60	94.60	94.60
50P:50S	95.10	95.50	95.60	94.60	94.95
70P:30S	94.50	94.60	92.70	94.60	94.50

FIGURE 4.26: MAXIMUM VISCOSITY TEMPERATURES OF BAMBARA PROTEIN-STARCH ADMIXTURES

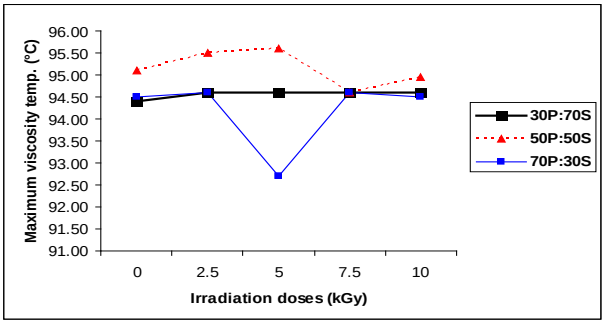


TABLE 4.6: MAXIMUM VISCOSITY

Gel Strength at Maximum Viscosity					
	Irradiation Doses (kGy)				
Blends (%)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
30P:70S	541.00	557.00	575.00	620.00	541.50
50P:50S	314.00	288.00	278.00	279.00	292.00
70P:30S	67.50	85.40	57.00	5.00	34.00

FIGURE 4.27: MAXIMUM VISCOSITY

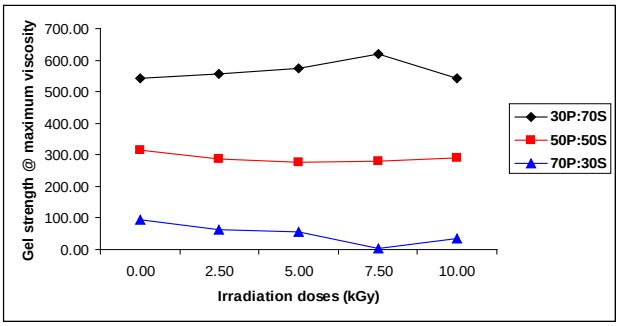


TABLE 4.7: GEL STRENGTH AT START OF HOLDING PERIOD

Gel Strength at start of holding Period					
	Irradiation Doses (kGy)				
Blends (%)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
30P:70S	618.50	522.00	479.00	471.00	461.00
50P:50S	311.00	277.00	271.00	270.00	274.00
70P:30S	105.54	54.00	24.00	26.75	23.00

FIGURE 4.28: GEL STRENGTH AT START OF HOLDING PERIOD

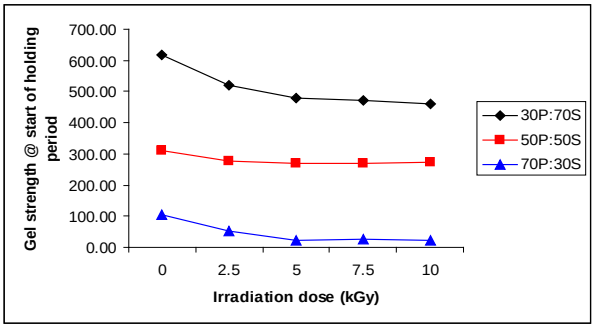


TABLE 4.8: GEL STRENGTH AT START OF COOLING PERIOD

Gel Strength at start of cooling Period					
	Irradiation Doses (kGy)				
Blends (%)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
30P:70S	532.50	554.00	575.50	620.50	541.00
50P:50S	272.00	253.00	255.00	254.00	269.50
70P:30S	70.50	61.50	39.00	2.00	33.00

FIGURE 4.29: GEL STRENGTH AT START OF COOLING PERIOD

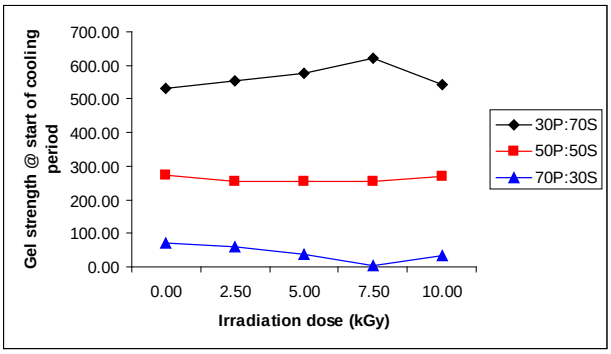


TABLE 4.9: GEL STRENGTH AT END OF COOLING PERIOD TEMPERATURES

Gel Strength at End Of Cooling Period					
	Irradiation Doses (kGy)				
Blends (%)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
30P:70S	862.00	840.00	974.00	997.50	896.00
50P:50S	376.00	356.50	344.00	340.50	351.50
70P:30S	126.00	109.00	82.00	12.00	78.00

FIGURE 4.30: GEL STRENGTH AT END OF COOLING PERIOD TEMPERATURES

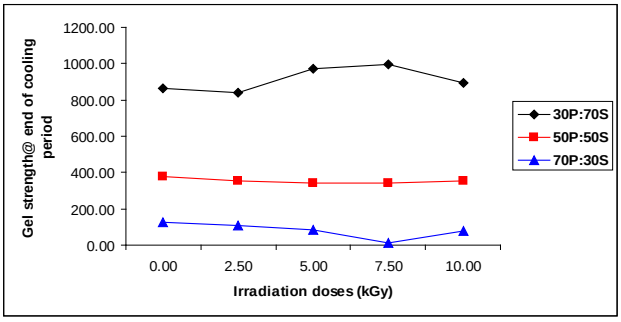


TABLE 4.10: GEL STRENGTH END OF FINAL HOLDING PERIOD

Gel Strength at end of final holding Period					
	Irradiation Doses (kGy)				
Blends (%)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
30P:70S	877.25	821.00	949.00	943.50	882.00
50P:50S	349.00	328.00	323.00	315.00	331.00
70P:30S	118.50	99.00	75.00	10.00	72.00

FIGURE 4.31: GEL STRENGTH END OF FINAL HOLDING PERIOD

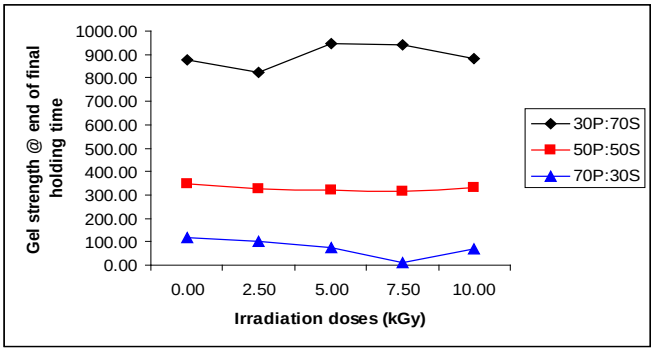


TABLE 4.11: BREAKDOWN VISCOSITY

Breakdown Viscosity					
	Irradiation Doses (kGy)				
Blends (%)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
30P:70S	4.00	4.00	1.00	0.00	2.50
50P:50S	42.00	37.00	21.00	24.00	21.50
70P:30S	1.00	2.00	17.00	1.00	1.00

FIGURE 4.32: BREAKDOWN VISCOSITY

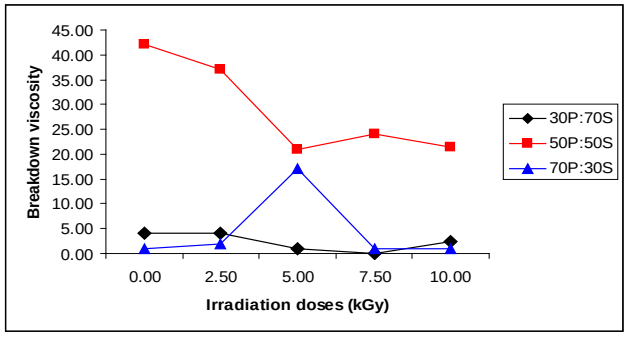


TABLE 4.12: SETBACK VISCOSITY

Setback Viscosity					
	Irradiation Doses (kGy)				
Blends (%)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
30P:70S	288.50	286.00	397.50	375.50	356.00
50P:50S	104.00	104.50	88.00	84.00	87.00
70P:30S	57.00	45.50	42.00	9.00	44.00

FIGURE 4.33: SETBACK VISCOSITY

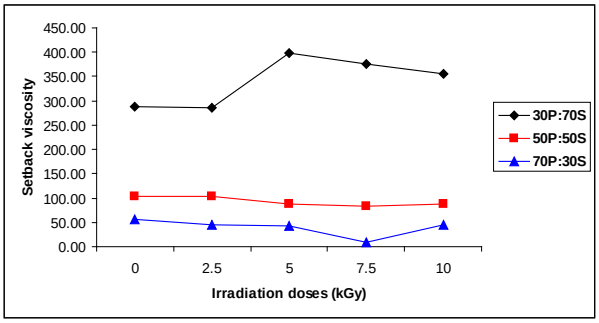


TABLE 4.13: CORRELATION COEFFICIENT *(r)* OF GEL STRENGTH BEGINNING OF GELATINIZATION WITH DECREASING STARCH AND INCREASING PROTEINS ACROSS IRRADIATION DOSES

Beginning of gelatinization temperature (ºC)					
Irradiated proteins within blends (kGy)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**

	Correlation coefficient (***r***)				
Decreasing Starch % 70→50→30	-0.92*	-0.46	-0.55	-0.94*	-0.91*
Increasing Protein % 30→50→70	0.92*	0.46	0.55	0.94*	0.91*

Values with asterisks (*) are significantly correlated ($p<0.05$)

TABLE 4.14: CORRELATION COEFFICIENT *(r)* OF GEL STRENGTH AT MAXIMUM VISCOSITY WITH DECREASING STARCH AND INCREASING PROTEINS ACROSS IRRADIATION DOSES

Gel Strength at Maximum Viscosity					
Irradiated proteins within blends (kGy)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
	Correlation coefficient (***r***)				
Decreasing Starch % 70→50→30	1.00	1.00	1.00	1.00	1.00
Increasing Protein % 30→50→70	-1.00	-1.00	-1.00	-1.00	-1.00

TABLE 4.15: CORRELATION COEFFICIENT *(r)* OF GEL STRENGTH START OF HOLDING PERIOD WITH DECREASING STARCH AND INCREASING PROTEINS ACROSS IRRADIATION DOSES

Gel Strength at start of holding period					
Irradiated proteins within blends (kGy)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
	Correlation coefficient (***r***)				
Decreasing Starch % 70→50→30	0.99	1.00	1.00	1.00	1.00
Increasing Protein % 30→50→70	-0.99	-1.00	-1.00	-1.00	-1.00

TABLE 4.16: CORRELATION COEFFICIENT *(r)* OF GEL STRENGTH START OF COOLING PERIOD WITH DECREASING STARCH AND INCREASING PROTEINS ACROSS IRRADIATION DOSES

Gel Strength at start of cooling period

Irradiated proteins within blends (kGy)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
	Correlation coefficient (***r***)				
Decreasing Starch % 70→50→30	1.00	0.99	0.99	0.99	1.00
Increasing Protein % 30→50→70	-1.00	-0.99	-0.99	-0.99	-1.00

TABLE 4.17: CORRELATION COEFFICIENT *(r)* OF GEL STRENGTH AT END OF COOLING PERIOD WITH DECREASING STARCH AND INCREASING PROTEINS ACROSS IRRADIATION DOSES

Gel Strength at End Of Cooling Period					
Irradiated proteins within blends (kGy)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
	Correlation coefficient (***r***)				
Decreasing Starch % 70→50→30	0.98	0.98	0.97	0.98	0.98
Increasing Protein % 30→50→70	-0.98	-0.98	-0.97	-0.98	-0.98

TABLE 4.18: CORRELATION COEFFICIENT *(r)* OF GEL STRENGTH END OF FINAL HOLDING PERIOD WITH DECREASING STARCH AND INCREASING PROTEINS ACROSS IRRADIATION DOSES

Gel Strength at end of final holding period					
Irradiated proteins within blends (kGy)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
	Correlation coefficient (***r***)				
Decreasing Starch % 70→50→30	0.98	0.98	0.97	0.98	0.98
Increasing Protein % 30→50→70	-0.98	-0.98	-0.97	-0.98	-0.98

TABLE 4.19: CORRELATION COEFFICIENT *(r)* OF BREAKDOWN VISCOSITY WITH DECREASING STARCH AND INCREASING PROTEINS ACROSS IRRADIATION DOSES

Breakdown Viscosity

Irradiated proteins within blends (kGy)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
	Correlation coefficient (***r***)				
Decreasing Starch % 70→50→30	0.07	0.05	-0.76*	-0.04	0.07
Increasing Protein % 30→50→70	-0.07	-0.05	0.76*	0.04	-0.07

Values with asterisks (*) are significantly correlated ($p<0.05$)

TABLE 4.20: CORRELATION COEFFICIENT *(r)* OF SETBACK VISCOSITY WITH DECREASING STARCH AND INCREASING PROTEINS ACROSS IRRADIATION DOSES

Setback Viscosity					
Irradiated proteins within blends (kGy)	**0.00**	**2.50**	**5.00**	**7.50**	**10.00**
	Correlation coefficient (***r***)				
Decreasing Starch % 70→50→30	0.96	0.96	0.92	0.95	0.92
Increasing Protein % 30→50→70	-0.96	-0.96	-0.92	-0.95	-0.92

CHAPTER FIVE

5.0 Conclusion and Recommendations

Irradiation of Bambara protein isolates caused some significant changes associated with their surface functional properties. Solubility, water and oil absorption capacities and foaming properties increased with increasing irradiation doses, whereas emulsifying properties rather decreased with increasing irradiation. The various modified protein samples however solubilized between pH of 5.0 to 9.02, a range in which most food preparations involving solvent environments that contain salt and pH fall within (i.e. pH 4.0 to 8.0).

Pasting characteristics on the other hand increased with increasing starch:protein ratios concluding that proteins could not contribute significantly to pasting properties mainly due to their inability to form strong gel networks with starches. This could be attributed to poor ionic interactions between the individual biopolymers and improper balance between protein-protein and protein-solvent interactions.

The moderately low water and oil absorption capacities of the gamma irradiated could be somewhat useful in flavour retention, improvement of palatability and extension of shelf life especially in meat products. Since foam contributes to smoothness, lightness, flavour dispersions, and the palatability, the moderately high foaming capacity and stability of the modified Bambara proteins could make them potential replacements of egg white proteins in foods like cakes, breads, marshmallow, toppings, and desserts. Again, the moderately high emulsion activity and stability of the non-irradiated Bambara protein sample indicates that native Bambara protein isolates could also be used as ingredients in many food formulations

most especially comminuted meats. Finally, protein-starch blends could also be useful in food systems which require low to moderately high viscosities, example baby foods.

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APPENDICES

APPENDIX 1- FORMULAE USED FOR CALCULATIONS

1. % Moisture = $\frac{\text{(Wet weight – dry weight)} \times 100}{\text{Initial weight of sample}}$

2. % Foam Capacity = $\frac{\text{(Volume after whipping – volume before whipping)} \times 100}{\text{Volume before whipping}}$

3. % Foam Stability = $\frac{\text{foam volume after time (t)} \times 100}{\text{Initial foam volume}}$

4. % Emulsifying Activity = $\frac{\text{height of emulsion} \times 100}{\text{Height of whole layer}}$

5. % Emulsifying Activity = $\frac{\text{height of emulsion after heating} \times 100}{\text{Height of whole layer}}$

6. % Water Absorption Capacity = $\frac{\text{volume of bound water} \times 100}{\text{Initial volume of water}}$

7. % Oil Absorption Capacity = $\frac{\text{volume of bound oil} \times 100}{\text{Initial volume of oil}}$

8. Equation for protein solubility: $Y = 6139X - 41.80$
   Y= absorbance
   X= soluble protein per gram of Bradford's solution

APPENDIX 2 – DATA ON PARAMETERS DETERMINED

APPENDIX 2A: Composite design table for protein solubility at optimum conditions

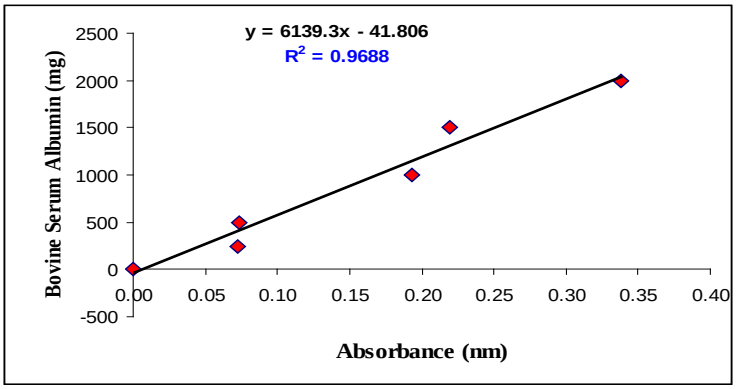
OPTIMUM CONDITIONS FOR PROTEIN SOLUBILITY			SAMPLE CODE
Buffer pH	**Protein slurry (g/20ml)**	**Vortexing Time (hrs)**	
Blank	0.00	0.00	**Blank**
6.50	1.75	2.00	**A1**
3.98	1.75	2.00	**B**
5.00	1.00	3.00	**C**
8.00	2.50	1.00	**D**
6.50	1.75	4.00	**E**
6.50	3.01	2.00	**F**
5.00	2.50	3.00	**G**
6.50	1.75	2.00	**A2**
6.50	0.50	2.00	**H**
8.00	1.00	3.00	**I**
6.50	1.75	2.00	**A3**
6.50	1.75	2.00	**A4**
5.00	1.00	1.00	**J**
9.02	1.75	2.00	**K**
8.00	2.50	3.00	**L**
6.50	1.75	2.00	**A5**
6.50	1.75	2.00	**A6**
5.00	2.50	1.00	**M**
8.00	1.00	1.00	**N**
6.50	1.75	0.50	**O**

** Replicate combinations are for estimation of the standard error*

APPENDIX 2B: A STANDARD ABSORBANCE TABLE FOR BSA

BSA standard	Mass of BSA(microgram)	Volume of BSA(ml)	Volume of water(ml)	Volume of Bradford reagent(ml)	Absorbance A	Absorbance B	Average Absorbance
Blank	**0**	1.000	0.000	2.000	0.000	0.000	**0.000**
1	**250**	1.000	32.000	2.000	0.073	0.071	**0.072**
2	**500**	1.000	16.000	2.000	0.075	0.073	**0.074**
3	**1000**	1.000	8.000	2.000	0.193	0.193	**0.193**
4	**1500**	1.000	5.300	2.000	0.219	0.219	**0.219**
5	**2000**	1.000	4.000	2.000	0.336	0.340	**0.338**

APPENDIX 2C: A STANDARD REGRESSION GRAPH FOR BOVINE SERUM ALBUMIN



APPENDIX 2D: Protein solubility of 0.00kGy irradiated Bambara protein sample

Optimum conditions	PROTEIN SOLUBILITY AT 0.00kGY					
	ABSORBANCE at 595.00nm				SOLUBLE PROTEINS (ml)	
CODE	REP 1	REP 2	REP 3	Average	X1	X2
A1	0.262000	0.261000	0.270000	0.264333	0.006852	0.171300
B	0.412000	0.395000	0.398000	0.401667	0.006874	0.171859
C	0.386000	0.404000	0.398000	0.396000	0.006873	0.171836
D	0.349000	0.351000	0.351000	0.350333	0.006866	0.171650
E	0.610000	0.584000	0.587000	0.593667	0.006906	0.172641
F	0.456000	0.429000	0.449000	0.444667	0.006881	0.172034
G	0.554000	0.544000	0.544000	0.547333	0.006898	0.172452
A2	0.285000	0.270000	0.273000	0.276000	0.006854	0.171347
H	0.482000	0.480000	0.502000	0.488000	0.006888	0.172210
I	0.398000	0.388000	0.397000	0.394333	0.006873	0.171829
A3	0.269000	0.247000	0.255000	0.257000	0.006851	0.171270
A4	0.285000	0.287000	0.292000	0.288000	0.006856	0.171396
J	0.389000	0.404000	0.409000	0.400667	0.006874	0.171855
K	0.182000	0.195000	0.187000	0.188000	0.006840	0.170989
L	0.604000	0.616000	0.594000	0.604667	0.006907	0.172686
A5	0.229000	0.240000	0.238000	0.235667	0.006847	0.171183
A6	0.257000	0.227000	0.235000	0.239667	0.006848	0.171199
M	0.483000	0.518000	0.486000	0.495667	0.006890	0.172242
N	0.288000	0.302000	0.300000	0.296667	0.006857	0.171431
O	0.226000	0.227000	0.206000	0.219667	0.006845	0.171118
BLANK	0.000000	0.000000	0.000000	0.000000	0.006809	0.170223

** X1 = Average absorbance + 41.81*
6139.30
**X1 values are the amount (mg) of soluble proteins in 0.04ml protein solution.*

**X2 = (Average absorbance + 41.81) / 6139.30*
0.04

**X2 values are the amount (mg) of soluble proteins in 1.00ml protein solution*

APPENDIX 2E: Protein solubility of 2.50kGy irradiated Bambara protein sample

Optimum conditions CODED	**PROTEIN SOLUBILITY AT 2.50kGY**					
	ABSORBANCE at 595.00nm				**SOLUBLE PROTEINS (ml)**	
	REP 1	**REP 2**	**REP 3**	**Average**	**X1**	**X2**
A1	0.147000	0.173000	0.175000	0.165000	0.006836	0.170895
B	0.092000	0.103000	0.103000	0.099333	0.006825	0.170628
C	0.176000	0.180000	0.187000	0.181000	0.006838	0.170960
D	0.085000	0.097000	0.104000	0.095333	0.006824	0.170611
E	0.079000	0.090000	0.088000	0.085667	0.006823	0.170572
F	0.276000	0.284000	0.290000	0.283333	0.006855	0.171377
G	0.276000	0.304000	0.308000	0.296000	0.006857	0.171429
A2	0.081000	0.112000	0.113000	0.102000	0.006826	0.170639
H	0.153000	0.165000	0.175000	0.164333	0.006836	0.170892
I	0.211000	0.223000	0.226000	0.220000	0.006845	0.171119
A3	0.110000	0.121000	0.123000	0.118000	0.006828	0.170704
A4	0.101000	0.115000	0.120000	0.112000	0.006827	0.170679
J	0.065000	0.071000	0.076000	0.070667	0.006820	0.170511
K	0.342000	0.362000	0.359000	0.354333	0.006867	0.171666
L	0.344000	0.337000	0.374000	0.351667	0.006866	0.171655
A5	0.075000	0.089000	0.090000	0.084667	0.006823	0.170568
A6	0.141000	0.162000	0.166000	0.156333	0.006834	0.170860
M	0.158000	0.157000	0.165000	0.160000	0.006835	0.170875
N	0.195000	0.216000	0.220000	0.210333	0.006843	0.171080
O	0.082000	0.082000	0.083000	0.082333	0.006822	0.170558
BLANK	0.000000	0.000000	0.000000	0.000000	0.006809	0.170223

APPENDIX 2F: Protein solubility of 5.00kGy irradiated Bambara protein sample

Optimum conditions	PROTEIN SOLUBILITY AT 5.00kGY					
	ABSORBANCE at 595.00nm				SOLUBLE PROTEINS (ml)	
CODE	REP 1	REP 2	REP 3	Average	X1	X2
A1	0.120000	0.130000	0.128000	0.126000	0.006829	0.170736
B	0.134000	0.136000	0.139000	0.136333	0.006831	0.170778
C	0.202000	0.213000	0.207000	0.207333	0.006843	0.171067
D	0.082000	0.080000	0.081000	0.081000	0.006822	0.170553
E	0.275000	0.282000	0.286000	0.281000	0.006855	0.171367
F	0.312000	0.316000	0.316000	0.314667	0.006860	0.171505
G	0.382000	0.388000	0.390000	0.386667	0.006872	0.171798
A2	0.082000	0.092000	0.092000	0.088667	0.006823	0.170584
H	0.244000	0.246000	0.245000	0.245000	0.006849	0.171221
I	0.304000	0.318000	0.313000	0.311667	0.006860	0.171492
A3	0.150000	0.162000	0.162000	0.158000	0.006835	0.170867
A4	0.128000	0.131000	0.137000	0.131000	0.006830	0.170757
J	0.233000	0.239000	0.240000	0.237333	0.006848	0.171190
K	0.247000	0.247000	0.244000	0.246000	0.006849	0.171225
L	0.359000	0.402000	0.402000	0.387667	0.006872	0.171802
A5	0.219000	0.219000	0.222000	0.220000	0.006845	0.171119
A6	0.133000	0.142000	0.145000	0.140000	0.006832	0.170793
M	0.290000	0.266000	0.291000	0.282333	0.006855	0.171373
N	0.304000	0.313000	0.312000	0.309667	0.006859	0.171484
O	0.119000	0.143000	0.145000	0.135667	0.006831	0.170776
BLANK	0.000000	0.000000	0.000000	0.000000	0.006809	0.170223

** X1 = $\frac{\text{Average absorbance + 41.81}}{\text{6139.30}}$*

**X1 values are the amount (mg) of soluble proteins in 0.04ml protein solution.*

**X2 = $\frac{\text{(Average absorbance + 41.81) / 6139.30}}{\text{0.04}}$*

**X2 values are the amount (mg) of soluble proteins in 1.00ml protein solution*

APPENDIX 2G: Protein solubility of 7.50kGy irradiated Bambara protein sample

Optimum conditions	PROTEIN SOLUBILITY AT 7.50kGY					
	ABSORBANCE at 595.00nm				SOLUBLE PROTEINS (ml)	
CODE	REP 1	REP 2	REP 3	Average	X1	X2
A1	0.242000	0.282000	0.279000	0.267667	0.006853	0.171313
B	0.215000	0.206000	0.219000	0.213333	0.006844	0.171092
C	0.526000	0.563000	0.549000	0.546000	0.006898	0.172447
D	0.268000	0.285000	0.289000	0.280667	0.006855	0.171366
E	0.272000	0.314000	0.298000	0.294667	0.006857	0.171423
F	0.330000	0.334000	0.320000	0.328000	0.006862	0.171559
G	0.442000	0.459000	0.462000	0.454333	0.006883	0.172073
A2	0.266000	0.277000	0.296000	0.279667	0.006854	0.171362
H	0.274000	0.276000	0.286000	0.278667	0.006854	0.171358
I	0.337000	0.368000	0.383000	0.362667	0.006868	0.171700
A3	0.249000	0.297000	0.304000	0.283333	0.006855	0.171377
A4	0.209000	0.199000	0.196000	0.201333	0.006842	0.171043
J	0.255000	0.306000	0.307000	0.289333	0.006856	0.171401
K	0.392000	0.407000	0.402000	0.400333	0.006874	0.171853
L	0.393000	0.424000	0.422000	0.413000	0.006876	0.171905
A5	0.296000	0.290000	0.274000	0.286667	0.006856	0.171391
A6	0.339000	0.373000	0.361000	0.357667	0.006867	0.171680
M	0.292000	0.313000	0.309000	0.304667	0.006859	0.171464
N	0.332000	0.351000	0.369000	0.350667	0.006866	0.171651
O	0.143000	0.173000	0.165000	0.160333	0.006835	0.170876
BLANK	0.000000	0.000000	0.000000	0.000000	0.006809	0.170223

** X1 = $\frac{\text{Average absorbance + 41.81}}{\text{6139.30}}$*

**X1 values are the amount (mg) of soluble proteins in 0.04ml protein solution.*

**X2 = $\frac{\text{(Average absorbance + 41.81) / 6139.30}}{\text{0.04}}$*

**X2 values are the amount (mg) of soluble proteins in 1.00ml protein solution*

APPENDIX 2H: Protein solubility of 10.00kGy irradiated Bambara protein sample

Optimum conditions	PROTEIN SOLUBILITY AT 10.00kGY					
	ABSORBANCE at 595.00nm				SOLUBLE PROTEINS (ml)	
CODE	REP 1	REP 2	REP 3	Average	X1	X2
A1	0.324000	0.348000	0.360000	0.344000	0.006865	0.171624
B	0.338000	0.349000	0.380000	0.355667	0.006867	0.171672
C	0.575000	0.522000	0.570000	0.555667	0.006899	0.172486
D	0.408000	0.413000	0.430000	0.417000	0.006877	0.171921
E	0.539000	0.550000	0.566000	0.551667	0.006899	0.172470
F	0.463000	0.468000	0.457000	0.462667	0.006884	0.172107
G	0.746000	0.881000	0.886000	0.837667	0.006945	0.173634
A2	0.522000	0.496000	0.517000	0.511667	0.006892	0.172307
H	0.321000	0.321000	0.307000	0.316333	0.006860	0.171511
I	0.420000	0.445000	0.457000	0.440667	0.006881	0.172018
A3	0.497000	0.511000	0.500000	0.502667	0.006891	0.172270
A4	0.396000	0.402000	0.413000	0.403667	0.006875	0.171867
J	0.379000	0.382000	0.365000	0.375333	0.006870	0.171752
K	0.512000	0.525000	0.532000	0.523000	0.006894	0.172353
L	0.403000	0.411000	0.409000	0.407667	0.006875	0.171883
A5	0.414000	0.419000	0.431000	0.421333	0.006878	0.171939
A6	0.367000	0.388000	0.369000	0.374667	0.006870	0.171749
M	0.407000	0.403000	0.403000	0.404333	0.006875	0.171870
N	0.370000	0.382000	0.397000	0.383000	0.006871	0.171783
O	0.209000	0.262000	0.288000	0.253000	0.006850	0.171253
BLANK	0.000000	0.000000	0.000000	0.000000	0.006809	0.170223

$$* X1 = \frac{\text{Average absorbance} + 41.81}{6139.30}$$

**X1 values are the amount (mg) of soluble proteins in 0.04ml protein solution.*

$$*X2 = \frac{(\text{Average absorbance} + 41.81) / 6139.30}{0.04}$$

**X2 values are the amount (mg) of soluble proteins in 1.00ml protein solution*

APPENDIX 3 – RESULTS ON THE FUNCTIONAL PROPERTIES OF THE IRRADIATED BAMBARA PROTEIN SAMPLES

APPENDIX 3A: Moisture, Emulsifying activity (EA) and Emulsion stability

Irradiation Doses	Moisture	EA	ES
0.00	5.81(0.44)	38.33(2.98)	42.50(2.50)
2.50	5.78(0.21)	45.83(1.44)	73.33(2.89)
5.00	5.81(0.48)	44.17(1.44)	65.00(5.00)
7.50	5.77(0.14)	41.67(1.44)	61.67(5.77)
10.00	5.81(0.04)	35.00(0.00)	49.17(1.44)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 3B: Foam capacity (FC), Foam stability at 30seconds and Foam stability at 10 minutes

Irradiation Doses	FC	FS at 30 sec	FS at 10 minutes
0.00	53.00(1.41)	92.16(1.78)	43.14(0.40)
2.50	65.00(1.41)	92.12(0.79)	52.73(0.41)
5.00	69.00(1.41)	89.94(0.92)	43.79(0.37)
7.50	77.00(4.24)	92.66(0.62)	51.98(1.95)
10.00	80.00(0.00)	96.67(0.00)	58.89(1.57)
EW	95.00(1.41)	97.44(0.71)	68.72(0.22)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 3C: Foam stability at 30 minutes, Foam stability at 60 minutes, and Foam stability at 120 minutes

Irradiation Doses	FS at 30 minutes	FS at 60 minutes	FS at 120 minutes
0.00	23.53(1.63)	15.03(2.91)	7.52(0.53)
2.50	27.88(2.82)	17.27(0.28)	12.12(0.10)
5.00	18.93(1.52)	17.16(1.82)	10.06(0.75)
7.50	40.68(4.17)	19.21(3.66)	11.86(1.08)
10.00	44.44(1.57)	19.44(3.93)	13.06(1.18)
EW	55.90(2.58)	44.10(0.32)	38.97(0.28)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 3D: Water absorption capacities (WAC) and Oil absorption capacities (OAC) for irradiated samples

Irradiation Doses	WAC	OAC
0.00	16.86(1.03)	9.01(0.02)
2.50	17.78(0.07)	9.11(0.05)
5.00	17.82(0.69)	9.31(0.15)
7.50	17.82(0.12)	9.58(0.24)
10.00	18.45(0.38)	10.09(0.21)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 4 – ANALYSIS OF VARIANCE RESULTS FOR PROTEIN SOLUBILITY FOR IRRADIATED BAMBARA PROTEINS UNDER OPTIMUM CONDITION

APPENDIX 4A: Solubility of 0.00kGy irradiated protein sample at pH 6.5, slurry concentration 1.75g/20ml and vortex time of two hours

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	5	1.031E-07	2.061E-08	14.89	<.001
Residual	12	1.661E-08	1.384E-09		
Total	17	1.197E-07			

APPENDIX 4B: Solubility of 2.50kGy irradiated protein sample at pH 6.5, slurry concentration 1.75g/20ml and vortex time of two hours

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	5	2.453E-07	4.906E-08	18.25	<.001
Residual	12	3.227E-08	2.689E-09		
Total	17	2.776E-07			

APPENDIX 4C: Solubility of 5.00kGy irradiated protein sample at pH 6.5, slurry concentration 1.75g/20ml and vortex time of two hours

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	5	5 4.733E-07	9.465E-08	197.90	<.001
Residual	12	5.739E-09	4.783E-10		
Total	17	4.790E-07			

APPENDIX 4D: Solubility of 7.50kGy irradiated protein sample at pH 6.5, slurry concentration 1.75g/20ml and vortex time of two hours

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	5	6.182E-07	1.236E-07	21.32	<.001
Residual	12	6.958E-08	5.799E-09		
Total	17	6.878E-07			

APPENDIX 4E: Solubility of 10.00kGy irradiated protein sample at pH 6.5, slurry concentration 1.75g/20ml and vortex time of two hours

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	5	1.149E-06	2.298E-07	96.52	<.001
Residual	12	2.857E-08	2.381E-09		
Total	17	1.178E-06			

APPENDIX 4F: Solubility of protein samples under condition ***A1*** across irradiation doses (0.00, 2.50, 5.00, 7.50 and 10.00kGy)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	1.573E-06	3.933E-07	187.24	<.001
Residual	10	2.101E-08	2.101E-09		
Total	14	1.594E-06			

APPENDIX 4G: Solubility of protein samples under condition ***A2*** across irradiation doses (0.00, 2.50, 5.00, 7.50 and 10.00kGy)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	5.869E-06	1.467E-06	519.79	<.001
Residual	10	2.823E-08	2.823E-09		
Total	14	5.898E-06			

APPENDIX 4H: Solubility of protein samples under condition ***A3*** across irradiation doses (0.00, 2.50, 5.00, 7.50 and 10.00kGy)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	4.473E-06	1.118E-06	286.91	<.001
Residual	10	3.898E-08	3.898E-09		
Total	14	4.512E-06			

APPENDIX 4I: Solubility of protein samples under condition ***A4*** across irradiation doses (0.00, 2.50, 5.00, 7.50 and 10.00kGy)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	2.878E-06	7.196E-07	860.91	<.001
Residual	10	8.359E-09	8.359E-10		
Total	14	2.887E-06			

APPENDIX 4J: Solubility of protein samples under condition ***A5*** across irradiation doses (0.00, 2.50, 5.00, 7.50 and 10.00kGy)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	2.940E-06	7.349E-07	706.90	<.001
Residual	10	1.040E-08	1.040E-09		
Total	14	2.950E-06			

APPENDIX 4K: Solubility of protein samples under condition ***A6*** across irradiation doses (0.00, 2.50, 5.00, 7.50 and 10.00kGy)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	2.391E-06	5.977E-07	202.57	<.001
Residual	10	2.950E-08	2.950E-09		
Total	14	2.420E-06			

APPENDIX 4L: Solubility of protein samples under condition ***B*** across irradiation doses (0.00, 2.50, 5.00, 7.50 and 10.00kGy)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	0.2122556	0.0530639	409.65	<.001
Residual	10	0.0012953	0.0001295		
Total	14	0.2135509			

APPENDIX 4M: Solubility of protein samples under condition ***C*** across irradiation doses (0.00, 2.50, 5.00, 7.50 and 10.00kGy)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	0.3841391	0.0960348	355.51	<.001
Residual	10	0.0027013	0.0002701		
Total	14	0.3868404			

APPENDIX 4N: Solubility of protein samples under condition ***D*** across irradiation doses (0.00, 2.50, 5.00, 7.50 and 10.00kGy)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	0.27374173	0.06843543	972.09	<.001
Residual	10	0.00070400	0.00007040		
Total	14	0.27444573			

APPENDIX 4O: Solubility of protein samples under condition ***E*** across irradiation doses (0.00, 2.50, 5.00, 7.50 and 10.00kGy)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	0.5312867	0.1328217	736.81	<.001
Residual	10	0.0018027	0.0001803		
Total	14	0.5330893			

APPENDIX 4P: Solubility of protein samples under condition ***F*** across irradiation doses (0.00, 2.50, 5.00, 7.50 and 10.00kGy)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	0.07933067	0.01983267	297.49	<.001
Residual	10	0.00066667	0.000066673		
Total	14	0.07999733			

APPENDIX 4Q: Solubility of protein samples under condition ***G*** across irradiation doses (0.00, 2.50, 5.00, 7.50 and 10.00kGy)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	0.518125	0.129531	95.53	<.001
Residual	10	0.013559	0.001356		
Total	14	0.531684			

APPENDIX 4R: Solubility of protein samples under condition ***H*** across irradiation doses (0.00, 2.50, 5.00, 7.50 and 10.00kGy)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	0.17245373	0.04311343	571.80	<.001
Residual	10	0.00075400	0.00007540		
Total	14	0.17320773			

APPENDIX 4S: Solubility of protein samples under condition ***I*** across irradiation doses (0.00, 2.50, 5.00, 7.50 and 10.00kGy)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	0.0858911	0.0214728	102.22	<.001
Residual	10	0.0021007	0.0002101		
Total	14	0.0879917			

APPENDIX 4T: Solubility of protein samples under condition ***J*** across irradiation doses (0.00, 2.50, 5.00, 7.50 and 10.00kGy)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	0.2077040	0.0519260	231.88	<.001
Residual	10	0.0022393	0.0002239		
Total	14	0.2099433			

APPENDIX 4U: Solubility of protein samples under condition ***K*** across irradiation doses (0.00, 2.50, 5.00, 7.50 and 10.00kGy)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	0.20774200	0.05193550	802.30	<.001
Residual	10	0.00064733	0.00006473		
Total	14	0.20838933			

APPENDIX 4V: Solubility of protein samples under condition ***L*** across irradiation doses (0.00, 2.50, 5.00, 7.50 and 10.00kGy)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	0.1175443	0.0293861	101.87	<.001
Residual	10	0.0028847	0.0002885		
Total	14	0.1204289			

APPENDIX 4W: Solubility of protein samples under condition ***M*** across irradiation doses (0.00, 2.50, 5.00, 7.50 and 10.00kGy)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	0.1943489	0.0485872	334.93	<.001
Residual	10	0.0014507	0.0001451		
Total	14	0.1957996			

APPENDIX 4X: Solubility of protein samples under condition ***N*** across irradiation doses (0.00, 2.50, 5.00, 7.50 and 10.00kGy)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	0.0512823	0.0128206	81.42	<.001
Residual	10	0.0015747	0.0001575		
Total	14	0.0528569			

APPENDIX 4Y: Solubility of protein samples under condition ***O*** across irradiation doses (0.00, 2.50, 5.00, 7.50 and 10.00kGy)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	0.0549397	0.0137349	31.04	<.001
Residual	10	0.0044247	0.0004425		
Total	14	0.0593644			

APPENDIX 5 - ANALYSIS OF VARIANCE RESULTS FOR FUNCTIONAL PROPERTIES OF IRRADIATED BAMBARA PROTEIN SAMPLES

APPENDIX 5A: percent moisture

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	0.00314	0.00079	0.01	1.000
Residual	5	0.48670	0.09734		
Total	9	0.48984			

APPENDIX 5B: Percent emulsifying activity (%EA)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	230.833	57.708	19.79	<.001
Residual	10	29.167	2.917		
Total	14	260.000			

APPENDIX 5C: Percent emulsion stability (%ES)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	1861.73	465.43	30.99	<.001
Residual	10	150.17	15.02		
Total	14	2011.90			

APPENDIX 5C: Percent Foam capacity (% FC)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	5	2057.667	411.533	94.97	<.001
Residual	6	26.000	4.333		
Total	11	2083.667			

APPENDIX 5D: Percent Foam stability (% FS at 30 seconds)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	5	85.2245	17.0449	18.55	0.001
Residual	6	5.5136	0.9189		
Total	11	90.7381			

APPENDIX 5E: Percent Foam stability (% FS at 10 minutes)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	5	961.714	192.343	170.22	<.001
Residual	6	6.780	1.130		
Total	11	968.494			

APPENDIX 5F: Percent Foam stability (% FS at 30 minutes)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	5	1981.530	396.306	60.32	<.001
Residual	6	39.423	6.570		
Total	11	2020.952			

APPENDIX 5G: Percent Foam stability (% FS at 60 minutes)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	5	1193.449	238.690	35.13	<.001
Residual	6	40.766	6.794		
Total	11	1234.215			

APPENDIX 5H: Percent Foam stability (% FS at 120 minutes)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	5	1349.7679	269.9536	462.29	<.001
Residual	6	3.5037	0.5839		
Total	11	1353.2716			

APPENDIX 5I: Percent Oil absorption capacity (% OAC)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	2.25599	0.56400	22.42	<.001
Residual	10	0.25160	0.02516		
Total	14	2.50759			

APPENDIX 5J: Percent water absorption capacity (% WAC)

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	3.9012	0.9753	2.86	0.081
Residual	10	3.4098	0.3410		
Total	14	7.3109			

APPENDIX 6 – GRAPHICAL REPRESENTATION OF COGELLED PROTEIN-STARCH ADMIXTURES

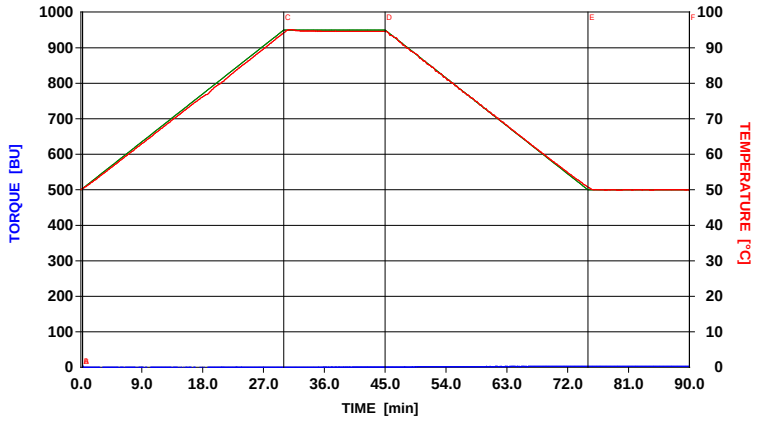
APPENDIX 6A: Cogelation at 30P:70S using 0.00kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	MR NAJAH		Date	:	7/17/2008	
Sample	:	0.0KGY 30P:70S		Method	:		
Moisture	:	11.35	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	38.8	[g]
Water	:	420	[ml]	Corr. to 14%	:	421.2	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	1000	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 1000 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:00:15	5	50.4
B	Maximum viscosity	00:00:15	5	50.4
C	Start of holding period	00:30:00	0	94.2
D	Start of cooling period	00:45:00	1	94.6
E	End of cooling period	01:15:00	3	50.8
F	End of final holding period	01:30:00	3	50.0
B-D	Breakdown		4	
E-D	Setback		2	

File : Measurement V: 2.3.16

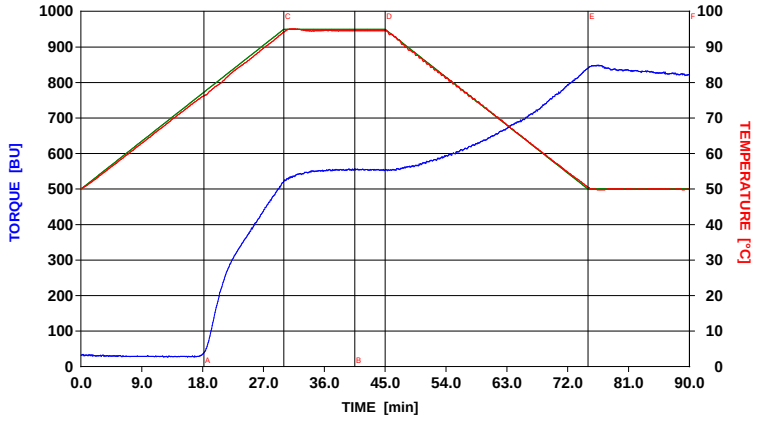
APPENDIX 6B: Cogelation at 30P:70S using 2.50kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	MR NAJAH		Date	:	7/30/2008	
Sample	:	2.5KGY 30P :70S		Method	:		
Moisture	:	10.51	[%]	Correction	:	11.06	[%]
Sample weight	:	40	[g]	Corr. to 11.06%	:	39.7	[g]
Water	:	420	[ml]	Corr. to 11.06%	:	420.2	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:18:10	39	76.2
B	Maximum viscosity	00:40:30	557	94.6
C	Start of holding period	00:30:00	522	94.2
D	Start of cooling period	00:45:00	554	94.6
E	End of cooling period	01:15:00	840	50.5
F	End of final holding period	01:30:00	821	49.9
B-D	Breakdown		4	
E-D	Setback		286	

File : Measurement V: 2.3.16

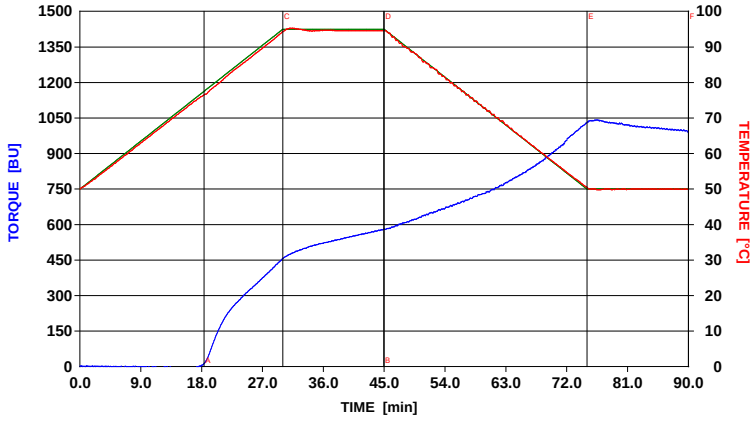
APPENDIX 6C: Cogelation at 30P:70S using 5.00kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	MR NAJAH		Date	:	8/4/2008	
Sample	:	5.0KGY 30P :70S		Method	:	METHOD 1	
Moisture	:	11.14	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	38.7	[g]
Water	:	420	[ml]	Corr. to 14%	:	421.2	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:18:20	13	76.5
B	Maximum viscosity	00:44:55	580	94.6
C	Start of holding period	00:30:00	457	94.3
D	Start of cooling period	00:45:00	581	94.6
E	End of cooling period	01:15:00	1031	50.5
F	End of final holding period	01:30:00	994	49.9
B-D	Breakdown		0	
E-D	Setback		448	

File : Measurement V: 2.3.16

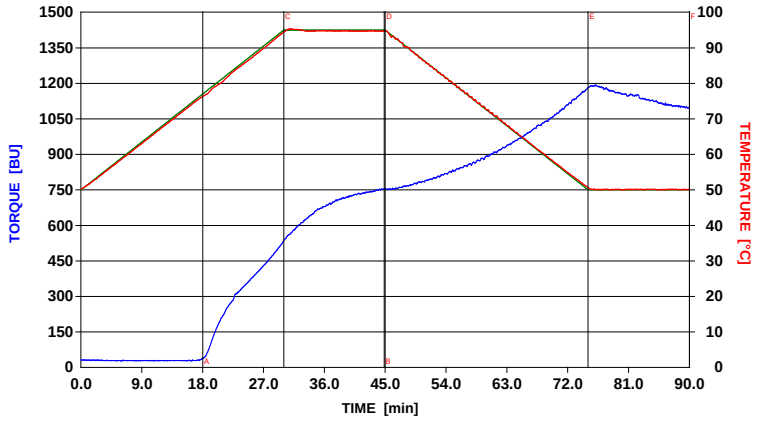
APPENDIX 6D: Cogelation at 30P:70S using 7.50kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	MR NAJAH		Date	:	7/31/2008	
Sample	:	7.5KGY 30P :70S		Method	:	METHOD 1	
Moisture	:	10.96	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	38.6	[g]
Water	:	420	[ml]	Corr. to 14%	:	421.3	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:18:00	36	76.2
B	Maximum viscosity	00:44:50	754	94.6
C	Start of holding period	00:30:00	535	94.3
D	Start of cooling period	00:45:00	755	94.6
E	End of cooling period	01:15:00	1180	50.5
F	End of final holding period	01:30:00	1092	49.9
B-D	Breakdown		0	
E-D	Setback		424	

File : Measurement V: 2.3.16

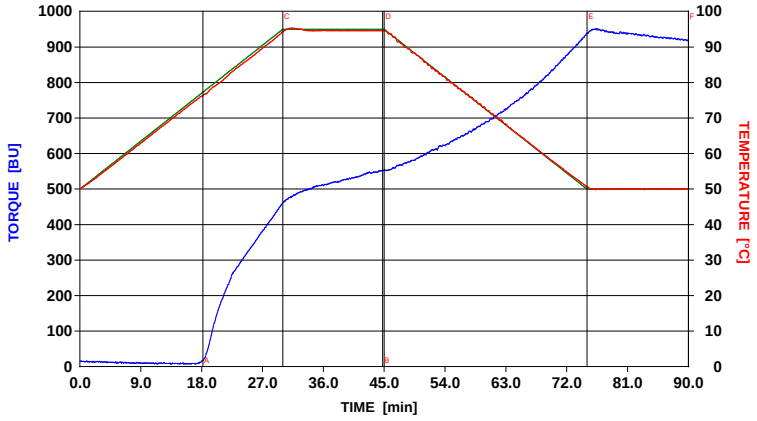
APPENDIX 6E: Cogelation at 30P:70S using 10.00kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	MR NAJAH		Date	:	8/4/2008	
Sample	:	10KGY 30P :70S		Method	:	METHOD 1	
Moisture	:	10.51	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	38.4	[g]
Water	:	420	[ml]	Corr. to 14%	:	421.6	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:18:10	18	76.3
B	Maximum viscosity	00:44:45	554	94.6
C	Start of holding period	00:30:00	461	94.2
D	Start of cooling period	00:45:00	553	94.6
E	End of cooling period	01:15:00	938	50.6
F	End of final holding period	01:30:00	921	49.9
B-D	Breakdown		2	
E-D	Setback		386	

File : Measurement V: 2.3.16

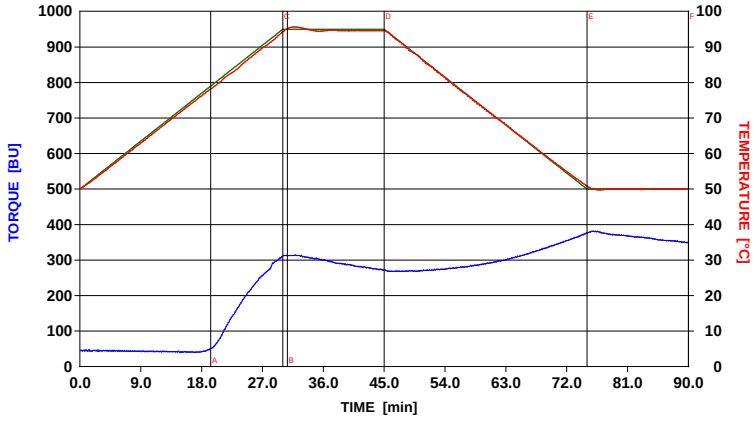
APPENDIX 6F: Cogelation at 50P:50S using 0.00kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	NII NORTEY		Date	:	7/29/2008	
Sample	:	0.00 KGY 50p:50s		Method	:	METHOD 1	
Moisture	:	10.51	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	38.4	[g]
Water	:	420	[ml]	Corr. to 14%	:	421.6	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:19:20	51	78.1
B	Maximum viscosity	00:30:40	314	95.1
C	Start of holding period	00:30:00	311	94.3
D	Start of cooling period	00:45:00	272	94.6
E	End of cooling period	01:15:00	376	50.8
F	End of final holding period	01:30:00	349	50.0
B-D	Breakdown		42	
E-D	Setback		104	

File : Measurement V: 2.3.16

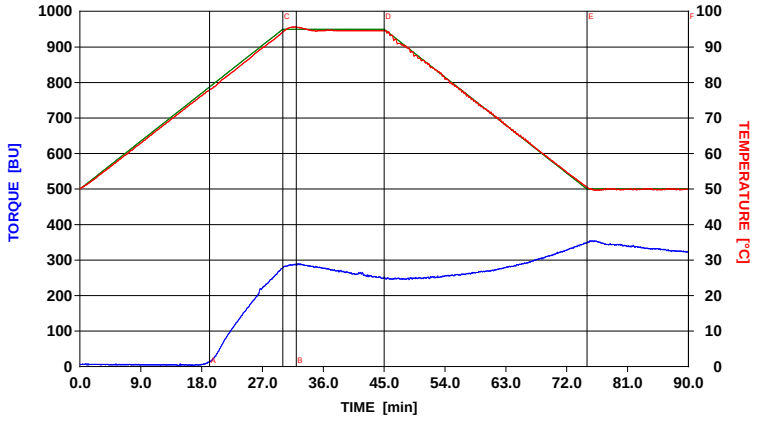
APPENDIX 6G: Cogelation at 50P:50S using 2.50kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	MR NAJAH		Date	:	8/6/2008	
Sample	:	2.5KGY 50P:50S		Method	:	METHOD 1	
Moisture	:	9.97	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	38.2	[g]
Water	:	420	[ml]	Corr. to 14%	:	421.7	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:19:10	14	77.9
B	Maximum viscosity	00:32:00	289	95.6
C	Start of holding period	00:30:00	278	94.2
D	Start of cooling period	00:45:00	252	94.6
E	End of cooling period	01:15:00	349	50.5
F	End of final holding period	01:30:00	322	50.0
B-D	Breakdown		40	
E-D	Setback		100	

File : Measurement V: 2.3.16

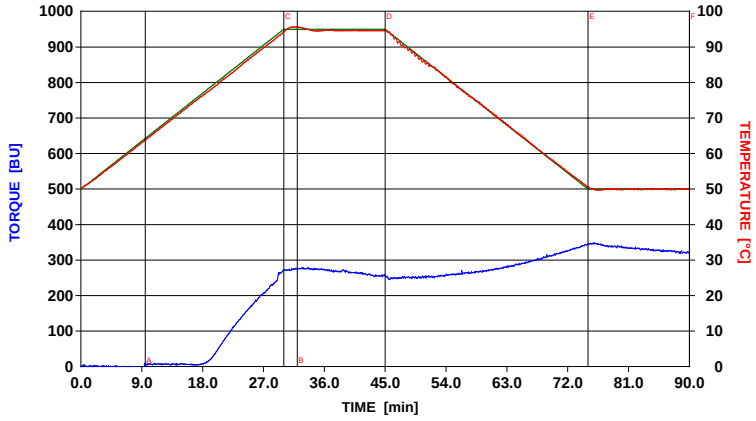
APPENDIX 6H: Cogelation at 50P:50S using 5.00kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	MR NAJAH		Date	:	7/31/2008	
Sample	:	5.00KGY 50P:50S		Method	:	METHOD 1	
Moisture	:	9.86	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	38.1	[g]
Water	:	420	[ml]	Corr. to 14%	:	421.8	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:09:30	8	63.8
B	Maximum viscosity	00:32:00	278	95.6
C	Start of holding period	00:30:00	271	94.3
D	Start of cooling period	00:45:00	255	94.6
E	End of cooling period	01:15:00	344	50.6
F	End of final holding period	01:30:00	323	49.9
B-D	Breakdown		21	
E-D	Setback		88	

File : Measurement V: 2.3.16

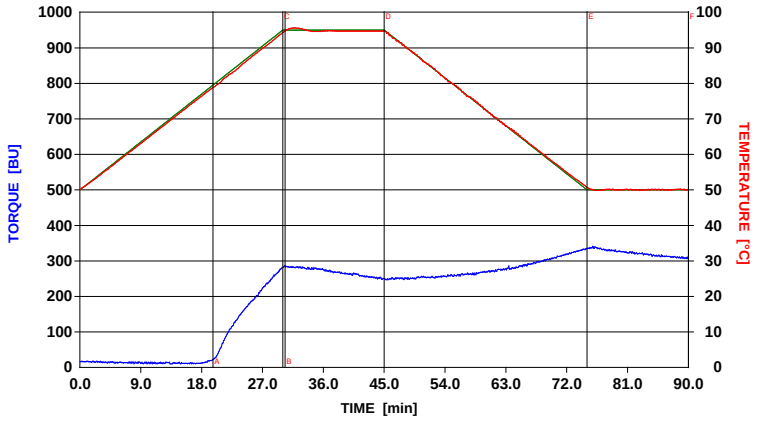
APPENDIX 6I: Cogelation at 50P:50S using 7.50kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	NII NORTEY		Date	:	7/28/2008	
Sample	:	7.50 KGY 50p:50s		Method	:	METHOD 1	
Moisture	:	10.18	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	38.2	[g]
Water	:	420	[ml]	Corr. to 14%	:	421.7	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:19:40	22	78.7
B	Maximum viscosity	00:30:20	286	94.6
C	Start of holding period	00:30:00	281	94.2
D	Start of cooling period	00:45:00	250	94.6
E	End of cooling period	01:15:00	335	50.6
F	End of final holding period	01:30:00	305	49.8
B-D	Breakdown		36	
E-D	Setback		83	

File : Measurement V: 2.3.16

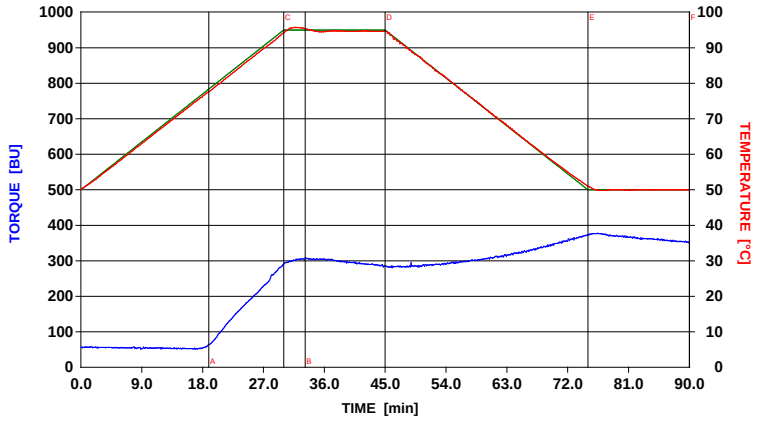
APPENDIX 6J: Cogelation at 50P:50S using 10.00kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	MR NAJAH		Date	:	8/6/2008	
Sample	:	10.00KGY 50P :50S		Method	:	METHOD 1	
Moisture	:	10.48	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	38.4	[g]
Water	:	420	[ml]	Corr. to 14%	:	421.6	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:18:55	64	77.6
B	Maximum viscosity	00:33:10	309	95.4
C	Start of holding period	00:30:00	291	94.2
D	Start of cooling period	00:45:00	285	94.6
E	End of cooling period	01:15:00	373	51.0
F	End of final holding period	01:30:00	353	50.0
B-D	Breakdown		23	
E-D	Setback		87	

File : Measurement V: 2.3.16

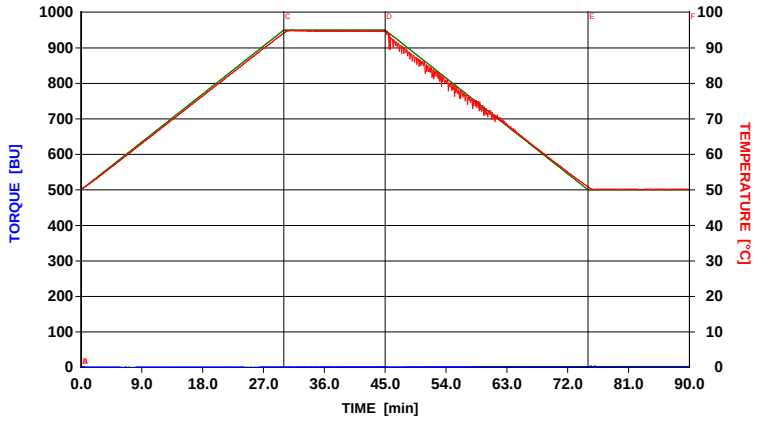
APPENDIX 6K: Cogelation at 70P:30S using 0.00kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	MR NAJAH		Date	:	7/17/2008	
Sample	:	0.00KGY 70P:30S		Method	:		
Moisture	:	10.07	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	38.2	[g]
Water	:	420	[ml]	Corr. to 14%	:	421.7	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	1000	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 1000 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:00:05	2	50.2
B	Maximum viscosity	00:00:00	2	50.0
C	Start of holding period	00:30:00	0	94.0
D	Start of cooling period	00:45:00	1	94.6
E	End of cooling period	01:15:00	2	50.7
F	End of final holding period	01:30:00	2	50.0
B-D	Breakdown		1	
E-D	Setback		1	

File : Measurement V: 2.3.16

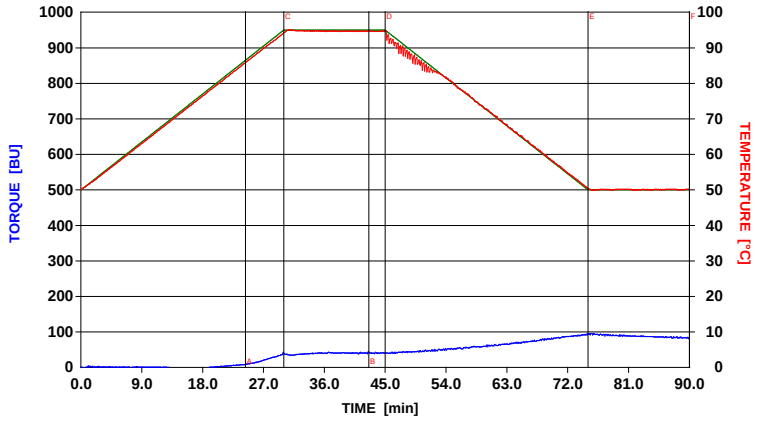
APPENDIX 6L: Cogelation at 70P:30S using 2.50kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	NII NORTEY		Date	:	7/28/2008	
Sample	:	2.5kgy 70p:30s		Method	:	METHOD 1	
Moisture	:	8.95	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	37.7	[g]
Water	:	420	[ml]	Corr. to 14%	:	422.2	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:24:20	8	85.8
B	Maximum viscosity	00:42:35	43	94.6
C	Start of holding period	00:30:00	40	94.1
D	Start of cooling period	00:45:00	40	94.5
E	End of cooling period	01:15:00	94	50.3
F	End of final holding period	01:30:00	82	50.0
B-D	Breakdown		3	
E-D	Setback		51	

File : Measurement V: 2.3.16

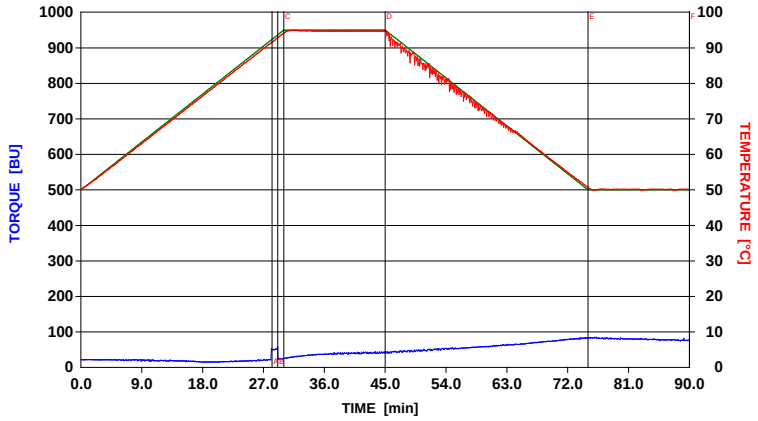
APPENDIX 6M: Cogelation at 70P:30S using 5.00kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	MR NAJAH		Date	:	7/21/2008	
Sample	:	5.00KGY 70P :30S		Method	:		
Moisture	:	8.90	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	37.7	[g]
Water	:	420	[ml]	Corr. to 14%	:	422.2	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:28:15	51	91.5
B	Maximum viscosity	00:29:05	57	92.7
C	Start of holding period	00:30:00	24	94.0
D	Start of cooling period	00:45:00	39	94.6
E	End of cooling period	01:15:00	82	50.6
F	End of final holding period	01:30:00	75	50.0
B-D	Breakdown		17	
E-D	Setback		42	

File : Measurement V: 2.3.16

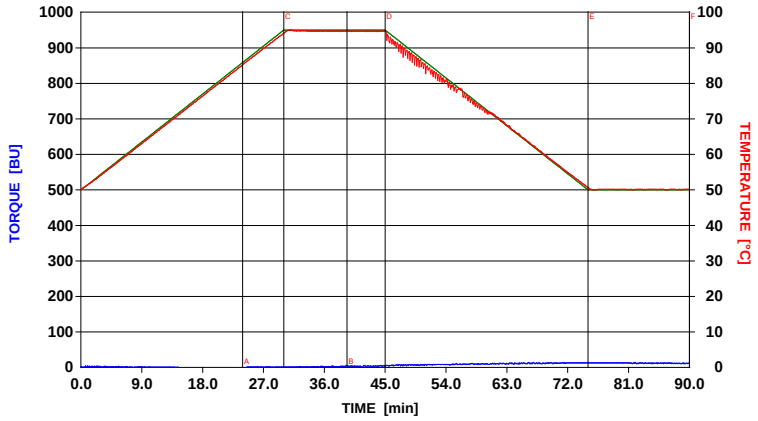
APPENDIX 6N: Cogelation at 70P:30S using 7.50kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	NII NORTEY		Date	:	7/29/2008	
Sample	:	7.5 KGY 70p:30s		Method	:	METHOD 1	
Moisture	:	8.90	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	37.7	[g]
Water	:	420	[ml]	Corr. to 14%	:	422.2	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:23:55	-1	85.1
B	Maximum viscosity	00:39:20	5	94.6
C	Start of holding period	00:30:00	0	94.0
D	Start of cooling period	00:45:00	2	94.6
E	End of cooling period	01:15:00	12	50.5
F	End of final holding period	01:30:00	10	50.0
B-D	Breakdown		1	
E-D	Setback		9	

File : Measurement V: 2.3.16

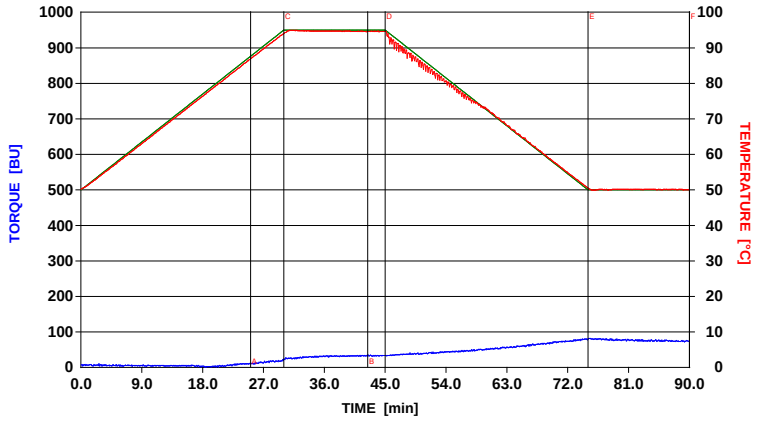
APPENDIX 6O: Cogelation at 70P:30S using 10.00kGy proteins

BRABENDER VISCOGRAPH

Parameter

Operator	:	NII NORTEY		Date	:	7/29/2008	
Sample	:	10.00 KGY 70p:30s		Method	:	METHOD 1	
Moisture	:	9.44	[%]	Correction	:	14	[%]
Sample weight	:	40	[g]	Corr. to 14%	:	37.9	[g]
Water	:	420	[ml]	Corr. to 14%	:	422.1	[ml]
Note	:						
Note	:						
Speed	:	75	[1/min]	Meas. range	:	250	[cmg]
Start temperature	:	50	[°C]	Heat./Cool. rate	:	1.5	[°C/min]
Max. temperature	:	95	[°C]	Upp. hold. time	:	15	[min]
End temperature	:	50	[°C]	Fin. hold. time	:	15	[min]

MEASURING RANGE : 250 [cmg]



Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:25:05	10	86.8
B	Maximum viscosity	00:42:25	34	94.5
C	Start of holding period	00:30:00	23	93.9
D	Start of cooling period	00:45:00	33	94.5
E	End of cooling period	01:15:00	78	50.4
F	End of final holding period	01:30:00	72	50.0
B-D	Breakdown		1	
E-D	Setback		44	

File : Measurement V: 2.3.16

APPENDIX 7 – ANALYSIS OF VARIANCE PASTING PROPERTIES

APPENDIX 7A: Beginning of gelatinization temperature at 30P:70S across irradiation doses

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	0.070	0.018	0.01	1.000
Residual	5	8.175	1.635		
Total	9	8.245			

APPENDIX 7B: Beginning of gelatinization temperature at 50P:50S across irradiation doses

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	460.49	115.12	1.52	0.325
Residual	5	379.33	75.87		
Total	9	839.82			

APPENDIX 7C: Beginning of gelatinization temperature at 70P:30S across irradiation doses

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	46.904	11.726	5.35	0.047
Residual	5	10.960	2.192		
Total	9	57.864			

APPENDIX 7D: Beginning of gelatinization temperatures within blends (30P:70S, 50P:50S, and 70P:30S) across irradiation doses

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments 1	4	114.16	28.54	1.07	0.405
Treatments 2	2	1240.27	620.13	23.26	<.001
TRTS 1. TRTS 2	8	393.31	49.16	1.84	0.146
Residual	15	399.91	26.66		
Total	29	2147.65			

APPENDIX 7E: Maximum viscosity temperatures within blends (30P:70S, 50P:50S, and 70P:30S) across irradiation doses

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments 1	4	1.12133	0.28033	6.95	0.002
Treatments 2	2	4.77800	2.38900	59.23	<.001
TRTS 1. TRTS 2	8	5.77867	0.72233	17.91	<.001
Residual	15	0.60500	0.04033		
Total	29	12.28300			

APPENDIX 7F: Gel strength at maximum viscosity temperature for 30P:70S blend

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	8598	2150	0.30	0.869
Residual	5	36306	7261		
Total	9	44905			

APPENDIX 7G: Gel strength at maximum viscosity temperature for 50P:50S blend

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	1697.6	424.4	3.13	0.121
Residual	5	678.0	135.6		
Total	9	2375.6			

APPENDIX 7H: Gel strength at maximum viscosity temperature for 70P:30S blend

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	8746.4	2186.6	12.37	0.008
Residual	5	884.0	176.8		
Total	9	9630.4			

APPENDIX 7I: Gel strength at the end of cooling period for 30P:70S blend

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	38152.	9538.	0.62	0.667
Residual	5	76638.	15328		
Total	9	114791			

APPENDIX 7J: Gel strength at the end of cooling period for 50P:50S blend

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	1556.6	389.1	1.77	0.271
Residual	5	1097.5	219.5		
Total	9	2654.1			

APPENDIX 7K: Gel strength at the end of cooling period for 70P:30S blend

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	15158.40	3789.60	41.92	<.001
Residual	5	452.00	90.40		
Total	9	15610.40			

APPENDIX 7L: Setback viscosity at 30P:70S

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	20777	5194	2.24	0.200
Residual	5	11610	2322		
Total	9	32386			

APPENDIX 7M: Setback viscosity at 50P:50S

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	788.00	197.00	23.18	0.002
Residual	5	42.500	8.50		
Total	9	830.50			

APPENDIX 7N: Setback viscosity at 70P:30S

Source of variation	*d.f.*	*s.s.*	*m.s.*	*v.r.*	*F pr.*
Treatments	4	2598.00	649.50	51.96	<.001
Residual	5	62.50	12.50		
Total	9	2660.50			

APPENDIX 8 – DATA ON THE PASTING CHARACTERISTICS OF PROTEIN-STARCH BLENDS

APPENDIX 8A: Beginning of gelatinization temperatures

BEGINNING OF GELATINIZATION TEMPERATURE (ºC)			
Irradiation dose (kGy)	PROTEIN-STARCH ADMIXTURES (%)		
	30P:70S	**50P:50S**	**70P:30S**
0.00	76.40(2.83)	78.10(0.00)	88.20(3.11)
2.50	76.20(0.00)	64.15(19.45)	86.60(1.13)
5.00	76.35(0.21)	63.80(0.00)	91.50(0.00)
7.50	76.45(0.35)	77.95(1.06)	85.10(0.00)
10.00	76.35(0.07)	77.40(0.28)	86.80(0.00)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 8B: Maximum viscosity temperatures

MAXIMUM VISCOSITY TEMPERATURE (ºC)			
Irradiation dose (kGy)	PROTEIN-STARCH ADMIXTURES (%)		
	30P:70S	**50P:50S**	**70P:30S**
0.00	94.40(0.00)	95.10(0.00)	94.50(0.42)
2.50	94.60(0.00)	95.35(0.35)	94.60(0.00)
5.00	94.60(0.00)	95.60(0.00)	92.70(0.00)
7.50	94.60(0.00)	94.60(0.00)	94.60(0.00)
10.00	94.60(0.00)	94.95(0.64)	94.50(0.00)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 8C: Gel strength at maximum viscosity temperatures

GEL STRENGTH at MAXIMUM VISCOSITY			
Irradiation dose (kGy)	PROTEIN-STARCH ADMIXTURES (%)		
	30P:70S	**50P:50S**	**70P:30S**
0.00	541.00(5.66)	314.00(0.00)	67.50(34.65)
2.50	557.00(0.00)	288.00(1.41)	85.40(0.57)
5.00	575.00(7.07)	278.00(0.00)	57.00(0.00)
7.50	620.00(189.50)	279.00(9.90)	5.00(0.00)
10.00	541.50(17.68)	292.00(24.04)	34.00(0.00)

APPENDIX 8D: Gel strength at the end of cooling period

GEL STRENGTH at END OF COOLING PERIOD			
Irradiation dose (kGy)	PROTEIN-STARCH ADMIXTURES (%)		
	30P:70S	**50P:50S**	**70P:30S**
0.00	862.00(0.00	376.00(0.00)	126.00(1.41)
2.50	840.00(0.00)	356.50(10.61)	109.00(21.21)
5.00	974.00(80.61)	344.00(0.00)	82.00(0.00)
7.50	997.50(258.09)	340.50(7.78)	12.00(0.00)
10.00	896.00(59.40)	351.50(30.41)	78.00(0.00)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 8E: Setback viscosities

SETBACK VISCOSITY			
Irradiation dose (kGy)	PROTEIN-STARCH ADMIXTURES (%)		
	30P:70S	**50P:50S**	**70P:30S**
0.00	288.50(2.12)	104.00(0.00)	57.00(1.41)
2.50	286.00(0.00)	104.50(6.36)	45.50(7.78)
5.00	397.50(71.42)	88.00(0.00)	42.00(0.00)
7.50	375.50(68.59)	84.00(1.41)	9.00(0.00)
10.00	356.00(42.43)	87.00(0.00)	44.00(0.00)

Values are means ± SD (in parenthesis) of at least two determinations (n = 2 or 3)

APPENDIX 9 – CORRELATION STUDIES ON FUNCTIONAL PROPERTIES OF PROTEINS, AND PASTING CHARACTERISTICS OF PROTEIN-STARCH BLENDS

APPENDIX 9A: correlation coefficients (*r*) of pasting properties of Protein-Starch blends

Protein-Starch Blends	Pasting Properties versus Irradiation Doses (I.D) at each blend			Correlation Coefficient (*r*)
30P:70S	Beginning of gelatinization (º C)	vs.	I.D	**0.25**
	Maximum viscosity (º C)	vs.	I.D	**0.71***
50P:50S	Beginning of gelatinization (º C)	vs.	I.D	**0.26**
	Maximum viscosity (º C)	vs.	I.D	**-0.46**
70P:30S	Beginning of gelatinization (º C)	vs.	I.D	**-0.28**
	Maximum viscosity (º C)	vs.	I.D	**0.00**

Values with asterisks (*) are significantly correlated ($p<0.05$)

APPENDIX 9B: correlation coefficients (*r*) of some functional properties irradiated Bambara proteins

FUNCTIONAL PROPERTIES	**FUNCTIONAL PROPERTIES**						
	SOL	WAC	OAC	FC	FS	EA	ES
	Correlation coefficients (*r*)						
SOL		**0.31**	**0.74**	**0.37**	**0.75**	**-0.98**	**-0.78**
WAC			**0.84**	**0.92***	**0.73**	**-0.14**	**0.31**
OAC				**0.87**	**0.92**	**-0.62**	**-0.21**

Values with asterisks (*) are significantly correlated ($p<0.05$)

ABSTRACT

This research was carried out to evaluate the surface functional properties of gamma irradiated Bambara groundnut proteins isolates and the pasting characteristics of the Bambara modified protein-native starch admixtures using the Brabender Viscoamylograph. Irradiation was done at five levels: 0.00, 2.50, 5.00, 7.50, and 10.00kGy; while starch-protein admixtures in three combinations: 30P:70S, 50P:50S, 70P:30S. Results showed significant ($p<0.05$) effects of increasing irradiation doses on protein related functional properties, while pasting characteristics of admixtures showed no dose-dependent significant ($p<0.05$) changes. However, pasting characteristics on the other hand increased significantly ($p<0.05$) with increasing starch:protein ratios. Conclusively, correlation studies proved the sole dependence of the pasting properties on the starch concentration within the blends, indicating the insignificant contribution of Bambara proteins to the pasting properties of the blends. Enhanced surface functional properties of the gamma irradiated proteins however makes them potential foaming, emulsifying, shelf life extension, and flavour retention agents for various foods such as cakes, comminuted meat products, and soups respectively. Admixtures may also serve as thickening agents for foods that require various degrees of viscosities.

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