

**Oil distribution, microstructure and *in vitro* starch digestibility of
bran-enriched *magwinya***

DOCTOR OF PHILOSOPHY IN AGRICULTURE (PHDAGR) THESIS

BY

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ABSTRACT

Magwinya, a cereal fried dough, is a popular traditional snack widely consumed across various ethnic groups in Sub-Saharan Africa, but little is known about its production and consumption in a scholarly context. Furthermore, its oil distribution, starch digestion and microstructure have not been documented. It is against this background that this research was carried out. Firstly, a survey of the production process, ingredient formulation, sales, characteristics and consumption of *magwinya* in Thohoyandou were investigated. Out of the 30 *magwinya* production sites visited and 650 consumer questionnaires distributed, data were obtained from 29 sites and 634 consumers: a response rate of 97% and 98%, respectively were realised. Results revealed, details on the formulation, ingredients, processing methods, sales, consumption patterns and consumer preference of *magwinya*; and proposed considerations for the development of a healthier *magwinya*. Cake wheat flour (55%) was the main ingredient used. Production process was non-automated as evidenced by manual mixing and fermentation processes (93%), coupled with low usage of electronic equipment (14%). With a daily turnover between ZAR500 – ZAR3000 (\$35 - \$210), there is a need to improve *magwinya* production through an automated production line, especially for large-scale producers of this product. The daily turnover indicated that *magwinya* production is a lucrative business contributing immensely to the livelihoods of, and financially empowering the producers, who were all females (100%). Consumer data revealed *magwinya* to be a moderately liked food (46%) consumed at least twice a week (32%), as either a snack or main meal; with taste as the most favoured characteristic (79%). About 93% of consumers fell within <20 and 21-30 age groups. A greater percentage of consumers (75%) disliked the oiliness of *magwinya*, and with increasing awareness of the health implications of frequent consumption of fried foods; 87% of consumers affirmed purchase of low-fat *magwinya* if made available. Development of low-fat, nutrient-rich *magwinya* is therefore recommended to offer consumers a healthier variety. The experiments in this study were constructed using a 2x2x7 factorial experimental design. Factors considered were two (2) bran types - wheat and oat bran; two (2) product types and seven (7) bran amounts (0, 1, 5, 8, 10, 15 & 20 g/ 100 g). Oil fractions of the total oil content of *magwinya* was categorized as surface oil (SO), penetrated surface oil (PSO) and structural oil (STO) using spectrophotometric method. Moisture loss reduced ($P < 0.05$) from 23.35% in control to 15.19% fried batter with 20% oat bran (OB), while a reverse trend was observed in fried dough. Reduction of total fat from 0.43 g/g in control to 0.38 g/g at 20% OB and 8 g wheat bran (WB) was observed. At 15% OB and 20% WB, total fat reduced from 0.41 g/g in control to 0.26 g/g of FD. An STO<SO<PSO trend was observed in fried dough while fried batter followed a SO<STO<PSO trend. Furthermore, oil penetration in crust and cross-sectional properties of fried dough were elucidated using confocal laser scanning microscopy (CLSM). Penetrated oil by image analysis (POia), porosity and pore features were quantified

from the cross-section micrographs; while crust surface roughness was measured using fractal metrics. Crumb porosity ranged between 54.94 – 81.84% and reduced ($P < 0.05$) with bran addition. Crumb pore sizes ranged from 0 – 475 μm with <1 circularity, indicating elliptical shape. POia values were notably higher ($P < 0.05$) than PO by soxhlet extraction (POsox) except for WB fried dough where the values of POia and POsox were closely ranked. Linear effect of initial moisture content and bran concentration showed significance on image properties. Mean fractal dimension (FD) decreased as initial moisture increased. addition of WB caused a significant reduction of FD of fried dough while an opposite effect was noted for its oat bran counterpart. Due to non-collinearity of image properties (FD, POia & porosity), data were fitted to cubic polynomial regression with r^2 values >0.70 . Confocal microscopy and image analysis were effective in measuring oil absorption and interpreting crumb properties of fried dough. Glucose, starch fractions and estimated glycemic index (eGI) of bran-enriched *magwinya* were estimated using *in vitro* starch digestion assay. Rapidly available glucose (RAG) of control fried dough (60.31 g/ 100 g) product was 33% less than fried batter (90.07 g/ 100 g) and more unavailable glucose (UG) less than fried batter. OB and WB significantly reduced RAG and increased UG of fried products. However, OB showed about 9% more reduction than wheat bran. A similar trend was observed for rapidly digestible starch and resistant starch of fried products. The eGI results showed control fried batter to be a high GI food (eGI = 80.02) and control fried dough to be medium GI food (eGI = 58.11). WB fried dough, fried batter and OB fried dough can be categorised as medium GI foods at eGI of 56.46 – 58.39, 65.93 – 68.84 & 56.34 – 57.27 respectively; while OB fried batter at 73.57 – 80.03 as high GI foods. RS showed negative significant correlation with eGI ($r = -0.866, -0.932, P < 0.01$) and fat content ($r = -0.618, -0.671, P < 0.01$) for OB and WB fried products. Finally, the distribution of chemical components of *magwinya* were visualised using NIR hyperspectral imaging (HSI). Principal component analysis (PCA) was applied to mean-centred data for pixel-wise classification using spectral scattering (standard normal variate) and 1st and 2nd derivatives of Savitsky-Golay method. There was little separation observed in the PCA score plots in the results due to a large similarity between classes. Prominent bands related to oil were featured at 1400, 2305 and 1716 nm, while those related to starch were featured at 1449, 1776, and 2261 nm. 1941 (related to moisture), and 2117 nm (related to protein). Bands related to protein were featured at 1509, 1994, 2223, 2229 nm and water at 1450, 1934 and 1940nm. Aromatics, phenols (1422 nm) and benzene were also identified, albeit minimum. The processing methods and some principal components assisted in mapping the chemical components of *magwinya*. Visualisation of *magwinya* chemical constituents using HSI shows good promise for further research in modelling and predictions. The protocols for oil distribution measurement and CLSM used herein can be applied to other thick deep-fried products for qualitative observation and quantitative measurement of specific physical or

chemical property. These results reveal that ingredient modification and processing is effective for oil uptake reduction, regulation of starch digestion and related eGI of deep-fried dough/batter foods.

Keywords: *Magwinya*, *in vitro* assay, starch digestion, oil distribution, fried dough, fried batter, hyperspectral imaging, production survey, consumption pattern, oil absorption.

DECLARATION

I, hereby declare that this submission is my work and to the best of my knowledge, it contains no material previously submitted, in whole or in part, to qualify for any other academic award; the intellectual content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program. Any contribution made to the research by others is explicitly acknowledged in the thesis.

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DEDICATION

To God Almighty, my enabler, way maker, beginning and ending.

Table of Contents

Abstract	ii
Declaration	v
Acknowledgements	vi
Dedication	viii
Table of Contents	ix
List of Tables	xii
List of Figures	xiii
List of Abbreviation	xv
List of Appendices	xvi
Publications and Conference Proceedings	xvii
CHAPTER 1: INTRODUCTION	19
1.1. Introduction	19
1.2. Problem statement	20
1.3. Hypotheses	21
1.4. Research objectives	22
1.4.1. Aim	22
1.4.2. Objectives	22
1.5. Outline and summary of the study	22
Chapter 3: Survey of <i>magwinya</i> production and consumption	23
Chapter 4: Quantification of oil fractions of a bran-enriched <i>magwinya</i>	24
Chapter 5: Microstructure and image analysis of <i>magwinya</i> for oil uptake, crumb and crust properties	24
Chapter 6. In vitro starch digestibility of <i>magwinya</i> enriched with oat and wheat bran	24
Chapter 7: Mapping oil, protein and carbohydrates in <i>magwinya</i> using near infra-red hyperspectral imaging technology	25
CHAPTER 2: LITERATURE REVIEW	26
2.1. Deep frying	26
2.1.1 Hydrolysis	27
2.1.2 Oxidation	27
2.2. Mechanism of oil uptake and distribution in fried food	28
2.2.1 Water replacement theory	30
2.2.2 Cooling phase theory	31
2.2.3. Surfactant theory	31
2.3. Oil reduction attempts in cereal fried dough	32
2.4. Food microstructure	35
2.4.1. Confocal laser scanning microscopy	35
2.4.2 Application of CLSM in the study of food microstructure	36
2.4.3 Advantages and limitations of CLSM	38
2.4.4 Image quantification	38
2.4.5 Pore characterisation of fried foods	39
2.5. Starch composition and structure	39
2.5.1. Starch gelatinisation	41
2.5.2. Starch retrogradation	41
2.6 Starch digestibility	42
2.6.1. Factors influencing starch digestibility	42
2.6.2. In vitro starch digestibility	44
2.6.3. Effect of frying on starch digestibility food	47
2.7 Hyperspectral imaging technology	47
2.8 Conclusion	52
CHAPTER 3: THE SOCIOECONOMIC BENEFITS, PRODUCTION AND CONSUMPTION STATISTICS OF <i>MAGWINYA</i> IN LIMPOPO PROVINCE, SOUTH AFRICA	54

Abstract	54
3.1. Introduction	54
3.2. Materials and Methods	56
3.2.1. Sample population and survey area	56
3.2.2. Questionnaire for producers and consumers	56
3.2.3. Data analysis	57
3.3. Results and Discussion	57
3.3.1. Demography of Producers and consumers	57
3.3.2. <i>Magwinya</i> production process	59
3.3.3. <i>Magwinya</i> sales	64
3.3.4. <i>Magwinya</i> consumption	66
3.3.5. Consumer purchase attributes	70
3.4. Conclusion	71
CHAPTER 4: QUANTIFICATION OF OIL FRACTIONS OF A DEEP-FRIED WHEAT DOUGH AS AFFECTED BY BRAN AND MOISTURE LEVELS	73
Abstract	73
4.1 Introduction	73
4.2. Materials and Methods	75
4.2.1 Materials	75
4.2.2 Experimental design	75
4.2.3 <i>Magwinya</i> production	75
4.2.5 Fresh weight	77
4.2.6 Moisture content and moisture loss determination	78
4.2.7 Surface oil	78
4.2.8 Penetrated surface oil and structural oil	78
4.2.9 Statistical analysis	79
4.3. Results and Discussion	79
4.3.1 Weight of <i>magwinya</i>	79
4.3.2 Moisture content and moisture loss	81
4.3.3 Total oil content	84
4.3.4 Surface oil of <i>magwinya</i>	89
4.3.5 Penetrated surface oil of <i>magwinya</i>	91
4.3.6 Structural oil of <i>magwinya</i>	93
4.3.7 Correlation of oil fractions and other properties of fried products	95
4.4 Conclusion	96
CHAPTER 5: CONFOCAL LASER SCANNING MICROSCOPY AND IMAGE ANALYSIS FOR ELUCIDATING OIL UPTAKE, CRUMB AND CRUST MICROSTRUCTURE OF BRAN-ENRICHED SOUTH AFRICAN FRIED DOUGH AND BATTER	97
Abstract	97
5.1. Introduction	97
5.2. Materials and method	99
5.2.1 Materials	99
5.2.2 <i>Magwinya</i> production and frying process	99
5.2.3 Confocal imaging of <i>magwinya</i>	99
5.2.4 Image segmentation and analysis	100
5.2.5 Quantification of surface roughness of <i>magwinya</i> using fractal analysis	101
5.2.6 Statistical analysis	101
5.3. Results and Discussion	102
5.3.1 Qualitative analysis of microstructure	102
5.3.2. Penetrated oil content by image analysis	108
5.3.3 Porosity and pore distribution	111
5.3.4 Pore distribution in <i>magwinya</i> crumb	113

5.3.5 Relationship between microstructural properties against Soxhlet fat extraction	118
5.3.6 Crust surface roughness of <i>magwinya</i>	121
5.3.7 Surface roughness using fractal metrics	125
5.3.8 Correlation of fractal dimension to surface oil and texture of fried products	126
5.4. Conclusion	127
CHAPTER 6: IN VITRO STARCH DIGESTIBILITY OF <i>MAGWINYA</i> ENRICHED WITH OAT AND WHEAT BRAN	129
Abstract	129
6.1 Introduction	129
6.2. Materials and methods	131
6.2.1. Materials	131
6.2.2. Sample preparation	131
6.2.3. Starch digestibility protocol	132
6.2.4. Glucose measurement in <i>magwinya</i> digest	133
6.2.5 Free sugar glucose content of <i>magwinya</i>	134
6.2.6 Digestible and total starch of <i>magwinya</i>	134
6.2.7 Expected glycemic index of <i>magwinya</i>	134
6.2.8 Statistical analysis	135
6.3. Results and discussion	135
6.3.1 Rapidly and slowly available glucose content of <i>magwinya</i>	135
6.3.2 Unavailable glucose content of <i>magwinya</i>	136
6.3.3. Digestible starch content of <i>magwinya</i>	139
6.3.4 Estimated glycemic index (eGI) of <i>magwinya</i>	143
6.3.5 Relationship between estimated glycemic index, and other variables in the study	146
6.4. Conclusion	151
CHAPTER 7: MAPPING OIL, CARBOHYDRATES AND OTHER CHEMICAL CONSTITUENTS OF <i>MAGWINYA</i> USING NEAR INFRA-RED HYPERSPETRAL IMAGING TECHNOLOGY	152
Abstract	152
7.1. Introduction	152
7.2. Materials and methods	153
7.2.1 <i>Magwinya</i> production	153
7.2.2 Scanning of <i>magwinya</i> using hyperspectral imaging system	153
7.2.3 Image analysis	154
7.2.4 Pre-processing of hyperspectral images of <i>magwinya</i>	154
7.3 Results and discussion	155
7.3.1 Comparison of control fried batter and fried dough	155
7.3.2 Mean centring and standard normal variate processing of bran enriched <i>magwinya</i>	161
7.3.3. Mean centring and Savitsky-Golay (SG) second derivative processing of bran- enriched <i>magwinya</i>	164
7.4. Conclusion	167
CHAPTER 8: GENERAL CONCLUSION	168
8.1. Conclusion	168
8.2. Contribution to knowledge and recommendations	169
REFERENCES	170
APPENDICES	189

List of Tables

Table 1. Relationship between mechanism of oil uptake and location in food	32
Table 2. Oil reduction in some wheat-based fried snacks using plant additives	34
Table 3. <i>In vitro</i> starch digestion process of some foods	46
Table 4. Food applications of hyperspectral imaging technology	51
Table 5. Demographic information of <i>magwinya</i> producers and consumers	59
Table 6. <i>Magwinya</i> production process	61
Table 7. <i>Magwinya</i> sales data	65
Table 8. <i>Magwinya</i> characteristics	67
Table 9. <i>Magwinya</i> consumer purchase attributes	71
Table 10. Pearson's correlation for oat bran enriched fried snack	95
Table 11. Pearson's correlation for wheat bran enriched fried snack	96
Table 12. Porosity & penetrated oil (PO) content determined by image analysis (POia) and PO by soxhlet	110
Table 13. Regression analysis showing the main and interaction effects of independent variables on porosity (%) and penetrated oil by image analysis	112
Table 14. Pore properties of <i>magwinya</i> samples as influenced by the addition of water and bran variation	116
Table 15. Cubic polynomial regression equation for plots of image properties and soxhlet extraction	121
Table 16. Fractal dimension (FD) values of fried products	125
Table 17. Cubic polynomial regression equation for plots of fractal dimension versus surface oil and crust hardness	127
Table 18. Available glucose content (g/ 100 g) of <i>magwinya</i> enriched with oat and wheat bran	138
Table 19. Multivariate linear regression for main and interaction effects of independent variables on glucose content of <i>magwinya</i>	140
Table 20. Digestible starch content of wheat bran <i>magwinya</i> (% wet weight)	141
Table 21. Digestible starch content of oat bran <i>magwinya</i> (% wet weight)	143
Table 22. Estimated glycemic index of <i>magwinya</i>	145
Table 23. Correlation coefficients of independent variables, glucose, digestible starch and glycemic index of wheat bran <i>magwinya</i>	148
Table 24. Correlation coefficients of independent variables, glucose, digestible starch and glycemic index of oat bran <i>magwinya</i>	150
Table 25. Specific wavelengths in mean centring pre-processing for control <i>magwinya</i> crumb	157

List of Figures

Figure 1. Hydrolysis of triacylglycerol to fatty acid and diacylglycerol	27
Figure 2. Thermal oxidation process during frying	28
Figure 3. Factors affecting oil uptake	30
Figure 4. Simultaneous heat and mass transfer in fried foods	31
Figure 5. Amylose and amylopectin structure	40
Figure 6. <i>Magwinya</i> from different locations in Thohoyandou area	63
Figure 7. Number of <i>magwinya</i> per kg of flour by producers in Thohoyandou area	64
Figure 8. Average daily sales by <i>magwinya</i> producers in Thohoyandou	66
Figure 9. <i>Magwinya</i> samples with various sizes and shapes	69
Figure 10. Flow chart for production of <i>magwinya</i>	76
Figure 11. Fresh weight of oat and wheat bran <i>magwinya</i>	80
Figure 12. Moisture content and moisture loss of <i>magwinya</i>	83
Figure 13. Total oil content (g/g) of <i>magwinya</i> samples.	85
Figure 14. Samples of fried products	88
Figure 15. Stained fried dough and batter showing the oil fractions	89
Figure 16. Surface oil of <i>magwinya</i>	90
Figure 17. Penetrated surface oil of <i>magwinya</i>	92
Figure 18. Structural oil of <i>magwinya</i>	94
Figure 19. Cross-section micrographs of fried dough enriched with oat bran	104
Figure 20. Cross-section micrographs of oat bran fried batter enriched with oat bran	105
Figure 21. Cross-section micrographs of fried dough enriched with wheat bran	106
Figure 22. Cross-section micrographs of fried batter enriched with wheat bran	107
Figure 23. Types of pores identified in <i>magwinya</i>	113
Figure 24. Pore size distribution in cross-section micrographs of fried dough and batter samples	115
Figure 25. Crust confocal micrographs of wheat bran fried batter and fried dough	123
Figure 26. Crust confocal micrographs of oat bran fried batter and fried dough	124
Figure 27. Whole and minced <i>magwinya</i>	132
Figure 28. Digested <i>magwinya</i> and digest	133
Figure 29. RGB images of control <i>magwinya</i> with no background	156
Figure 30. Score image (PC4) for control <i>magwinya</i> using mean centring and standard normal variate processing	158
Figure 31. PC1 score image f using mean centring and Savitzky-Golay 2nd derivative processing or control <i>magwinya</i>	159
Figure 32. Clean RGB images of oat bran and wheat bran <i>magwinya</i>	160

Figure 33. Score image (PC2) using mean centring and standard normal variate for bran enriched fried dough	161
Figure 34. Score image (PC4) using mean centring and standard normal variate for bran enriched fried batter	163
Figure 35. Score image (PC1) using mean centring and Savtzky-Golay 2nd derivative processing for bran enriched fried dough	165
Figure 36. Score image (PC1) using mean centring/ Savtzky-Golay 2nd derivative processing for bran enriched fried batter	166

List of Abbreviation

CLSM	Confocal laser scanning microscopy
eGI	Estimated glycemic index
FD	Fractal dimension
FITC	Fluorescein-5-isothiocyanate
GI	Glycemic index
HSI	Hyperspectral imaging
NIR	Near-infrared
OB	Oat bran
OBFB	Oat bran fried batter
OBFD	Oat bran fried dough
PCA	Principal component analysis
PSO	Penetrated surface oil
RAG	Rapidly available glucose
RDS	Rapidly available starch
RS	Resistant starch
SAG	Slowly available glucose
SDS	Slowly available starch
SO	Surface oil
STO	Structural oil
TAG	Triacylglycerol
UG	Unavailable glucose
WB	Wheat bran
WBFB	Wheat bran fried batter
WBFD	Wheat bran fried dough

List of Appendices

Appendix I	Survey sample size calculation	186
Appendix II	<i>Magwinya</i> production survey	187
Appendix III	<i>Magwinya</i> consumption survey	189
Appendix IV	Consumer responses across age groups	192
Appendix V	Preliminary findings for proofing temperature of magwinya	195
Appendix VI	Standard curve of Sudan III dye concentrations against absorbance	196
Appendix VII	Segmented pores of <i>magwinya</i> cross-section micrographs	197
Appendix VIII	Multivariate analysis showing main and interaction effects of independent variables on fractal dimension	201
Appendix IX	Measurement of glucose using glucose-oxidase reagent kit	202

Publications and Conference Proceedings

Publications

1. Onipe, O. O., Beswa, D., & Jideani, A. I. O. (2018). I love fat cakes (*magwinya*) but hate the fat; is there an option for me? *FST Magazine*, 27(2), 19-20.
2. Onipe, O. O., Beswa, D., & Jideani, A. I. O. (2019). The socioeconomic benefits, production and consumption statistics of *magwinya* in Limpopo Province, South Africa. *African Journal of Food, Agriculture, Nutrition and Development*, 19(4), 15007-15028.
3. Onipe, O. O., Beswa, D., & Jideani, A. I. O. (2020). Confocal laser scanning microscopy and image analysis for elucidating oil uptake, crumb and crust microstructure of bran-enriched South African fried dough and batter. *Foods*, 9(5), 605.

Submitted papers

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Conference proceedings

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4. Onipe, O.O., Beswa, D., & Jideani, A.I.O. September 2018. Fried foods in developing Countries: Consumption, enrichment, and optimisation for fat reduction. Oral session moderation and presentation, *Institute of Food Technologists (IFT) Food Congress*, Chicago, USA.

5. Onipe O.O. & Jideani A.I.O. (2018) Development of a low-fat, high-fibre snack: effect of bran particle sizes and processing conditions. Poster and oral presentation, 5th International ISEKI Food Conference, Stuttgart, Germany.

CHAPTER 1: INTRODUCTION

1.1. Introduction

Frying is a complex process that alters the physicochemical, textural and sensory properties of food. Most changes that take place in foods during frying are both at the cellular and sub-cellular level (Bouchon and Aguilera, 2001). *Magwinya* – a wheat-based, yeast-leavened deep-fried snack which contains about $\leq 14\%$ oil (Onipe *et al.*, 2019), and is one of the most consumed street snacks in South Africa. However, statistics on consumption and production process is lacking. Previous research in *magwinya* on fortification with wheat bran (Onipe *et al.*, 2018; 2019), psyllium husk fibre (Kwinda *et al.*, 2018), guar gum (unpublished), resulted in about 22 - 64% oil uptake reduction and increased fibre and mineral content.

Study of deep-fried foods at the microstructural level is important in modelling heat and mass transfer, thereby unveiling their mechanisms (Ngadi *et al.*, 2009). Besides, changes that occur at macroscopic and microscopic levels in fried foods can provide relevant information needed in characterizing pore structure and optimizing textural properties, in turn improving sensory properties of such foods (Fazaeli *et al.*, 2012). Since *magwinya* processing involves physical and chemical interactions that take place at the cellular and sub-cellular level, it is imperative to study *magwinya* microstructure. Among the microscopic techniques that have facilitated the study of oil uptake in fried food microstructure are scanning electron microscopy for cellular characterisation (Kim *et al.*, 2012), confocal laser scanning microscopy for observation of fat distribution within the food structure (Moreno and Bouchon, 2013), X-ray computed tomography for surface core geometry (Alam and Takhar, 2016) and hot-stage video microscope for real-time observation of the frying process (Bouchon and Aguilera, 2001; Ovalle *et al.*, 2013).

Magwinya can be categorized as a high carb, high-fat food because its main ingredient refined wheat flour, coupled with the processing method- deep frying which contributes to the caloric density of *magwinya*. The surge in metabolic diseases like obesity and type II diabetes (now a global epidemic called diabetes) in sub-Saharan Africa, has been linked, for the most part to dietary changes coupled with personal, economic and socio-cultural transitions (Parada & Santos, 2016; Atun *et al.*, 2017). Obesity is a diet-based physiological problem that stems from consumption of foods, high in carbohydrates and /or unhealthy fats coupled with little or no active lifestyle (Wenhold *et al.*, 2014). Overweight/ obese individuals are prone to the development of insulin resistance which is a precedence to other metabolic diseases such as hyperlipidemia, hyperinsulinemia and type II diabetes (Kelishadi, 2007). Glycemic index (GI) is a model of classification of starchy food into groups based on their impact on blood glucose

after ingestion of a carbohydrate meal (Singh *et al.*, 2010). The GI of *magwinya* is not reported in the data repository of GI of South African foods, hence a need to estimate the GI *magwinya* and the effect of bran addition thereof.

The bran of cereal grain has been reported to improve the overall health of the gut and also offer potential protection against diseases such as colon cancer and cardiovascular diseases (Prückler *et al.*, 2014). Health benefits of wheat bran (WB) is linked to its insoluble dietary fibre content that increases digesta viscosity (Onipe *et al.*, 2015) and phenolic compounds which inhibit the absorption of cholesterol in the small intestine (Hemdane *et al.*, 2015). Wheat bran is especially known for its insoluble fibre which imparts health properties in the gastrointestinal system. Oat bran (OB) is popular for its high content of β -glucan known for potential protective mechanism, such as, lowering plasma cholesterol and glucose, which may indirectly affect the metabolism of bile acids and neutral sterols in the intestine and liver; it also possesses anti-mutagenic properties (Fardet, 2010).

1.2. Problem statement

Magwinya has a high consumption prevalence in South Africa as evidenced by its presence in townships, supermarkets, bus parks, and franchises like Fat Cake City. Despite its probable prevalence, there are no scholarly studies to support this assertion. Transformation of flour into *magwinya* involves frying – a processing method that increases the energy density of food due to high oil uptake (about one-third of food weight). Regular consumption of deep-fried foods coupled with inactive lifestyle has been linked to obesity. Obese or overweight individuals are at a high risk for various metabolic diseases such as diabetes, colon cancer, cardiovascular disease, etc. Statistics show that 1 in 8 children in Africa are obese/overweight 2020 and childhood obesity is reported at 17 - 23% in South African children (Otitoola *et al.*, 2020). The percentage of obese/overweight individuals with above 25 BMI was 55% for men and 77% for women with overall at 70% in South Africa. Limpopo stood at 39% for men and 70% for women. From this report, morbidity due to obesity showed more than half (51%) had high blood pressure (hypertension) and about 16% had diabetes mellitus (STATSSA, 2019). Obesity is associated with increased comorbidity, giving rise to an increase in both direct and indirect socioeconomic costs such as increased healthcare costs, decreased functionality, lower life expectancy, increased comorbidity, and general quality of life. Ability to predict digestion and absorption of glucose upon ingestion of starch-based foods is of vital to health concerns all over the world, giving special attention to diabetes and obesity (Singh *et al.*, 2010). High GI foods raise postprandial blood glucose thereby increasing the risk of overeating and in turn being overweight. Due to its high carbohydrate content, *magwinya* may be categorised as a high GI food, however, this has not been established in an empirical study.

1.3. Hypotheses of the study

- (1) With no standardised method of *magwinya* production coupled and variation in consumer preferences;

Alternative hypothesis 1: Production process will vary from one producer to the other and consumer pattern will differ based on demography.

Null hypothesis 1: Production will not vary from one producer to the other and consumer pattern will differ based on demography.

- (2) Intrinsic factors such as food composition, additives, moisture contents affect oil distribution within a food, oil uptake and consequentially its reduction if these factors are well managed. Also, there is a simultaneous relationship between moisture loss and oil uptake in fried foods. (Ziaifar *et al.*, 2008).

Alternative hypothesis 2: Bran type, concentration and level of initial moisture content will reduce oil uptake and affect oil distribution in *magwinya*.

Null hypothesis 2: Bran type, concentration and level of initial moisture content will reduce oil uptake and affect oil distribution in *magwinya*.

- (3) Crust microstructure, surface roughness, food shape and size are factors linked to product features that affect oil distribution and uptake in the product (Ziaifar *et al.*, 2008). Moreover, higher crust roughness and initial moisture content have been linked to increased oil uptake in fried products (Moreno *et al.*, 2010).

Alternative hypothesis 3: Addition of OB and WB will improve crust microstructure and consequentially reduce oil uptake of *magwinya* and improve crumb properties (porosity, pore formation).

Null hypothesis 3: Addition of OB and WB will not improve crust microstructure and consequentially reduce oil uptake of *magwinya* and improve crumb properties (porosity, pore formation).

- (4) Oat and wheat bran contain considerable amounts of soluble and insoluble dietary fibres, respectively. Dietary fibres delay enzymatic hydrolysis of starch-based foods (Singh *et al.*, 2010; Onipe *et al.*, 2015).

Alternate hypothesis 4: Incorporation of OB and WB in *magwinya* formulation will reduce glucose release during *in vitro* starch digestion and as a result reduce digestible starch, estimated glycemic index and increase the resistant starch portion of *magwinya*.

Null hypothesis 4: Incorporation of OB and WB in *magwinya* formulation will not reduce glucose release during *in vitro* starch digestion and as a result reduce digestible starch, estimated glycemic index and increase the resistant starch portion of *magwinya*.

- (5) The process of deep-frying results in the transformation of existing constituents of a food product and the formation of new flavour and colour compounds in the products (Ghidurus *et al.*, 2010).

Alternate hypothesis 5: Incorporation of WB and OB will cause a variation in the distribution of *magwinya* chemical properties detectable using NIR HSI spectroscopy.

Null hypothesis 5: Incorporation of WB and OB will not cause a variation in the distribution of *magwinya* chemical properties detectable using NIR HSI spectroscopy.

1.4. Research objectives

1.4.1. Aim

The present research was executed to understand the oil absorption in *magwinya* and, most importantly the effect of ingredient modification and the role they play in oil distribution, microstructure, starch digestion and the distribution of the food components within *magwinya*. To carry this out, the following specific objectives are set out:

1.4.2. Objectives

- (1) To generate information on the production and consumption of *magwinya* in Thohoyandou area using semi-structured interviews and questionnaires.
- (2) To quantify oil fractions (surface, penetrated surface, structural and total oil content) of *magwinya* as influenced by bran and moisture levels.
- (3) To characterise crust and crumb properties (porosity, pore size, depth of oil penetration, surface roughness) of *magwinya* using confocal laser scanning microscopy;
- (4) To estimate glucose (rapidly available, slowly available and unavailable glucose), digestible starch (rapidly digestible, slowly digestible, resistant and total starch) and GI of bran-enriched *magwinya* using *in vitro* assay.
- (5) To visualise oil, protein and carbohydrates distribution in *magwinya* using near-infrared hyperspectral imaging technology

1.5. Outline and summary of the study

Frying is an intricate process resulting in oil uptake at various depths in the food, mostly linked to intrinsic and extrinsic factors. This complexity, coupled with physical, chemical and lack of

information on oil distribution, pore variation, starch digestion and chemical distribution promoted the research described in this thesis. The results obtained in this research were in the form of journal papers from each result-based chapter with the status 'published', 'under review' or 'in preparation' as follows:

- The socioeconomic benefits, production and consumption statistics of *magwinya* in Limpopo province, South Africa (*published*).
- Quantification of oil fractions of a deep-fried wheat dough as affected by bran and moisture levels (*Revised paper submitted to Journal of Food Measurement and Characterisation for second review*).
- Confocal laser scanning microscopy and image analysis for elucidating oil uptake, crumb and crust microstructure of bran-enriched South African fried dough and batter (*Revised paper submitted for a second review in Foods journal*).
- *In vitro* starch digestibility of *magwinya* enriched with oat and wheat bran (*Submitted to Foods journal*).
- Mapping chemical components variation of bran-enriched *magwinya* using near-infrared hyperspectral imaging technology (*in preparation*).

A synopsis of the result chapters is presented in the following section.

Chapter 3: Survey of *magwinya* production and consumption

Owing to the lack of information on the *magwinya* production and consumption pattern, the results of this chapter serves as a backdrop to the necessity of *magwinya* research. The information gathered from the survey revealed that *magwinya* is a frequently consumed snack amongst various age groups, especially high school and university students. *Magwinya* production process on a commercial level is still quite basic in that most producers do not use up-to-date equipment, thereby opening up an opportunity for Food Engineers to design *magwinya* production machine. This is because manual production limits the number that is produced by the reason of fatigue. While most consumers attributed their disinterest in *magwinya* to its high-fat content; some consumers, in fact, like the product for the same reason. Similarly, this study confirmed that consumers are becoming increasingly aware of the health-diet association and show a willingness to purchase low-fat *magwinya* if made available commercially. Production and availability of low-fat *magwinya* substitutes will, to an extent, contribute to alleviate obesity and other diet-related problems. This chapter has been published as a paper in the *African Journal of Food, Nutrition and Development*.

Chapter 4: Quantification of oil fractions of a bran-enriched *magwinya*

This chapter presents the first study on the quantification of oil fractions of thick deep-fried foods through ingredient modification. Three oil fractions were identified and quantified thus: surface oil (SO), penetrated surface oil (PSO) and structural oil (STO). Bran type (wheat and oat bran) and its concentration impacted each product type (batter and dough) uniquely. The PSO fraction was similar for both product type in that it ranked highest in all the samples; meaning oil uptake in *magwinya* occurs mostly during the cooling period. However, STO was higher in fried dough while SO was higher in fried batter. Total oil-reduction effect of OB was evident from 5 g inclusion in fried dough, and 10 g in fried batter; while for WB-enriched products, oil reduction was noticed from 10 g concentration. Products with initial low moisture content had significantly lower oil content than products with high initial moisture content. The outcome of this study confirms oil uptake reduction in *magwinya* is possible through ingredient formulation. A manuscript from this chapter has been reviewed by the Editor of *the Journal of Food Measurement and Characterisation* and is currently being reviewed by the journal reviewers.

Chapter 5: Microstructure and image analysis of *magwinya* for oil uptake, crumb and crust properties

This chapter presents the first qualitative and quantitative description of crumb grain features including porosity and pore properties of *magwinya*. Oil depth and penetration in the products were observed and described using a non-invasive double staining protocol and confocal imaging. Pore sizes, porosity, oil uptake and grain features were estimated from the confocal micrographs with the aid of image analysis software (ImageJ) and the results were correlated to conventional measurements. Out of all the products, only the result of WB fried dough was ranked linearly for oil uptake by Soxhlet and image analysis. Other products followed a third-order polynomial relationship. Finally, the surface roughness of fried products was estimated using fractal metrics and the results were linked to crust texture and oil uptake. Surface roughness of WB products showed no significant variation, however, that of OB fried dough was higher than fried batter. Product modification is useful for controlling crust permeability and consequently oil uptake. A manuscript from this chapter has published in *Foods* journal.

Chapter 6. *In vitro* starch digestibility of *magwinya* enriched with oat and wheat bran

In vitro starch digestion of *magwinya* as influenced by bran and moisture levels is presented in this chapter. Bran type, bran concentration and moisture level in products showed significant effect on glucose fractions, digestible starch and estimated glycemic index (eGI) of *magwinya* - all of which have not been previously documented. *Magwinya* with no bran had high amounts of rapidly digestible starch, rapidly available glucose contents and high eGI value. Increments

to bran concentration reduced the RDS and RAG values. *Magwinya* with high moisture content (fried batter) were high in eGI than fried dough products. Reduction in RAG values and concomitant increase in UG values of WB and OB fried products points to the delayed effect of the fibres in the release of glucose of the fibres during enzymatic hydrolysis. WB and OB are easily accessible additives that can be incorporated in *magwinya* at commercial and household levels for medium GI *magwinya*. A manuscript from this chapter is currently in preparation.

Chapter 7: Mapping oil, protein and carbohydrates in *magwinya* using near-infrared hyperspectral imaging technology

The use of hyperspectral imaging technology in mapping chemical components of *magwinya* is presented in this chapter. NIR absorption bands featuring oil, starch, water, cellulose and protein were identified in *magwinya* crumb, as well as other peaks featuring aromatic and phenolic compounds. Although not quantified, these compounds were detected which points to the potential of HIS in future research for quantitative and prediction models for quality evaluation of *magwinya*. A manuscript from this chapter is currently in preparation.

CHAPTER 2: LITERATURE REVIEW

Introduction

This chapter presents a review of the general concept and mechanism of oil uptake, microscopic techniques for quantitative and qualitative analysis of fried foods and starch digestibility of fried foods. The main aspects covered include an overview of deep frying and oil reduction attempts of fried dough snacks as well as a review of food microstructure and starch digestion process. The effects of cereal bran on oil uptake, microstructure and starch digestion are also reviewed. Finally, the use of hyperspectral imaging technology in foods are reviewed and specific applications in foods are elucidated.

2.1. Deep frying

There is an increasingly high consumption of fried foods because of its palatability, distinct flavour, crispiness/texture and appearance (Jeon *et al.*, 2013). Deep frying is a longstanding method of food preparation which dates to 1600 BC where fragments of food were cooked in a heated pot of oil (Zhang *et al.*, 2012). This method of cooking is used at both household and industrial levels because of its ease of use and fast method of preparation (Dobarganes, 2009). Deep frying is a complex and the least understood process involving physical changes of food, chemical reactions, as well as mass and heat transfer characterised by the evaporation of water from a food product which is then replaced by the migration of oil into the food. Other processes like colour/crust formation, starch gelatinisation, water vaporisation, and protein denaturation are some of the changes that occur in food during the frying process. These chemical reactions lead to the formation of new compounds in the frying oil (Rimac-Brnčić *et al.*, 2004; Bou *et al.*, 2012).

The frying process occurs in four stages (a) initial heating stage which lasts a few seconds where food products heat to boiling temperature of surface water, and heat transfers from oil to the product through natural convection; (b) surface boiling stage is characterized by a loss of surface moisture due to evaporation. It occurs during the first 60 secs of the frying process whereby vaporisation starts at the surface and a crust begins to form. In the heat transfer layer in the oil, at the surface of the product, boiling increases the surface heat transfer coefficient; (c) falling rate stage is the longest stage where most foods are sufficiently cooked, and the crust of the food thickens. Internal core temperature rises, and more internal moisture migrates outwards from the food. At this stage, starch gelatinisation, protein denaturation and cooking occur. (d) Bubble endpoint is the stage where there are no more vapour bubbles and moisture loss become negligible coupled with the reduction of heat transfer to the core of the food. Stages 2 – 4 represent the boiling regime, with most foods adequately cooked at stage 3

(Ahmed and Rahman, 2012). Various foods that can be cooked using the frying method include vegetables, fish, meat, pasta, noodles, tubers (Sobukola *et al.*, 2013), French fries, doughnuts, and potato chips (Yang *et al.*, 2012). During frying, the triacylglycerol (TAG) structure of oil/fat is used simultaneously undergoes alteration through hydrolysis and oxidation in the presence of air, moisture and high temperature ($\geq 170^{\circ}\text{C}$).

2.1.1 Hydrolysis

Hydrolysis which occurs in the presence of heat and water (the moisture content of the food) is known to be the only reaction that causes a breakdown of ester bonds of the TAG molecule (Figure 1) resulting in the release of free fatty acids (FFA), glycerol mono- and diacylglycerol (Choe and Min 2007; Velasco *et al.*, 2008).

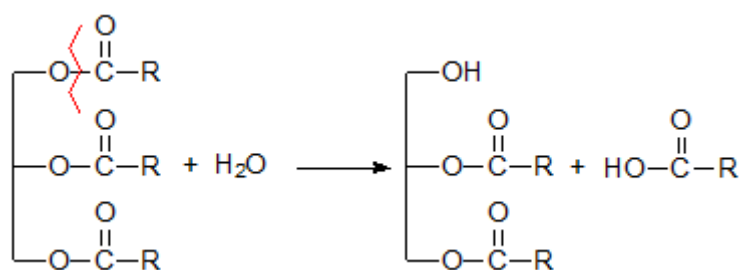


Figure 1. Hydrolysis of triacylglycerol to fatty acid and diacylglycerol. Source: Dobarganes (2009)

These new compounds influence the sensory, nutritional and technological quality of the food and oil. From a technological point of view, the release of FFA causes a decrease in interfacial tension of the oil (which decreases the shelf life of the oil), the formation of some off-flavour and volatile compounds as well as a decrease of the smoke point of the oil (Nawar, 1998; Dobarganes, 2009). Diacylglycerol and FFA increases with continuous re-use of the same oil (Romero *et al.*, 1998), hence, the quality of frying oil is measured by an estimation of its FFA content. As frying progresses, moisture is changed into steam, but unlike steam, water hydrolyses oil faster (Dana *et al.*, 2003). The hydrolysis of oil, however, can be slowed down by intermittent substitution or addition of used oil with/to fresh oil. Percentage polar fractions of FFA and diacylglycerol in replenished oils were significantly higher than the amount in non-replenished oils used to fry potatoes (Romero *et al.*, 1998).

2.1.2 Oxidation

Oxidation of lipids is known to be a complex chemical reaction that occurs in the presence of oxidative and thermal reactions which takes place concurrently (Dobarganes, 2009). Thermal and auto-oxidative reactions follow the same free radical mechanism of chain reactions,

except that the former progresses faster than the latter. As frying progresses, the temperature increase in the frying medium causes a rapid progression in oxidative reactions coupled with a sharp decrease in solubility of oxygen (Choe and Min 2007; Zhang *et al.*, 2012). In the thermal oxidation process, which occurs in 3 stages - initiation, propagation and termination - one of the three unsaturated fatty acyl groups of the TAG molecule (RH) undergoes oxidation. At the initial stage, a hydrogen radical separates either from its allylic or bis allylic position of an unsaturated fatty acid (Figure 2). The separation causes a formation of an alkyl radical. In the propagation stage, the alkyl radical reacts with oxygen to form peroxy radicals which react with new TAG molecules to form hydroperoxides and new alkyl radicals which further propagate the reaction chain. In the termination stage, stable non-radical species are formed from a reaction between the radicals formed in the propagation stage (Velasco *et al.*, 2008; Zhang *et al.*, 2012).

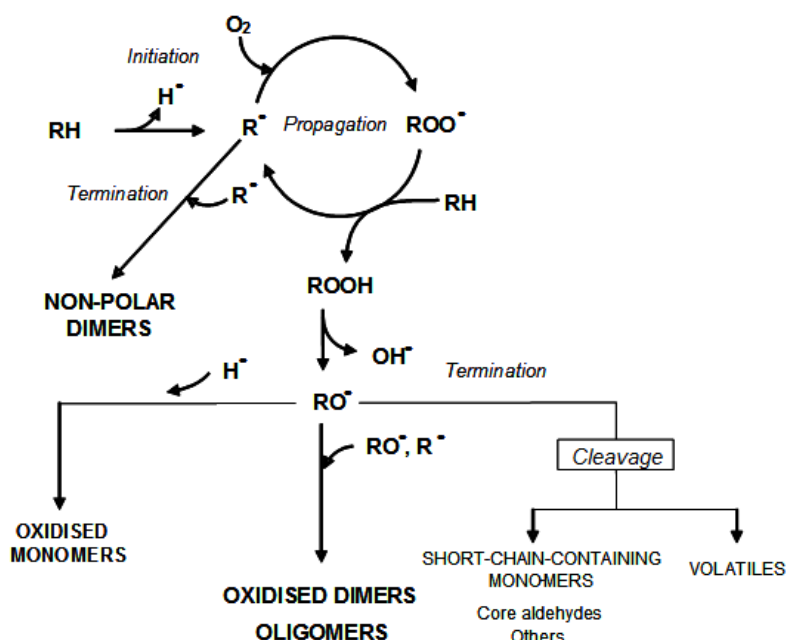


Figure 2. Thermal oxidation process during frying. Source: Dobarganes (2009)

In summary, the frying stages coupled with the reactions taking place in the frying medium all culminate in the amount of oil absorbed by the food as well as the nutrients gained or lost in the process. All these processes are influenced by frying time, temperature and the number of cycles in which the oil has been used.

2.2. Mechanism of oil uptake and distribution in fried food

Oil uptake is a surface and pressure-driven phenomenon which is mediated by atmospheric, vapour and capillary pressures (Saguy and Dana, 2003; Sandhu *et al.*, 2013). Frying is a unit operation that is described as a dehydration process that causes a moving boundary layer

which leads to a distinction between the crispy crust and moist core (Sandhu *et al.*, 2013). Due to this difference in texture, oil distribution in fried foods led to the classification as surface, structural and penetrated surface oil (Bouchon *et al.*, 2003). This classification introduced by Bouchon *et al.* (2001) was based on the various mechanisms of oil uptake mentioned in literature. Surface oil (SO) represents the oil that adheres to the food surface after frying – this is greatly influenced by interfacial tension of the oil. Penetrated surface oil (PSO) is the oil suctioned into the crust pores during the cooling period of the food and is regarded as the most important oil fraction in the study of oil uptake. Structural oil represents the oil absorbed into the core of the food during frying (Bouchon *et al.*, 2003; He *et al.*, 2013a). Adhered oil and absorbed oil are interrelated in the sense that the amount of absorbed oil during cooling depends on the amount of adhered oil at the end of the frying process (Thanatuksorn *et al.*, 2005).

Oil absorption occurs both during and post frying and this makes it easier to quantify oil absorbed in food and monitor the stages of oil absorption during and after the frying process. Bouchon *et al.* (2003) concluded in their estimation of oil-uptake location in fried potatoes that a significant amount of oil in the potato was absorbed after its removal from the oil. Out of the three oil fractions, they reported PSO as the highest amount because of the highly porous structure of the crust caused by mechanical abrasion during cutting of the potato as well as crispiness caused by total dehydration of the crust due to high thermal action at the food surface. Oil uptake in fried foods is dependent on some of the intrinsic and extrinsic factors which make frying a complex phenomenon because of the number of variables involved in its quantification. Among the factors that affect oil uptake are (1) product composition, such as, water content, starch content and leavening agents (Dana and Saguy 2006), (2) product characteristics including size, shape, surface area, roughness (Moreno *et al.*, 2010), density, porosity (Falcone *et al.*, 2005) and crust microstructure (Bouchon *et al.*, 2001); and (3) processing methods including pre-, and post-processing methods. Other factors involved in the frying operation and their influence on oil uptake is presented in Figure 3.

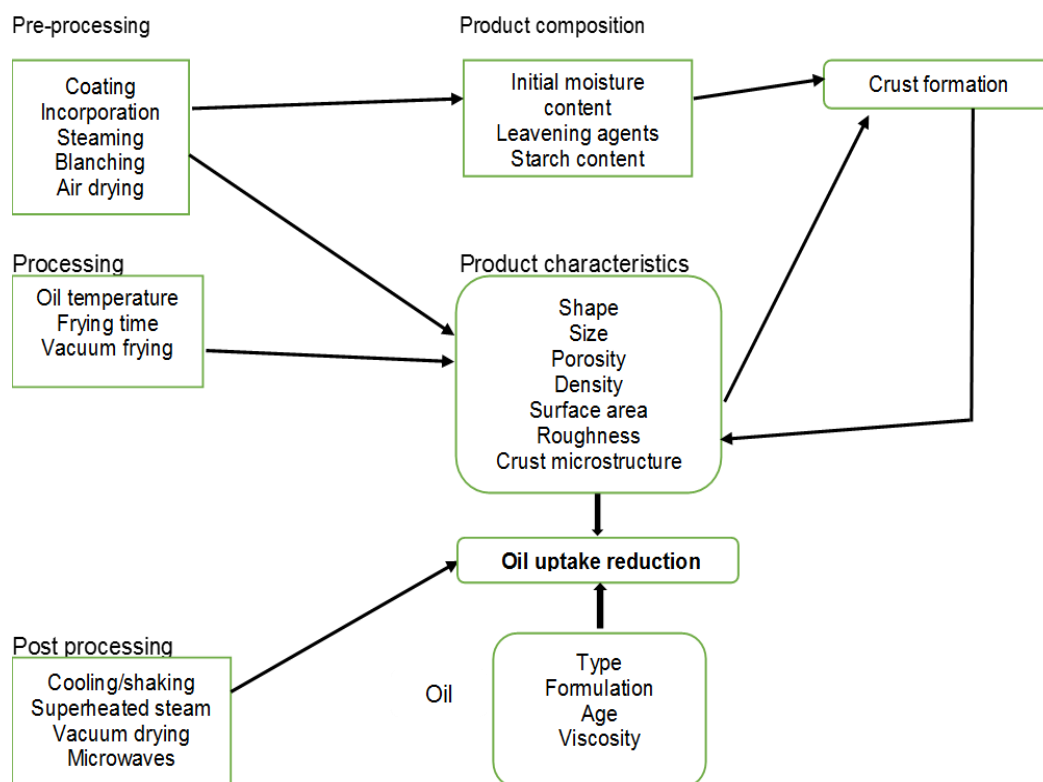


Figure 3. Factors affecting oil uptake. Source: Ziaifar *et al.* (2008)

During frying, the formation of the crust influences heat and mass transfer (Ziaifar *et al.*, 2008) that is why oil uptake is regarded as a surface phenomenon; meaning that it occurs on the surface of the food. Three major theories of oil uptake which have been elucidated in literature are water replacement, cooling phase and surfactant theories.

2.2.1 Water replacement theory

As food is placed in frying oil, there is immediate conversion of water to steam and the formation of a positive pressure gradient inside the food as a result of the rise in the temperature. As frying progresses, the surface of the food becomes dehydrated and a dry crust is formed which acts as a barrier or protective covering to the core of the food (Brannan *et al.*, 2014). Cracks and pores are formed in the crust of the food as a result of the escape of water from the surface of the food (Mellema, 2003). In a situation where the crust voids formed are large enough, the vapour pressure causes entry of oil into the food. Mass transfer (oil influx and water migration) in the food does not take place simultaneously, but sequentially. This begins with the migration of water from the product at the water replacement phase (Figure 4), followed by an influx of oil which usually takes place during the cooling phase (He *et al.*, 2013a; Brannan *et al.*, 2014). Sandhu *et al.* (2013) reported that negative values of pressure recorded in potato discs and chicken nuggets during and after frying may have enhanced rapid oil uptake in these products.

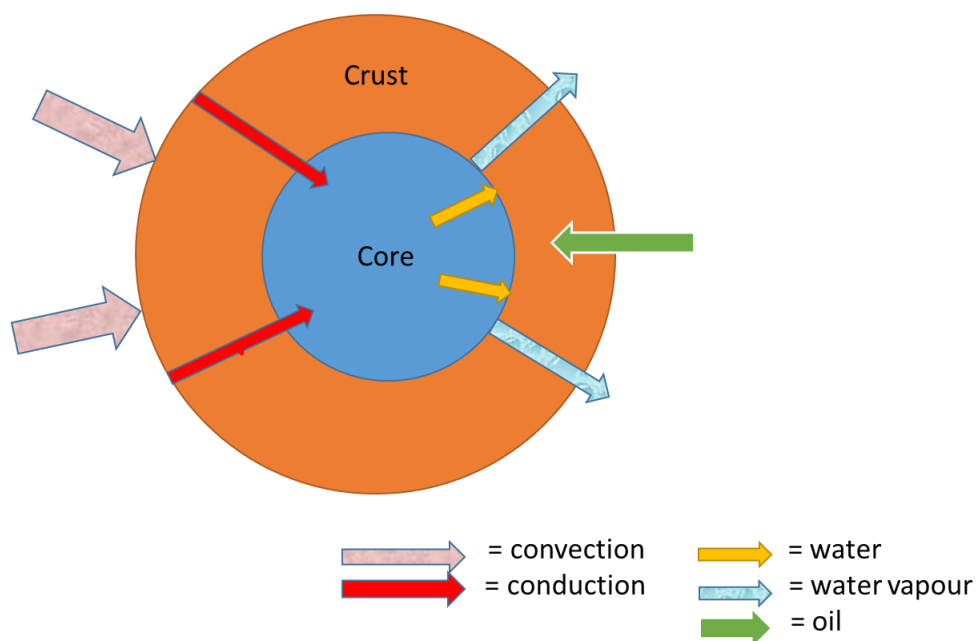


Figure 4. Simultaneous heat and mass transfer in fried foods. Source: Bouchon (2009)

2.2.2 Cooling phase theory

Upon removal of the food from the frying oil, the temperature of the core gradually reduces coupled with a subsequent reduction in the internal pressure at the core of the food and the influx of oil into the food (Saguy and Dana, 2003). Surface oil is sucked up by drainage forces into the food through the pores or capillaries, by adhesive forces between oil and the pore wall simultaneously through cohesive forces in the oil (Mellema, 2003; Ziaifar *et al.*, 2008). The mechanism of oil uptake following this theory is that during cooling, there is an equilibrium between adhesive and drainage forces, between oil and food which causes oil absorption. Oil uptake is thus regarded as a surface phenomenon (Ufheil and Escher, 1996; He *et al.*, 2013a).

2.2.3. Surfactant theory

As frying progresses, there is degradation of oil from the basic TAG molecule to hundreds of compounds including monoglycerides, diglycerides and free fatty acids. This change causes a lower interfacial tension between oil and the food which acts as surface-active agents or surfactants. The formation of surface-active agents leads to excessive absorption of oil by the food. From the theories explained above, a summary of the mechanisms of oil uptake in relationship with the location of oil in the food is presented in Table 1.

Table 1. Relationship between the mechanism of oil uptake and location in food

Oil location	Mechanism of oil uptake	Reference
Surface oil	Oil adsorption caused by the relationship between capillary forces and interfacial tension.	Pinthus <i>et al.</i> (1993).
Structural oil	A continuous replacement of moisture by fat and absorption of fat at the cooling stage through vacuum effect at the core of the food. Oil suction through a vacuum effect caused by condensation of steam	Gamble <i>et al.</i> (1987); Saguy and Dana (2003); Sandhu <i>et al.</i> (2013).
Penetrated surface oil	An equilibrium between adhesive and drainage forces of oil.	Ufheil and Escher (1996).

The myriad of studies in the literature which focused mainly on fried potato products have established oil uptake as a surface phenomenon which takes place in the crust of the product. The mechanism of oil uptake in *magwinya*, however, has not been established, hence, one of the objectives of this study. Through measurement of the surface, penetrated and structural oil, this research proposes to unveil which of the mechanisms earlier elucidated best explains the oil uptake process in *magwinya*.

2.3. Oil reduction attempts in cereal fried dough

One of the methods of oil reduction in fried foods is the use of functional food ingredients such as cereal bran (Kim *et al.*, 2012; Yadav and Rajan, 2012), legume hulls (Lee *et al.*, 2008), hydrocolloids (Annapure *et al.*, 1999; Funami *et al.*, 1999; Sakhale *et al.*, 2011; Yazdanseta *et al.*, 2015) and fruit fibre (Prakongpan *et al.*, 2002). The effect of other additives on oil content reduction of wheat-based fried foods are highlighted in Table 2.

Magwinya, also known as 'fat cake', is a wheat-based, yeast-raised deep-fried snack which may be categorised as a variant of a doughnut (Feeley *et al.*, 2011). It is a household and commercial snack consumed at any time of the day in South Africa and as party finger foods in Nigeria. The amount of water in *magwinya* dough vary according to individual preference, although *magwinya* dough usually has a runny consistency containing about 80 – 100% (w/w) of water. Due to unavailable scientific studies on *magwinya*, our previous study focused on oil reduction in *magwinya* through optimisation of its production process (Onipe *et al.*, 2018;

2019). The ingredients used were 100 g bread wheat flour, 62 – 71 ml water, 15 g sugar, and 1 g yeast. WB was incorporated in wheat flour at 1 – 15 g per 100 g wheat flour. Based on the water replacement theory of oil uptake, a high initial moisture content of product being fried increases moisture evaporation; cracks and pores are formed in the product crust which in turn increased oil absorbed. For this reason, the initial water content of *magwinya* was reduced from 80 ml to 71 ml. Another pre-processing factor that was considered for oil reduction in *magwinya* was the incorporation of WB as a fibre source. WB is rich in insoluble dietary fibre which is hydrophilic in nature (Onipe *et al.*, 2015) and acted as a barrier to oil absorption in doughnuts (Kim *et al.*, 2012). In addition to other variables such as fermentation and frying time, incorporation of WB significantly reduced oil content of *magwinya* by at least 44.96%. Due to the lack of study on oil distribution in *magwinya*, this current study focused on quantifying different oil fractions in *magwinya* and how moisture levels and bran addition affected the distribution.

Table 2. Oil reduction in some wheat-based fried snacks using plant additives

	Cereal food	Additive for oil reduction	Amount of additive (g)	Percentage oil reduction	Reference
1	<i>Poori</i>	Oat and wheat bran	3 – 12	A 20% oil reduction was observed at 3 g wheat bran and 11 g oat bran additions.	Yadav and Rajan (2012).
2	Doughnut	Pineapple dietary fibre (PDF) and pineapple core	3	PDF had the lowest oil content of 14.1% compared to control- 23.8% and doughnut formulated with pineapple core- 18.3%	Prakongpan <i>et al.</i> (2002).
3	Doughnut	Wheat bran (WB)	1 - 10	Fat content of doughnut by reduced 9% at 10% WB inclusion	Kim <i>et al.</i> (2012).
3		Pre-gelatinized rice flour (RF)	20 – 50	At 50% RF addition, oil content reduction of 64% was recorded.	Shih and Daigle (2002).
4		Soybean hull	1 – 10	35.8% oil reduction was reported at 10% soybean hull addition.	Lee <i>et al.</i> (2008).
5		Rice flour and soybean hull	10 – 30	30% oil reduction was recorded for rice flour and soy-bean hull doughnuts.	Lee <i>et al.</i> (2012).
6		PGA	0.25 – 1.00	Oil reduced the most at 1 % PGA	Lim <i>et al.</i> (2012).
7	<i>Magwinya</i>	Wheat bran	1 – 15	Up to 50% fat reduction at 15% bran addition	Onipe <i>et al.</i> (2018; 2019).
		Oat bran		36% fat reduction at 1 g oat bran inclusion	Kwinda <i>et al.</i>
		Psyllium husk fibre		58% reduction at 5 g inclusion	(2018).

PGA - Poly-γ-glutamic Acid

2.4. Food microstructure

Microstructural studies of food reveal the way elements in food are arranged at the microscopic level as well as their interactions concerning food properties such as physical, thermal, chemical and sensory properties (Aguilera, 2005). Food microstructure is observed using powerful microscopes at micro and nanoscale. Imaging probes have been employed in food research, and especially in understanding oil distribution in fried foods. These microscopes include light microscopes such as confocal laser scanning microscope (Bousquieres *et al.*, 2014); electron microscopes such as transmission electron, scanning electron, cryo- and environmental scanning electron microscopes (Kim *et al.*, 2012; Moreno and Bouchon 2013; Albertos *et al.*, 2016); x-ray microscope (Schoeman *et al.*, 2016), atomic force microscope and hot stage video microscope (Molina *et al.*, 2016). Food microstructure is related to nutritional, chemical, textural (Fazaeli *et al.*, 2012), physical, transport properties and product engineering (Aguilera, 2005). Study of food microstructure is essential to design processes that enhance food quality (Aguilera, 2005); reveal basic mechanisms, predict cereal product texture via mechanical modelling and thus reveal opportunities for product development (Guessasma *et al.*, 2011; Schoeman *et al.*, 2016); model food process design (Schoeman *et al.*, 2016); understand and model mass transfer in fried foods (Bouchon and Aguilera, 2001; Dueik *et al.*, 2012); and study product textural properties (Fazaeli *et al.*, 2012). Each microscopic technique to be used in this study will be reviewed subsequently in terms of their principles, data analysis, application in fried foods, and limitations. One of the aims of this research is to characterise *magwinya* crust and crumb microstructure using confocal laser scanning microscopy to understand the depth of oil penetration, quantify oil uptake and pore properties of *magwinya* using image analysis which is currently a research gap.

2.4.1. Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) is a powerful technique used for observation of specific components and their interactions in food microstructure. It works on the principle that image formation is achieved when a light source from a laser beam is focused on a bulk sample at a specific depth where the information is detected from a pinhole projection. Image information is dependent on the depth of penetration of the laser beam which is a function of its wavelength (Bouchon *et al.*, 2003). An important feature that makes CLSM unique is the production of an optical section- which contains information from one specific focal plane (Dürrenberger *et al.*, 2001).

Confocal microscopy can be used both in fluorescence and reflection modes (Lorén *et al.*, 2007); but in the study of the structural component of foods, fluorescence takes precedence as evidenced by several studies (Pedreschi and Aguilera, 2002; Moreno and Bouchon, 2013;

Bousquieres *et al.*, 2014). A molecule is said to be fluorescent when it absorbs light at a specific wavelength followed by subsequent emission at a longer wavelength in a space of a very short time (Lorén *et al.*, 2007). This type of molecule is also known as a fluorophore. Fluorescent dyes used in staining food samples are also known as fluorochromes with examples, such as, Nile red, Nile blue, Magneta dye, Calcofluor white, Acridine orange, Congo red, to mention a few. Fluorochromes for specific food component are excited at specific wavelengths different to each other; allowing viewing more than one food component in the same food sample. Despite its less-frequent use, reflection mode can be used to observe and quantify the surface topography of foods. For instance, using CLSM in reflective mode, Pedreschi and Aguilera (2002) collected topographic images of potato cells before and after frying. Their results revealed that most of the potato cells were broken during the cutting operation, which in turn affected oil migration into the product after frying.

A confocal laser scanning microscope is made up of a stage, detector, pinhole, laser, objective, beam splitter and a stage which holds the stained specimen. Light is emitted at specific wavelengths through the laser and the wavelength delivered is dependent on the food component being analysed, such as protein or fat. Lasers are regarded as the perfect light source for a CLSM due to some of its features such as plane-polarized emission, monochromaticity, high brightness, to mention a few (Lorén *et al.*, 2007). The beam splitter contains some filters with wavelength-distinguishing mirrors which can differentiate one wavelength from the other and then decides which light to pass on. Additionally, the beam splitter directs light in two ways: the incoming laser light is directed to the food sample and light re-emitted from the food sample is directed through the pinhole to the detector. The pinhole allows the light re-emitted from the sample at the same time to prevent out-of-focus light reaching the detector. The objective helps determine the informational content of the image by ensuring a constructive interference at the focal point as the objective focuses the laser beam it collects (Lorén *et al.*, 2007).

2.4.2 Application of CLSM in the study of food microstructure

Simultaneous determination of the spatial distribution of the components contained in a sample is possible through CLSM by staining the food sample with fluorochromes that have well-separated emission wavelengths. The microstructure of various foods have been studied using CLSM; some of which include characterisation of dough microstructure (Jekle and Becker, 2011); quantification of void spaces in fat layers of Danish pastry (Bousquieres *et al.*, 2014); characterisation of fat structures in meat gels (Liu and Lanier, 2015); quantification of bubble size and rheological properties of wheat dough as affected by water addition (Upadhyay *et al.*, 2012); structural properties of maize bread (Falade *et al.*, 2014);

characterisations of protein properties and starch network in the wheat dough as well as the cellular structure of yam parenchyma (Dürrenberger *et al.*, 2001); microstructural changes in noodle dough (Chewangkul *et al.*, 2001); fat distribution in fried potato and gluten-based matrix (Moreno and Bouchon, 2013), fat distribution in fried chicken (Adedeji *et al.*, 2011) and observation of structural properties of ice cream model mix containing carboxymethyl cellulose (Cheng *et al.*, 2015). Observation of microstructure is not limited to non-fried foods alone as is corroborated by the number of studies cited here. However, application of CLSM in the study of fried food microstructure has been around for some years, but increasingly in the last decade (Aguilera, 2013) with more focus on potato products than other food products; these studies include observation of surface morphology in potato chips during frying (Pedreschi and Aguilera, 2002).

Pedreschi and Aguilera (2002) observed oil location and cell wall of potato chips as affected by the frying process. It was observed that the distinct orthogonal structure of potato cell wall showed some abrasions due to the mechanical effect of cutting before frying, which left the surface of the potato with openings that contributed to the entry of oil during and post-frying. Oil absorption is predominantly a surface phenomenon. Bouchon *et al.* (2003) demonstrated this phenomenon by observing a fried-potato slice under a CLSM, and it was noticed that oil absorbed post frying was in the crust microstructure. To reduce artefacts during observation, Moreno and Bouchon (2013) developed a non-invasive double staining where the oil and dough were both stained with Nile red and fluorescein-5-isothiocyanate (FITC), respectively before frying. A relationship was established between the microstructure of the products and oil absorption. From the images, porosity, oil absorption and pore size were calculated, and a direct proportional relationship was established between porosity and oil absorption in a gluten-based product; the higher the porosity, the higher the oil absorption. Most of the oil absorbed post frying were distributed in the crevices of the crust structure. From the review above, it can be established that a study of the microstructure of fried products is key to understanding and modelling oil uptake and distribution in the products. This study, therefore, will examine the relationship between oil absorption and microstructure of *magwinya*, as there is a dearth of information on the microstructure of cereal-fried snacks concerning oil uptake.

Adedeji *et al.* (2011) studied the fat distribution, porosity and pore size distribution in fried chicken nuggets using CLSM technology. The samples were stained with Nile A and images were collected in the reflective and fluorescent mode for determination of surface topography and fat distribution in the chicken nuggets. There was a good correlation between the results from CLSM image analysis and conventional Soxhlet fat extraction.

2.4.3 Advantages and limitations of CLSM

Unlike other microscopic techniques, CLSM has the following advantages: (i) visualisation of images from the in-focus region only while the out-of-focus region is blacked out (Pedreschi and Aguilera, 2002); (ii) sample preparation requires no special treatment such as fixation, gold plating as required in scanning electron microscopy, de-fatting, freeze-drying or milling, as samples can be stained in their current state and prepared under ambient conditions (Liu and Lanier, 2015); (iii) samples can be observed at any thickness as CLSM application is not limited to thin samples (Dürrenberger *et al.*, 2001); (iv) more than one component of food sample can be viewed simultaneously through double staining as each component will emit light at separate spectra (Lorén *et al.*, 2007). For instance, Pedreschi and Aguilera (2002) viewed structural changes in potato cells during frying as well as oil distribution/absorption in the potato samples; (v) and finally, optical sectioning reduces the tendency of fat to migrate during sample preparation (Aguilera and Stanley, 1999) and also allows collection of images in 2D, which can be stacked to produce 3D images using a computer software. Staining of samples, however, has to be done for samples to be visible and staining of fried food has been reported to hamper image results through the production of artefacts, such as smearing of oil in the sample and swelling of sample core by the solvent used. To minimise artefacts during imaging, Moreno and Bouchon (2013) developed a non-invasive double staining protocol before frying by staining dough with fluorescein-5-isothiocyanate and the frying oil with Nile red. This double fluorescent staining protocol allowed safe observation of oil absorption in potato with minimal error. In our previous study (Onipe *et al.*, 2016), staining and acquisition of *magwinya* CLSM was carried out post-frying which presented numerous artefacts because of the immiscibility of water and stained oil. In this study, however, the double staining protocol will be employed for quantification of *magwinya* microstructure and porosity.

2.4.4 Image quantification

Conversion of microscopic images of food samples to quantifiable data is essential to obtain a better understanding of the various factors and interactions that contribute to the microstructural properties of food. This data quantification is attainable using image analysis software, such as, ImageJ for segmentation, measurement of geometrical features, such as, porosity (Schoeman *et al.*, 2016) and SRFRA[®] which is used to analyse surface topography through measurement of fractal dimensions (Dueik *et al.*, 2012). Other commercial software include VGStudio, MAVI-Fraunhofer ITWM, Avizo-VSG and Max (Schoeman *et al.*, 2016). Image data quantification has been achieved through statistical analysis of the grey values of an image (Aguilera, 2005). Similar densities in food samples is quantifiable by similar image grey values (Schoeman *et al.*, 2016). Jekle and Beckler (2011) used ImageJ to generate

quantifiable data from dough images acquired by CLSM. Some of the protein properties of the dough microstructure analysed included circularity, total area, perimeter, and fractal dimension. Data generated were correlated to water absorption, and a high linear correlation was found between the microstructure of dough and its rheological properties. Besides, Moreno and Bouchon (2013) utilised ImageJ for analysis (deconvolution, segmentation and quantification).

2.4.5 Pore characterisation of fried foods

Porous structure of fried foods is formed from capillary pathways created during moisture evaporation during frying. The pores may either be empty or filled with oil. Pore development is greatly influenced by crust formation - a process causes a rise in pressure at the core of the food which enlarges the pores in the food which in turn results in the expansion (Mellema, 2003; Ngadi *et al.*, 2009; Sandhu *et al.*, 2013). In the case of leavened dough products with pre-existing pores as a result of CO₂ production from leavening agents, upon frying, the starch gelatinises thus strengthening the dough structure which then causes expansion of existing pores and formation of new pores in the product (Llorca *et al.*, 2007). According to Dullien (1992), three types of pore structures have been identified in fried foods: (a) interconnected pores which are accessible from various points and greatly influence the flow of oil due to the continuous paths formed by the interconnection of the pores (b) blind pores which are accessible from just one direction and have limited influence on oil flow; and (c) non-interconnected pores which are inaccessible and do not influence the flow of oil through the food matrix. Factors that affect pore development in fried foods have been identified as including initial moisture content of food, frying temperature and pre-processing conditions, such as, coating (Ngadi *et al.*, 2009).

The microstructure of *magwinya* should be studied to (a) understand the effect of food composition on oil penetration in *magwinya*, and (b) quantify the structural properties of *magwinya* crust and crumb.

2.5. Starch composition and structure

Starch is a major polysaccharide stockpile in plants and the largest source of dietary carbohydrates in human food. Starch are packed into single or compound granules, which varies from one plant species to another (Tester *et al.*, 2004a; Dona *et al.*, 2010) in size (<1 - 100 µm), size distribution (uni- or bi-modal), shape (oval, spherical, polyhedral, lenticular or irregular), and composition (lipid, moisture, protein and mineral). In cereals, starches are known as amyloplasts (Jane, 1999; Van Der Merwe, 1999). Two types of anhydroglucose polymers make up a starch granule: amylose and amylopectin (Figure 5) which are both linked

by α (1 \rightarrow 4) glycosidic bonds at a linear level and α (1 \rightarrow 6) bonds at branching point (Dona *et al.*, 2010). Amylopectin makes up for 70 – 80 % and amylose about 20 – 30% of total starch content (Jane, 2009).

Amylose is a long, linear molecule with an approximate molecular weight ranging between 100000 – 1000000 Da and the molecules (Figure 5) are linked by α - (1 \rightarrow 4) glycosidic bonds (Singh *et al.*, 2010). Amylose is synthesized by granular-bound starch synthase. In the starch granule, amylose is radially arranged in the amorphous layer while forming a single or double helical configuration with lipophilic hydrogen atoms positioned on the inside of the helices and hydroxyl group on the outside of the coil (Van Der Merwe, 1999; Tester *et al.*, 2004b; Tang *et al.*, 2006). Although amylose does not contribute to the crystalline structure of starch granules, it is intertwined with amylopectin because amylose synthesis lays a foundation for the initial stages of crystallisation (Ziegler *et al.*, 2005). Amylopectin is a much larger and highly branched molecule linked by α -D-(1 \rightarrow 4) bonds linearly and α -D-(1 \rightarrow 6) at the branched points. Its molecular weight has been reported to be about 500000 KDa. The crystalline structure of starch granules is attributed to the amylopectin component, due to its branched structures which are composed of three chains- 'A', 'B' and 'C' chains. There is one 'C' chain within the amylopectin molecule and it carries the reducing end of the molecule while linked to 'B' chains which are then linked to 'A' chains (which are unbranched) through α - (1 \rightarrow 6) glycosidic bonds (Whistler and BeMiller, 1997; Jane, 2009). The crystalline structure of amylopectin is formed from double helices which arise from clustered branches in the molecule.

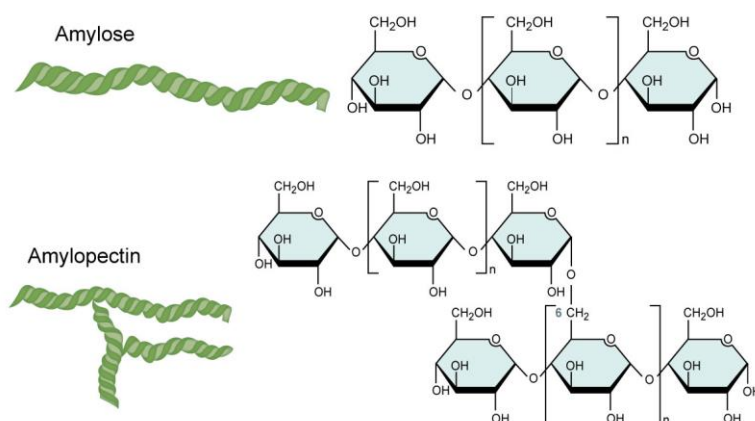


Figure 5. Amylose and amylopectin structure. Source:

<https://sites.google.com/a/aisr.org/mun-ib/biology/molecular-biology/topic-2-3-carbohydrates-and-lipids>

Three crystalline forms of the native starch structure have been identified by X-ray diffractometry viz: type A, B and C (Tang *et al.*, 2006) and this crystalline structure points to

intact amylopectin structure because amylose is easily leached out when the starch is hydrated (Van Der Merwe, 1999). Type 'A' crystallinity is predominant in cereals while type 'B' in tubers. Although starch granule organisation is not solely dependent on its crystalline structure; crystallinity, however, plays a major role in starch architecture and functional properties, such as its hydration and susceptibility to enzymes (Tester *et al.*, 2004a; Tang *et al.*, 2006). Wheat starch is said to contain about 75% amylopectin and 25% amylose. Physicochemical and functional properties of starch are influenced by details such as size, morphology and amylose-amylopectin ratio (Ellis *et al.*, 1998).

2.5.1. Starch gelatinisation

In its native form, starch granules do not dissolve in cold water, and to make homogenous starch-water mixtures, when heat is applied a non-equilibrium swelling in the crystalline regions of the starch granules occurs. This change in the molecular structure/order of starch granule when heated in the presence of water is termed gelatinisation (Whistler & BeMiller, 1997; Donald, 2004; Dona *et al.*, 2010). The gelatinisation process occurs in stages. Upon contact with water, the starch granule swells as it absorbs water, causing the crystalline regions to become soft or rubbery, thereby, increasing the viscosity of the starch suspension. As the temperature increases to around 60°C, crystallinity is lost due to the disruption of the hydrogen bonds in the crystalline region and swelling becomes irreversible, thereby, breaking the starch granule into smaller aggregates (Donald, 2004). Loss of crystallinity and molecular order during gelatinisation has been studied using differential scanning calorimetry and nuclear magnetic resonance (Tester *et al.*, 2004a). Amylose is leached out at temperatures below or higher than the gelatinisation temperature because amylose is found in the non-crystalline section of the starch granule (Dona *et al.*, 2010). Further heating causes an increase in solubility and viscosity of starch and total disruption of the granules to form a thick mass called 'paste'. This process of total dissolution of starch, following gelatinisation, is known as 'pasting'. Both gelatinisation and the pasting properties of starch affect the textural and functional properties of food (Mason, 2009).

2.5.2. Starch retrogradation

As cooling progresses, gelatinized starch molecules undergo inter- and intra-molecular reorganisation. The hydrogen bonds in linear amylose molecule are realigned and an insoluble precipitate is formed. The reorganisation of starch molecules is known as 'retrogradation' (Van Der Merwe, 1999). Amylose retrogradation occurs faster (within minutes or hours) than amylopectin which could take hours or days, thus, the first stage of retrogradation depends on the amylose content of starch molecule. Furthermore, the presence of a higher molecular weight amylose in a starch granule causes retrogradation to progress faster than low

molecular weight amylose and this determines the temperature at which retrogradation advances (Lii and Tsai, 2000). Retrogradation does not reverse gelatinisation but has been shown to improve the glycemic index of food as will be explained in the subsequent sections.

2.6 Starch digestibility

Carbohydrate digestion starts in the mouth. Food is broken down into small granules through the process of chewing, also known as 'mastication'. Food is mixed with saliva which contains enzyme α -amylase secreted from the salivary glands. The enzyme acts on the food by breaking down amylose and amylopectin into dextrin and maltose which are smaller chains of glucose. The digestion continues in the small intestine by the action of pancreatic α -amylase. Glucose is released into the bloodstream by the liver (Van Der Merwe, 1999). According to Englyst *et al.* (1999), digestibility of starch can be classified into three categories: (1) rapidly digestible starch (RDS) – the amount of glucose released after 20 min; slowly digestible starch (SDS) – the amount of glucose released between 20 – 120 min; and resistant starch (RS) – which is the uncooked starch portion that is resistant to hydrolysis by digestive enzymes (van der Merwe, 1999; Singh *et al.*, 2010; Anyasi *et al.*, 2013). The ability to predict digestion and absorption of glucose upon ingestion of starch-based foods is of great importance in the context of health concerns all over the world, with special attention on diabetes and obesity (Singh *et al.*, 2010). Food classification based on the measurement of postprandial blood glucose has been possible through the concept of the glycemic index (GI). GI is defined as the postprandial incremental area under the blood glucose response curve elicited by 50 g available carbohydrate portion of food, expressed as a percentage of the response to an equal amount of a reference food (such as glucose or white bread) taken by the same subject (Jenkins *et al.*, 1987; Wolever *et al.*, 2006; Singh *et al.*, 2010). Starchy foods such as puffed/extruded breakfast cereal release their glucose slowly and have their $GI \leq 50$ while foods such as pasta and legumes release their glucose rapidly with $GI \geq 70$. An international table of glycemic load values of 51 foods was first released by Jenkins *et al.* (1981) and a follow-up publication by Foster-Powell *et al.* (2002) but only a GI value of a total of 750 foods was published. The GI values of 76 and 108 were recorded for cake doughnut using reference samples of glucose and white bread, respectively.

2.6.1. Factors influencing starch digestibility

Factors that affect starch digestibility can be categorised into two: intrinsic and extrinsic factors. Intrinsic factors have to do with the food product/composition, such as starch characteristics, enzyme inhibitors, amylose/amylopectin ratio, particle size of starch granule and fibre content. Extrinsic factors are also known as processing factors, like the cooking

method, starch gelatinisation, retrogradation, and presence of other ingredients such as lipids, proteins and minerals.

The biological origins of starch determine their shape and size. As earlier discussed, starch has been classified based on their X-ray diffraction patterns. Starch with type B crystallinity, such as tubers are less susceptible to enzyme hydrolysis than starch with type A crystallinity, such as cereals. This implies that cereals are more easily digested than tubers. Also, foods with the same botanical origin can exhibit different digestion rates. For instance, sweet potatoes have lower GI value (<50) than potatoes (GI >70), despite that both are tubers (Brand-Miller and Foster-Powell, 2002; Tester *et al.*, 2004a; Vosloo, 2005). The size of starch granules also directly influences their rate of digestion and size reduction is directly proportional to starch digestibility. Larger starch granules have a lower digestibility rate compared to small granules. The rationale behind this is the increase in surface area in smaller granules makes the starch surface more accessible to digestive enzymes. For instance, amaranth starch was reported to have a higher hydrolysis rate when digested *in vitro*, and this was linked to its small granule size of $\leq 3 \mu\text{m}$. The nature of the starch granule surface also affects enzymatic digestion. In comparison to tuber and legume starch, the surface of cereal starch has been reported to have pores through which enzymes can penetrate and digest the starch from inside out (Dona *et al.*, 2010). High-amylose starch tends to digest more slowly than low-amylose starch and this may be due to the relatively high proportions of very long chains in the structure (Yoshimoto *et al.*, 2000; Tester *et al.*, 2004). High-fibre foods tend to have lower glycemic response due to delayed effect of fibre on digestibility because digestive enzymes do not affect fibre, delayed hydrolysis of polysaccharides, reduction of glucose absorption in the small intestine and water uptake by fibre in the food, making less water available for starch gelatinisation (Noort *et al.*, 2010; Sozer *et al.*, 2014). The presence of fibre in food can slow down enzymatic attack by increasing viscosity. The addition of 15 g psyllium fibre to white bread reduced its GI value from 100 to 48 (Frati *et al.*, 1998). Finally, the presence of phytochemicals such as tannins, phytic acids, and lectins inhibit starch digestibility (Frati *et al.*, 1998).

Various food processing techniques are used in the food industry and these techniques influence the textural, sensorial, and nutritional properties of food, including starch digestibility. Some of these processing factors include the degree of starch gelatinisation and the formation of retrograded starch. Gelatinised starch is easily hydrolysed because of the loss of crystallinity which makes the granules porous and accessible to digestive enzymes. Starch granules not completely gelatinised have delayed the rate of hydrolysis. The amount of water available to the starch molecules determines the extent to which the granules swell. If the

water available is less, starch digestibility through enzyme hydrolysis will reduce. Biscuits for example generally have low GI (< 50) due to the low water content available to starch granules, thereby leading to incomplete gelatinisation and in turn, reduced GI. Percentage digestible starch content of biscuit enriched with wheat bran, significantly, reduced with an increase in wheat bran addition (Sozer *et al.*, 2014). As amylose molecules structures are re-ordered, during retrogradation, it forms a thermos-resistant gel which is resistant to digestive enzymes, and this, in turn, lowers the GI value (Chung *et al.*, 2006; Dona *et al.*, 2010).

The presence of other ingredients such as fat and protein also influences starch digestibility. Some lipids present in native starch are known to form complexes with amylose molecules thereby resulting in behavioural changes in starch molecules such as increased gelatinisation, delayed retrogradation, decreased solubility, and resistance to digestive enzymes (Tester *et al.*, 2004b; Singh *et al.*, 2010). In cereals such as wheat, the presence of protein-starch interaction also has a major effect on starch digestibility. When wheat flour is hydrated, a protein matrix is formed around the starch granules, thus limiting starch digestibility. The protein encapsulates the starch granules and limits the action of digestive enzymes on starch hydrolysis. The starch-protein interaction, enhanced by compact structure of for example pasta, as well as the encapsulation of starch granules by protein and lipids, reduce the accessibility of starch to enzyme hydrolysis as has been reported (Jenkins *et al.*, 1987; Kim *et al.*, 2008).

2.6.2. *In vitro* starch digestibility

Various factors are involved in conducting *in vivo* studies to measure GI of foods such as (a) food portion size, (b) reference standard used (Foster-Powell *et al.*, 2002), (c) method, frequency and time length of blood sampling, (d) fasting glucose level of test subjects (Vosloo, 2005), and (e) method of GI calculation (Van Der Merwe, 1999). Also, *in vivo* studies are laborious and expensive. As a result, *in vitro* digestibility studies have been the alternative with a link established between *in vitro* digestibility rate and GI of food (Singh *et al.*, 2010). To determine starch digestibility *in vitro*, the procedure should mimic *in vivo* conditions as much as possible. A plethora of studies on *in vitro* starch digestibility have been published; Dona *et al.* (2010) did an extensive review of starch-digestion studies spanning 3 decades. Method of determining *in vitro* starch digestibility varies in sample preparation, type of enzymes used, incubation conditions (temperature, time and restriction), and technique for starch quantification.

The first step of *in vitro* starch digestion is the mechanical disruption of the food sample. Chewing was a method developed by Granfeldt *et al.* (1992), which allows salivary α -amylase

to mix with the food before further analysis. Other sample preparation methods are highlighted in Table 2. Proteolytic enzymes and amylases are used in the digestion process. Amyloglucosidase is an enzyme often used, although it is not of mammalian origin. Incubation conditions encompassed the temperature ranging from 37°C (normal body temperature) to 40°C depending on the enzyme used. Incubation time ranges from, but not limited to 0 to 240 min which is the approximate time taken for food to pass through the small intestine. A form of motion is needed to mimic the peristaltic movement of the gut, such as agitation, and a shaking or stirring water bath, although this might not be needed as seen in some studies (de la Hera *et al.*, 2014; Jang *et al.*, 2015). The last step in the process is to measure products of starch digestion such as glucose, maltose, maltotriose and maltotetraose (Dona *et al.*, 2010). Some of the methods used are, hexokinase, phenol and sulfuric acid, and 3, 5-dinitrosalicylic acid colourimetric methods (Van Der Merwe, 1999; Soong *et al.*, 2014). Conditions involved during *in vitro* starch digestion of various foods are highlighted below in Table 3.

Table 3. *In vitro* starch digestion process of some foods

Food	Sample preparation	Enzymes used	Conditions (temp, time and agitation used)	Measurement of digestion product	Reference
Flatbread	None	Porcine α -amylase, pepsin, pancreatin, amyloglucosidase	37°C 0, 20, 45, 60, & 120 min Reticulating water bath	Enzymatic-colourimetric method	Yousif <i>et al.</i> (2012)
Oat starch	None	Porcine pancreatin, amyloglucosidase, pepsin	37°C, 0, 10, 20, 30, 60, 90, 120 & 180 min Shaking water bath	Glucose oxidase-peroxidase assay kit	Kim and White (2013)
Gluten-free bread	Grinding	Nil	30, 90 & 120 min	None	de la Hera <i>et al.</i> (2014)
Muffin	Crumbling	Pepsin, α -amylase, pancreatin, invertase, amyloglucosidase	37°C 20, 60, 90 & 120 min Stirred water bath	Dinitrosalicylic acid colorimetric method	Soong <i>et al.</i> (2014)
Bread	Grinding	Pepsin, α -amylase	37°C 15, 30, 60, 120 & 180 min Shaking water bath to stir dialysis bag	High-performance liquid chromatography	Srichamroen (2014)
Millet	Starch isolation	Pancreatin, invertase, amyloglucosidase	37°C 20 & 120 min	Total starch kit (Megazyme)	Annor <i>et al.</i> (2015)
Puffed wheat kernel	Mincing	Salivary α -amylase, porcine pepsin, trypsin, lipase, and pancreatic amylase	37°C Peristaltic movement by gastro-small intestinal model	High-performance liquid chromatography	Cattaneo <i>et al.</i> (2015)
Bread	Homogenisation	Pepsin, α -amylase, amyloglucosidase	40°C 30, 60, 90, 120, 150 & 180 min Shaking water bath	Spectrophotometry	Ho <i>et al.</i> (2015)
Noodles	Milling	Pancreatin, amyloglucosidase	37°C, 30, 60, 90, 120 & 180 min	Glucose assay kit	Jang <i>et al.</i> (2015)
Foxtail millet	Grinding	Pancreatin, invertase, amyloglucosidase	37°C 20 & 120 min	Glucose oxidase-peroxidase assay kit	Ren <i>et al.</i> (2016)
Short-dough biscuit	None	Pepsin, lipase, trypsin, pancreatin	37°C, 20, 60, 120, 180 & 240 min Peristaltic movement by gastro-small intestinal model	Enzymatic kit	Villemejeane <i>et al.</i> (2016)

2.6.3. Effect of frying on starch digestibility food

As earlier established, the degree of gelatinisation is directly proportional to starch digestibility. Deep frying has been reported to reduce resistant starch content of potato products due to the abundance of fat available for lipid-starch complex formation. Yadav (2011) showed that deep-frying had a more pronounced effect (41.6%) on the reduction of resistant starch content of potatoes than shallow frying (37.6%). On the contrary, Mahmood *et al.* (2006) reported that shallow frying had a more pronounced effect on resistant starch of fried potatoes than shallow frying at rates of 77.1% and 65.4% decrease, respectively. Frying is a dehydration process; water loss in fried foods could directly affect the retrogradation process which will reduce/inhibit re-crystallisation of amylopectin molecules after frying and in turn reduce resistant starch content (Goñi *et al.*, 1997; Mangala *et al.*, 1999). Recently, Contardo *et al.* (2016) studied the effect of vacuum and atmospheric frying on gelatinisation and *in vitro* starch digestibility of laminated dough with some interesting results. As opposed to just measuring the amount of digested starch (Sozer *et al.*, 2014), amounts of rapidly available glucose (RAG), slowly available glucose (SAG) and unavailable glucose (UG) were measured. Degree of starch gelatinisation, amount of RAG and UG in vacuum-fried dough was significantly lower than dough fried under atmospheric pressure. UG values of vacuum-fried dough was similar to UG values of raw dough. The UG values of dough fried at atmospheric pressure although lower than vacuum fried dough, the amount of resistant starch in the fried dough was 48 – 50 g/100 g. Despite these interesting results, there are some limiting factors when the methodology is scaled up to doughnut or *magwinya*. Firstly, the dough is reconstructed, not having the same gluten and starch content as normal wheat flour. Secondly, sample shape and size which could affect frying time and temperature was not highlighted. Thirdly, laminated dough was used which might not be the case in other doughnut-like products. One of the focus of this research is to fill the highlighted research gaps by studying the effect of bran addition on starch digestibility of *magwinya*.

2.7 Hyperspectral imaging technology

Vibrational spectroscopy is a valuable non-invasive imaging technology for quality observation of food products through data acquisition based on the interaction between incident light and molecules in the food sample (Sun, 2010; Cheng and Sun, 2014; Mahajan and Kamalapur, 2019). However, NIR hyperspectral technology combines spectroscopy and digital imaging to produce a three-dimensional image data called a hypercube containing 2D spatial and 1D spectral information. The pixel of a multispectral image has a discrete spectrum of less than 10 bands, while that of a hyperspectral image has a continuous spectrum containing hundreds of bands (Mahajan and Kamalapur, 2019). Apart from spatial and spectral information gotten from a hypercube, when compared to other forms of spectral imaging such as computer vision,

multispectral imaging, and near infra-red spectroscopy; HSI holds the advantage in respect of building chemical images, offering multi constituent information, and flexible extraction of spectral information (Feng & Sun, 2012).

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2.7.1. Fundamentals of NIR spectroscopy

The electromagnetic spectrum consists of waves such as UV, gamma and X rays, micro, radio and infra-red (IR) waves. The wavelength of IR radiation may be classified as far IR (25000 - 1 000 000 nm), mid-IR (2500 - 25000 nm) and near (NIR) which ranges from 780 to 2500 nm on the spectrum. NIR is also known as short wave infra-red (SWIR). As radiation interacts with any material, there is absorption of energy resulting in molecular responses. In the IR region, such responses are termed molecular vibrations (Mahajan and Kamalapur, 2018).

Vibrations displayed by molecules differ and can either be deformation (twisting, wagging, scissoring, rocking) and/or stretching (symmetric and asymmetric). Due to continuous vibrations within molecules with covalent bonds (C=C, C-H, O-H, N-H, S-H and C-O), the transition from one vibration level to another occurs and this response is termed overtone. The higher the overtone, the less the intensity of the vibration. Transition within molecules appears as wavebands which can be measured using NIR spectroscopy. Wavebands with higher wavelengths have less overtone and the higher the transition levels, the smaller and weaker the wavelength (Nawrocka and Lamorska, 2013; Fei *et al.*, 2017).

When a material is exposed to NIR light source, the light could either be reflected, transmitted or absorbed by the sample. Absorption of light (energy) by a sample is dependent on factors like physical properties, of the sample such as density, size, and composition. On the one

hand, light absorbed by the sample gives information about the chemical composition of the sample. On the other hand, light reflected by the sample gives information about the compounds on the surface of the sample. The quality or chemical composition of a sample can be explored from the spectra of light absorbed by the sample (Millar, 2008; Mahajan and Kamalapur 2019).

2.7.2. NIR data analysis

Data analysis from the spectral information of samples can be performed based on the purpose of the analysis. Spectral data analysis is categorised as follows:

- (a) Exploratory analysis – used for differentiating or exploring relationships between samples based on physical and chemical property. Principal component analysis (PCA) is an unsupervised method used for exploratory analysis. In the case where the end goal of data analysis is calibration and model construction; PCA is used in the early stages to identify variation and similarities in the spectral data of the samples. New variables can be constructed through data compression which helps observe the association between samples in multivariate space through principal components (Manley, 2014).
- (b) Multivariate data analysis – prediction of a specific component of a material based on model construction which can either be quantitative for estimation of specific compounds in samples or categorical for classification of samples into physical or chemical groups.

Since adequate (hundreds) of sample is required to build models, exploratory analysis will be utilised for identification of similarities and differences in *magwinya*.

2.7.3. Food applications of hyperspectral imaging

There are exhaustive reviews and studies on HSI application to the field of Food Science. A few of these studies are reviewed in Table 4. These include but are not limited to quality evaluation of fruits and vegetables (Lu *et al.*, 2017), detection of contamination and quality examination of fish products (Menesatti *et al.*, 2010; Cheng and Sun, 2014; Cheng *et al.*, 2014a) detection of defects and contamination to ensure food safety (Feng and Sun, 2012), texture-based classification of maize kernels (McGoverin and Manley, 2012; Williams and Kucheryavskiy, 2016), physicochemical analysis of fish products (Cheng *et al.*, 2014b), safety evaluation of cereals (Sendin *et al.*, 2018a), measurement of moisture content of bread (Liu and Møller, 2011), and maize kernels (Zhang and Gao, 2020), to name a few. There are currently no documented efforts on HSI in quality evaluation of *magwinya*. Therefore, one of the objectives of this research is to investigate the potential of using short wave near-infrared

hyperspectral imaging for non-destructive exploratory analysis of chemical components of *magwinya* enriched with varying amounts of bran and moisture.

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2.8 Food surveys

Survey is a useful tool in gathering important information on a subject area. Food consumption and production survey, however, can be used to obtain information on (a) demographic characteristics of the sample population, (b) quantity of food consumed by individuals (c) perception and attitudes to food consumption, (d) nutritional composition of the diet; (e) anthropometric data as they relate to food consumption, and (f) processing ingredients, methods and tools (Food, 2004). The traditional survey approach collects information from the research participants, such as face-to-face interviews, telephone and printed questionnaires (Phellas *et al.*, 2011).

A survey may either be administered to a sample of individuals (or to the entire population) at a single point in time (cross-sectional survey), or the same survey may be administered to different samples from the population at different time points (repeat cross-sectional). Surveys have been carried out on food processing (Jideani *et al.*, 2001) and food purchasing/consumption practices about nutrition and health (Food, 2004; Labadarios *et al.*, 2005; Steyn *et al.*, 2005; Temple *et al.*, 2006; Steyn *et al.*, 2011; Boylan *et al.*, 2017). Data obtained from food consumption surveys can be an effective tool in development and management of policies, formulation of strategies to ensure healthy food practices, food security boost, as well as nutrition surveillance (Food, 2004; Sedibe *et al.*, 2014).

Table 4. Food applications of hyperspectral imaging technology

Food product	Properties measured	Major findings	Reference
Maize kernels	Moisture content	Development of model using (PLSR) model with NIR spectra for a single maize variety was better than multiple varieties.	Zhang and Guo, 2020
	Texture-based classification	Pixel-wise differentiation between soft and hard maize using PCA and PLS-DA models	Manley <i>et al.</i> , 2011; Williams & Kucheryavskiy, 2016
	Detection of defects	A clear distinction in good maize and undesirable materials via object-wise classification using the PLS-DA model yielding high Q ² values (≤ 0.99).	Sendin <i>et al.</i> , 2018b
Wheat kernel	Detection of <i>Fusarium</i> damage	Identification of good and damaged wheat kernels using NIR absorption spectra deoxynivalenol.	Peiris <i>et al.</i> , 2009
Bread	Water content of bread	Moisture content of NIR images comparable to conventional moisture values	Liu and Møller, 2011
Bread dough	Characteristics of dough during mixing	Physicochemical mechanisms in bread dough development	Kaddour, and Cuq, 2011
Bakery products	Moisture migration in bakery products	Mapping chemical components such as fat in doughnuts, moisture in bread using NIR spectroscopy.	Millar, 2008
Fish	Texture changes in grass carp fish as a function of frozen storage	Texture firmness prediction was established through genetic algorithms of PLSR model	Cheng <i>et al.</i> , 2014b
	Moisture distribution in Salmon	Moisture content values of Salmon was comparable to conventional values using PLSR model.	He <i>et al.</i> , 2013b; Wu and Sun, 2013
Fruits	Defect detection in apples	PLSR models for detection of defect in apples	Qin <i>et al.</i> , 2017

PLSR - partial least square regression, PCA- principal component analysis, PLS-DA - partial least square discriminant analysis, NIR- near-infrared

2.8.1 Questionnaire as a tool in survey research

A questionnaire is a research tool needed to gather meaningful data in a survey. Contained in a questionnaire is a series of questions to gather information from target respondents or population of interest. Questionnaires is a type of written interview which can be carried out face to face, via post, by telephone, or computer. To design a questionnaire for survey research, it is important, bearing in mind the aim of gathering the information, to choose what type of question will be asked whether open-ended or closed-ended questions. In an open-ended questionnaire, respondents are expected to answer the questions in their own words, hereafter the responses are coded into smaller responses which are then counted for statistical analysis. Whereas in a close-ended questionnaire, the respondents are given a list of pre-determined answers to the question being asked and are expected to choose from this list. A Likert scale is commonly used as responses for these questions. Closed-ended questions are usually preferred in survey research because of the ease of counting the frequency of each response. In this research, close-ended questions will be used for ease of data collation and analysis.

There is a dearth of information on *magwinya* production and much less on consumption. Mamabolo *et al.* (2006) reported *vetkoek* was reported as one of the thirty major foods consumed by children aged 3 years. Their survey was geared towards foods and snacks consumed by primary school learners with no interest in *magwinya* as the focus of the study. Gathering data on *magwinya* consumption and production will be important to assist future researchers to have a foundation to build upon. Hence, one of the objectives of this research is to fill this knowledge gap.

2.9 Conclusion

The frying stages coupled with the reactions taking place in the frying medium all culminate in the amount of oil absorbed by food as well as the nutrients gained or lost in the process. All these processes are influenced by frying time, temperature and number of frying cycles. Furthermore, the abundant studies in literature which focused mainly on fried potato products have established oil uptake as a surface phenomenon which takes place in the crust of the product. The mechanism of oil uptake in *magwinya*, however, has not been established, which is one of the objectives of this study. Through the measurement of the surface, penetrated and structural oil, this research proposes to unveil which of the mechanisms earlier explained best explains oil uptake in *magwinya*. Finally, from this review, the role of food microstructure in *magwinya* processing can be summarised as (a) to study the effect of food composition and frying process on oil uptake in *magwinya* (Aguilera, 2005); (b) to understand the link between oil uptake and starch digestion (Schoeman *et al.*, 2016); (c) to observe the digestion process

at sub-cellular level (Aguilera, 2005); and (d) to understand the mechanism of mass transfer in *magwinya* (Dueik *et al.*, 2012). Wheat bran showed some promising results in oil reduction of *magwinya* from previous studies (Onipe *et al.*, 2018; 2019), however, other additives could be incorporated to find the best formulation for optimum oil reduction. The question to be asked will be: How will these additives affect starch digestibility of *magwinya*? The focus of starch digestibility of fried foods has been mainly on potato products than wheat-based products. The gaps identified in the literature include:

1. *Magwinya* production and consumption survey
2. Starch digestion process of *magwinya*
3. Influence of fibre incorporation on starch digestibility of fried foods
4. Mechanism of oil uptake in *magwinya*
5. Oil distribution in *magwinya*

Filling the above-listed knowledge gaps will be beneficial to academics working on fried foods, nutritionists and individuals living with metabolic disorders such as diabetes and obesity.

CHAPTER 3: THE SOCIOECONOMIC BENEFITS, PRODUCTION AND CONSUMPTION STATISTICS OF *MAGWINYA* IN LIMPOPO PROVINCE, SOUTH AFRICA

Abstract

Magwinya, a cereal fried dough, is a popular traditional snack widely consumed across various ethnic groups in Sub-Saharan Africa, but little is known about its production and consumption in a scholarly context. It is against this background that the survey was carried out. This study examined production process, ingredient formulation, sales, characteristics and consumption of *magwinya* in Thohoyandou (South Africa), intending to develop a healthier form of *magwinya*. Out of the 30 *magwinya* production sites visited and 650 consumer questionnaires distributed, data were obtained from 29 sites and 634 consumers: a response rate of 97% and 98%, respectively were realised. Results revealed, details on the formulation, ingredients, processing methods, sales, consumption patterns and consumer preference of *magwinya*; and proposed considerations for the development of a healthier *magwinya*. Cake wheat flour (55%) was the main ingredient used. Production process was non-automated as evidenced by manual mixing and fermentation processes (93%), coupled with low usage of electronic equipment (14%). With a daily turnover between ZAR500 – ZAR3000 (\$35 - \$210), there is a need to improve *magwinya* production through an automated production line, especially for large-scale producers of this product. The daily turnover indicated that *magwinya* production is a lucrative business contributing immensely to the livelihoods of, and financially empowering the producers, who were all females (100%). Consumer data revealed *magwinya* to be a moderately liked food (46%) consumed at least twice a week (32%), as either a snack or main meal; with taste as the most favoured characteristic (79%). About 93% of consumers fell within <20 and 21-30 age groups. A greater percentage of consumers (75%) disliked the oiliness of *magwinya*, and with increasing awareness of the health implications of frequent consumption of fried foods; 87% of consumers affirmed purchase of low-fat *magwinya* if made available. Development of low-fat, nutrient-rich *magwinya* is therefore recommended to offer consumers a healthier variety.

Keywords: *Magwinya*, *vetkoek*, *magwinya* production, South African cereal fried dough, low-fat *magwinya*, fried cereal snack

3.1. Introduction

Surveys have been carried out on food processing (Jideani *et al.*, 2001) and food purchasing/consumption practices about nutrition and health (FAO, 2004; Labadarios *et al.*,

2005; Steyn *et al.*, 2005; Temple *et al.*, 2006; Steyn *et al.*, 2011; Boylan *et al.*, 2017). Data obtained from food consumption surveys can be an effective tool in the development and management of policies, formulation of strategies to ensure healthy food practices, boost food security, as well as having surveillance on nutrition (FAO, 2004; Sedibe *et al.*, 2014).

Magwinya also known as 'fat cakes' or 'vetkoek' in the Southern African region, is a deep-fried wheat dough and a popular traditional food in Africa (Benard, 2001; Bhana, 2005; Feeley *et al.*, 2009; Hyslop, 2012; Trujillo, 2014). It is usually sold by vendors on street corners (Feeley *et al.*, 2011), in high school tuck shops (Kearney *et al.*, 2011; Sedibe *et al.*, 2014) and university cafeterias. It has different nomenclature in other Sub-Saharan African countries. For instance, it is called *puff puff* in Nigeria, *bofrot* in Ghana, *beinye* in Cameroon, *mandazi* in Malawi, *mikate* in Congo, *kala* in Liberia (Onipe *et al.*, 2018). *Magwinya* can be eaten plain or with sweet/ savoury fillings such as cheese, curry, minced beef, snoek fish, jam, butter, polony, and/or *boerewors*. *Magwinya* is high in fat, sodium, calories and low in fibre (Temple *et al.*, 2006; Kearney *et al.*, 2011), and consumed by various age groups. Mamabolo *et al.* (2006), reported that *magwinya* was one of the thirty major foods consumed by children aged 3 years. Furthermore, Kearney *et al.* (2011) developed nutrient-rich *vetkoek* as a school feeding project for children between the ages of 6 – 13 years.

Production of *magwinya* begins with the mixing of the ingredients -wheat flour, yeast, sugar, salt and water; followed by fermentation of the dough, extrusion or cutting (based on the consistency of the dough) into the desired shape and finally frying until golden brown (Kwinda *et al.*, 2018). Processing of *magwinya* is mostly manual - this includes mixing and piping the dough into the oil with hands. Preparation method and recipe is usually at the discretion of the producer, thereby, leaving the method un-standardised. There are few available scholarly articles on *magwinya* processing and consumption, with the available ones focusing on oil uptake reduction through optimisation of processing conditions and product formulation using wheat bran (Onipe *et al.*, 2018), psyllium husk fibre and oat bran (Kwinda *et al.*, 2018), which improved nutritional quality of *magwinya*. However, there are no scholarly articles on *magwinya* production and consumption in South Africa. Therefore, this study was carried out to generate data and provide information on *magwinya* processing, sales, and consumption. The data would be useful in policy decisions, food consumption statistics and further research on *magwinya*.

3.2. Materials and Methods

3.2.1. Sample framework and survey area

The sampling frame was designed to be an enumeration of producers in major streets of Thohoyandou. The producers sampled were based on the ones that could be found in the representative streets in Thohoyandou. The sampling frame included as many information from the producers as possible; that is, it defaulted in favour of including records rather than excluding them. Consumers were sampled across age groups, starting from age 16 and above. Inclusive in the sampling frame of consumers was formal education was a pre-requisite for consumers as the questionnaires were self-administered. Permission was taken from the school principal before questionnaires were administered to learners. The cross-sectional survey was carried out over a period of 4 months in Thohoyandou (22.8785° S, 30.4818° E) – the town with the largest population (> 64 000) in Vhembe District, of Limpopo Province, (South Africa). Sample size for the consumers was calculated using an online sample size calculator (Appendix I).

Thohoyandou was selected for the following reasons: (a) it has the largest population in the district; (b) the institution of this study is situated there; and (c) it has a diverse population as a result of University – a centre of attraction for people of various ethnicity, language and nationalities. Using street addresses recovered from Google maps, the sampling sites were re-categorised into 8 locations and coded as follows: University road (UVR), P-West (PW), P-East (PE), Block F (BF), Venda casino road (VCR), Venda sun road (VSR) Capricorn plaza (CP), and Shayandima (SH). Thirty production sites were purposefully selected as representative of the major streets in Thohoyandou. Out of the 30 *magwinya* production sites sampled, one producer was unavailable for an interview. Thus, data were obtained from 29 sites yielding a response rate of 97%. The 29 producers interviewed were pooled from the locations as follows: 13 at SH, 7 at UVR, 3 at PW, 2 at PE and 1 each at BF, VCR, CP and VSR (Table 5). These sites included major and minor streets, supermarkets, high school tuck shops, and university cafeteria. Consumers (650) were purposefully selected around the production sites where *magwinya* is produced and sold. This was done to capture the consumers of the producers sampled.

3.2.2. Questionnaire for producers and consumers

Two pre-tested structured questionnaires were developed to target producers and consumers, respectively. The consumer questionnaires contained close-ended questions. The production questionnaires were administered in the form of interviews in the local language, a majority of the producers have little or no formal education and only speak the local language. Research assistants were trained, and they performed the interviews in the presence of the researcher.

Information sourced from *magwinya* producers were demography (gender, age, level of education, employment, and location), production process (ingredients, measurement, mixing mode, fermentation, frying time and use of electronic equipment) as well as sales of *magwinya* (Appendix II). A self-administered questionnaire was given to consumers who were regular consumers of *magwinya*, could read and write, and purchased from producers sampled or elsewhere. The questionnaire entailed questions about demographic information, consumption pattern, purchase habit and favourite characteristics of *magwinya* (Appendix III). A total of six hundred and fifty (650) consumer questionnaires were distributed. The Research Ethics Committee of the University of Venda (project number: SARDF/17/FST/03) approved this study.

3.2.3. Data analysis

Data extracted from questionnaires were analysed by descriptive statistics, cross-tabulations and presented as frequencies and percentages using SPSS version 24 (IBM Inc., Chicago, IL). Kruskal-Wallis non-parametric one-way analysis of variance was used for a test of significance of the data among the producers (Gaffa *et al.*, 2002). Pearson's Chi-square test of independence was used to measure the relationships between consumer data across age groups.

3.3. Results and Discussion

3.3.1. Demography of Producers and consumers

Magwinya producers

All producers were females (100%); this implies that *magwinya* production is a women-dominated business/ trade. Four age groups (in years) were captured and ranked in the following order: 51 – 60 > 41 – 50 > 31 – 40 > 61+ with age group 51 – 60 at the highest (37.9%) and 61+ years the least (10.3%). Over half (62.1%) of the producers were without formal education; this affected collection of specific information in terms of measurements, and flour-*magwinya* ratio output per day. In terms of production experience, 48.3% have above 15 years' experience while only 3.4% have less than 1-year experience. There were significant differences ($P < 0.05$) among the responses in each demographic parameter. Omemu and Aderoju (2008) reported up to 20-year experience amongst street food vendors in Abeokuta, Nigeria. Omemu and Aderoju (2008) reported up to 20-year experience amongst street food vendors in Abeokuta, Nigeria.

Magwinya consumers

From a total of 650 questionnaires administered to consumers, 634 (275 males and 359 females) were recovered – a response rate of 98% (Table 5). The age group of the

respondents ranged from <20 to above 60 years. The highest consumption percentage was found among the age group 21 – 30 at 49.2%. Six locations were covered including UVR (67.5%), SH (25.6%), PW (3.3%), VSR (1.4%), PE (1.3%), and BF (0.9%). Overall, 89.3% were high school and university students, 42.9% had Grade 12 qualification and 7.6% had a postgraduate qualification. This implies that *magwinya* consumption is popular amongst school learners. *Magwinya* is a common delicacy, among students because it is an affordable, ready-to-eat food which requires no preparation by the consumer. This is evidenced by long queues at University cafeterias, high schools and other retail outlets in the mornings and lunchtime. In the process of developing a nutrient-dense food product for a primary school feeding project, *vetkoek* was chosen because it was classified as a food frequently consumed in the community (Kearney *et al.*, 2011). There were significant differences among the responses in each demographic parameter.

Table 5. Demographic information of *magwinya* producers and consumers

Information	Producers (n = 29)	Consumers (n = 634)	Significance
Location			
University road	7	428	<0.001
P-West	3	21	
P-East	2	8	
Block F	1	6	
Venda casino road	1	-	
Capricorn Plaza	1	-	
Venda sun road	1	9	
Shayandima	13	162	
Age			<0.001
17 - 20	-	278	<0.001
21 - 30	-	312	
31 - 40	5	31	
41 - 50	10	10	
51 - 60	11	1	
61+	3	2	
Gender			
Male	-	275	<0.001
Female	29	359	
Education			<0.001
No formal schooling	18	-	<0.001
Grade 10 - 12		242	
Grade 12	7	272	
Diploma	3	12	
Degree	1	60	
Postgraduate		48	
Experience (yr)			
<1	1	-	<0.001
1 - 5	6	-	
6 - 10	5	-	
11 - 15	3	-	
15+	14	-	
Occupation			<0.001
Student	-	566	<0.001
Worker	-	64	
Unemployed	-	4	

*Kruskal-Wallis one-way ANOVA (P < 0.05)

3.3.2. *Magwinya* production process

In this study, 79.3% (Table 6) learned *magwinya* production at home and the distribution of this response significantly (P < 0.05) varied from 4.3% at VCR and PE to 30.4% at UVR and 47.8% at SH. The results showed that cake flour is the most significantly used type of flour (55.2%), followed by bread flour (34.5%) while only a handful of producers use a mixture of both (10.3%). It is evident from this survey that the use of non-wheat flour is non-existent.

About 96.6% of producers cup measurement (69%), weighing scale (10.3%) and 20.7% instinctively to measure their ingredients which can be attributed to several years of experience as confirmed by a high percentage (66.4%) of producers with over 15 years production experience. This makes the process a motor memory, and as such, they do not need to measure using any formal measurement tool. *Magwinya* is produced from wheat flour, sugar, yeast, salt and water in varying proportions among producers. Other ingredients like margarine, coffee creamer, eggs, and baking powder are added by a few producers to distinguish the taste of their product from others. Some producers use a combination of two or three of the listed ingredients. A significantly greater proportion of producers (93.1%) mix their *magwinya* dough manually (by hand); while the use of electric mixer falls on the lower side (6.9%). This reveals that manual mixing is predominant in *magwinya* production, even at big stores that use up to 25 kg of flour daily. Dough fermentation time differs from one producer to another, and it significantly ($p < 0.001$) ranged from ≤ 30 min to overnight fermentation (48.3%).

Out of producers that ferment overnight, 71.4% have >15 yr production experience and fall within ages 31 – 60. This implies that overnight fermentation seems to be a long-standing method. Fermentation is largely carried out on countertop (93.1%), while 6.9% make use of electric proofer. When asked about the duration of the frying process, some producers had no idea or had never timed the process.

Table 6. *Magwinya* production process

Information	Frequency	Percentage	Significance*
Where <i>magwinya</i> production was learnt			
Catering school	1	3.4	<0.001
Home	23	79.3	
Place of work	3	10.3	
Self-taught	2	6.9	
Flour type used			
White bread flour	10	34.5	
Cake flour	16	55.2	
Bread + cake flour	3	10.3	
Ingredient measurement			
Yes	28	96.6	< 0.001
No	1	3.4	
Measurement type			
Cup	20	69.0	<0.001
Weighing scale	3	10.3	
Instinct	6	20.7	
Use of other ingredients			
Margarine	1	16.7	0.20
Eggs	1	16.7	
Premix	1	16.7	
Marg/egg/premix/Creamer	1	16.7	
Marg/egg/premix	1	16.7	
Baking powder	1	16.7	
Type of mixing			
Manual	27	93.1	<0.001
Electronic	2	6.9	
Fermentation time (min)			
≤30	7	24.1	<0.001
45	3	10.3	
60	3	10.3	
120 – 180	2	6.9	
Overnight	14	48.3	
Frying time (min)			
<10	3	10.3	<0.001
10	8	27.6	
15	3	10.3	
20+	2	6.9	
Until brown	13	44.9	
Proofing mode			
Countertop	27	93.1	<0.001
Proofer	2	6.9	
<i>Magwinya</i> shape			
Round	27	93.1	<0.00
Flat	2	6.9	
Use of electronic equipment			
Yes	4	13.8	<0.00
No	25	86.2	
Equipment type			
Deep fryer	3	75.0	0.10
Cutter/fryer/proofer	1	25.0	

*Kruskal-Wallis one-way ANOVA (P < 0.05)

This is evidenced by the number of producers (44.9%) who fry the dough 'until brown' as they could not give an approximate frying time. The others ranged from < 10 min to 'over 20 min'. The frying time is also dependent on the *magwinya* size. It takes about <10 min, 10 min and over 15 min for small, medium and large sizes of *magwinya* to be cooked, respectively. The shape of *magwinya* were predominantly round at 93.1% (Figure 6), while only a few produced flat-shaped *magwinya* (6.9%). On the use of any electronic equipment, 13.8% responded 'yes' while 86.2% said 'no'. The equipment used in *magwinya* production include dough cutter, proofer and deep fryer. Of the 29 sites visited, only one location had an automated *magwinya* production line. This commences with the mixing of dough with a dough mixer, fermentation in a proofer, cutting into shapes with a dough cutter and finally frying in a deep fryer. A research gap needs to be filled through the development of *magwinya*-production machine that can mix, proof and fry all in one unit. Moreover, most large-scale producers interviewed commented that if a production machine is available, they will opt for it in place of manual method of production. Development of a *magwinya* production machine is recommended.



(A)



(B)



(C)



(D)



(E)



(F)

Figure 6. Large-sized round (A, B, C) and flat (D, E, F) *Magwinya* from different locations

3.3.3. *Magwinya* sales

The price of *magwinya* in the survey area depends on its size. The pricing varies from ZAR0.50 – ZAR0.90 (\$0.07) for small *magwinya*, ZAR1.00 – ZAR2.00 (\$0.14) for medium, and ZAR2.50 – ZAR3.00 (\$0.20) Due to the lack of a standardized equipment, *magwinya* yield per kg of flour varied from one producer to the other. The number of *magwinya* pieces per kg of flour made by producers is presented in Figure 7. Six producers made about 11 – 20 *magwinya* loaves (ranging from medium to large sizes) per kg of flour; while only one producer made over 50 small *magwinya* loaves per kg of flour. The variation in yield may be linked to processing losses and *magwinya* size differences. The information obtained from 24 producers, revealed that 17 make a daily turnover of ZAR500.00 (\$35); 5 make between ZAR500.00 – ZAR1000.00 (\$35 – \$70) per day; and 1 each make between ZAR2000.00 – ZAR3000.00 (\$140 – \$210) per day (Figure 8). According to producers, *magwinya* production is a lucrative small-scale business which has empowered and sustained their livelihoods, and in turn, participated in the economic growth of South Africa. Some of the roadside and school market producers commented that it is a good source of revenue for them, from which they have built houses, bought cars and raised their children.

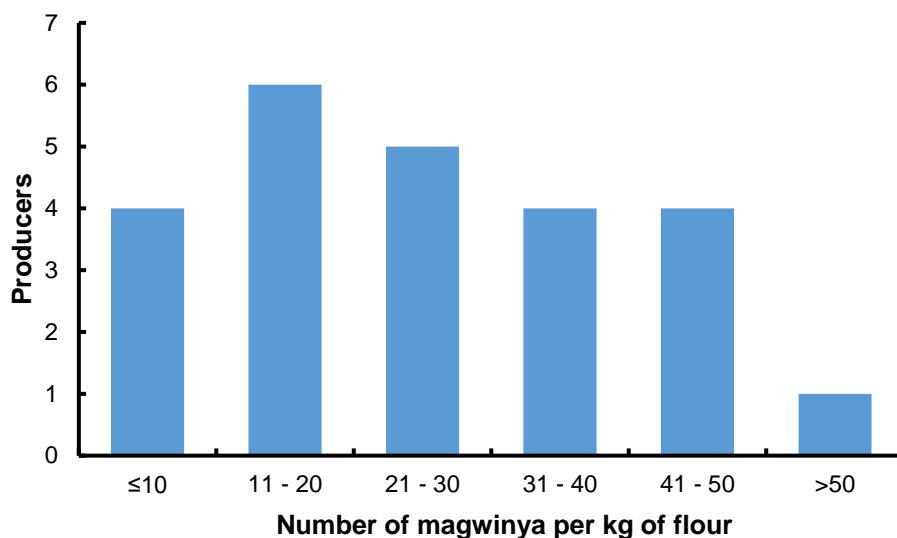


Figure 7. Number of *magwinya* per kg of flour by producers in Thohoyandou area (n = 24)

About 69% produce from Monday to Friday, most especially producers in high school tuck shops 13.8% on Monday to Saturday (supermarkets and roadside vendors); and 17.2% every day (supermarkets, University cafeterias and roadside vendors). A greater percentage of producers (82.8%) report peak sales in the morning (Table 7) because some consumers, especially students take *magwinya* as breakfast.

Table 7. *Magwinya* sales data

Information	Frequency	Percentage (%)	Significance*
Days of production/ sales			
Everyday	5	17.2	<0.001
Mon-Fri	20	69.0	
Mon-Sat	4	13.8	
Peak sales			
Morning	24	82.8	<0.001
Morning+ noon	5	17.2	
Meet sales target			
Yes	25	86.2	<0.001
No	4	13.8	
After-sales demand			
Yes	23	79.3	<0.001
No	1	3.5	
Sometimes	5	17.2	
Production limitation reasons			
Manpower	7	29.2	<0.001
Finance	8	33.3	
Tiredness	4	16.7	
Other snacks	2	8.3	
Time	3	12.5	
Leftovers			
Yes	16	55.2	<0.001
No	10	34.5	
Sometimes	3	10.3	
What happens to leftovers			
Thrown away	1	5.3	<0.001
Resold	4	21.1	
Given away	14	73.7	

*Kruskal-Wallis one-way ANOVA (P < 0.05)

Overall, 86.2% of producers met their daily sales target; while 79.3% answered ‘yes’ to after-sale demand (Table 7). However, the after-demand sales did not necessarily compel producers to make more *magwinya* due to some factors like inadequate manpower, (29.2%), financial constraints (33.3%), tiredness (16.7%), production of other snacks (8.3%), and time limit (12.5%). Despite these reasons, about 55.2% of producers had leftovers which were either thrown away (5.3%), resold (21.2%) or given away to homeless or hungry people (71.7%). One of the producers reported that leftover *magwinya* were stored in the refrigerator and microwaved before re-sale the following day. The reasons why some producers throw away is probably due to a lack of refrigerators for storage. Only one of the production sites packaged their *magwinya* in an air-tight cling film wrap with a “sell-by date” of two days post-production (Figure 6F). This is because *magwinya* has a short shelf life of two days (Kearney *et al.*, 2011) and can go stale rapidly as a result of oil rancidity and retrogradation of starch granules (Singh *et al.*, 2010).

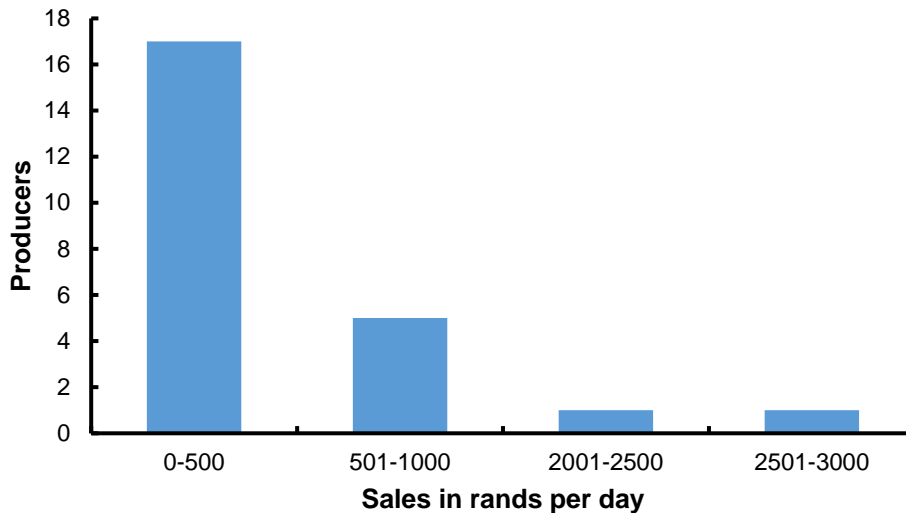


Figure 8. Average daily sales by *magwinya* producers in Thohoyandou (n = 24)

3.3.4. *Magwinya* consumption

Respondents were asked about their consumption habits relative to *magwinya* characteristics. About 46.1% reported 'like moderately' for *magwinya* (Table 8), with the highest significant ratings by age groups ≤ 20 and 21-30 ($P = 0.001$). On the frequency of *magwinya* consumption per week, 31.9% eat twice a week, 27% once a month, 22.9% every day and 18.3% thrice a week. Across all age groups, the frequency of *magwinya* consumption was significantly different ($P < 0.001$, Appendix IV). *Magwinya* is consumed either as a snack (39.9%), main meal (31.5%) and/or side dish (19.7%). Majority of consumers that ate *magwinya* as breakfast were in the ≤ 20 age group. About 28.5% ate *magwinya* as breakfast and 13.7% as lunch. Quite some high school learners and University students rely so much on *magwinya* as one / two of the meals of the day. Temple *et al.* (2006) reported fat cakes as one of the unhealthy foods purchased by about 10% of their survey population. The size consumed depended on what is available to buy. Overall, 58% consume large size, especially at the UVR where a large chunk of the data pool was obtained.

Roadside and high school market producers sell small and medium size (Figure 9) because of the cheap price (less than ZAR1) which meant learners could afford it. In terms of how *magwinya* is consumed, 34.5% reported plain consumption; while 33.9% sometimes consumed it plain or with another food; and 26.8% served it with either of the following: mango atchar (17.8%), French fries/ potato chips (11.4%), polony (9.8%), sausage also known as 'Russian' (5.0%), fish (2.1%), cheese (1.9%), meat (1.1%), curry (0.8%), and avocado (0.2%). Some of these garnishes are unhealthy combinations with *magwinya*.

Table 8. *Magwinya* characteristics

Information	Frequency	Percentage (%)
<i>Magwinya</i> likeness		
Like extremely	97	15.3
Like very much	168	26.5
Like moderately	292	46.1
Neither like nor dislike	77	12.1
Consumption frequency		
Everyday	145	22.9
Twice a week	202	31.9
Thrice a week	116	18.3
Once a month	171	27.0
How <i>magwinya</i> is eaten		
Main meal	200	31.5
Snack	253	39.9
Side dish	125	19.7
All of the above	56	8.8
Main meal type		
Breakfast (B)	181	28.5
Lunch (L)	87	13.7
Dinner (D)	4	0.6
B + L + D	27	4.3
B + L	8	1.3
Size consumed		
Small	83	13.1
Medium	167	26.3
Large	368	58.0
All of the above	16	2.5
Eat <i>magwinya</i> plain		
Yes	219	34.5
No	170	26.8
Sometimes	215	33.9
Most of the time	30	4.7
<i>Magwinya</i> garnish (n = 317)		
Curry	5	0.8
Fish	13	2.1
Meat	7	1.1
Polony	62	9.8
Cheese	12	1.9
Russian	32	5.0
Atchar	113	17.8
Potato chips	72	11.4
Avocado	1	0.2
Accompanying drink (n = 629)		
Soft drink/Juice	321	50.6
Coffee	46	7.3
Tea	127	20.0
Energy drink	17	2.7
Water	118	18.6

For instance, a combination of potato chips and *magwinya* is not a healthy dietary option, because both are high in fats, sodium and calories (Temple *et al.*, 2006). This reflects an unhealthy diet choice among school learners. From a list of drinks taken alongside *magwinya*, sodas/fruit juice top the chart at 50.6% followed by tea (20%) – taken mostly during winter months, because consumers eat *magwinya* as a comfort food during the cold season.



A



B



C

D

Figure 9. *Magwinya* samples (A) Flat vs round (B) various sizes and shapes (C) big and (D) small size

3.3.5. Consumer purchase attributes

Consumers purchased based on their location and producers in their environment. These purchase points included school cafeteria (30.6%), tuck shops (31.9%), high school markets (22.1%), roadside vendors (12.5%) and supermarkets (2.8). Reasons for purchase at a location included proximity to the seller (31.9%), cleanliness of the vendor (25.1%), price (20.3%), size (17.5%), better taste (3.3%), and perceived less fat (1.3%). In comparison to other snacks, consumers chose *magwinya* for personal likeness (39.9%), satisfaction/ satiety (32.8%), and price (26.5%). On the list of favourite *magwinya* characteristics, taste topped the chart at 79.3%, smell – 9.9%, shape – 6.5%, and oiliness – 3.0% (Table 9). On the overall likeness of *magwinya* oiliness, 74.4% responded 'no', 18.0% yes and 7.6% were undecided. This justifies a need for oil reduction in *magwinya*. Some consumers, especially university students, squeeze the oil out with tissue paper to remove the excess fat before consumption. From previous studies (Temple *et al.*, 2006; Kearney *et al.*, 2011), *magwinya* is reported to be energy-dense (183 kcal), low in fibre and high in fat (about 8 – 14 g per 50 g serving), and sodium (14 mg per 50 g serving). As earlier reported that consumers are increasingly becoming aware of the relationship between their health and diet, about 70.5% of our respondents responded 'yes' to their awareness of the health implications related frequent consumption of fried foods like *magwinya*; and as such, 74.9% said they would prefer a low-fat *magwinya* ($P < 0.001$, Appendix 1). To further affirm this, 86.6% said they would purchase low-fat *magwinya* if available. It is recommended that nutrient-rich and acceptable low-fat *magwinya* be developed. If this can be achieved and the product commercialised, healthier *magwinya* options will be available for the consumers. Recent studies from our research group on oil uptake reduction of *magwinya* via fibre enrichment has shown some promising results which can be scaled up for commercialisation (Kwinda *et al.*, 2018; Onipe *et al.*, 2018).

Table 9. *Magwinya* consumer purchase attributes

Responses	Frequency	Percentage (%)
Where <i>magwinya</i> is purchased		
School cafeteria	194	30.6
School tuck shop	202	31.9
Roadside vendors	79	12.5
School market	140	22.1
Supermarket	18	2.8
Reasons for purchase		
Proximity	202	31.9
Price	129	20.3
Cleanliness	159	25.1
Size	111	17.5
Better taste	21	3.3
Less fat	8	1.3
<i>Magwinya</i> purchase compared to other snacks		
Price	168	26.5
Personal likeness	253	39.9
Satisfaction	208	32.8
Cravings	3	0.5
Favourite <i>magwinya</i> characteristics		
Taste	503	79.3
Shape	41	6.5
Smell	63	9.9
Oiliness	19	3.0
Like <i>magwinya</i> oiliness		
Yes	114	18.0
No	472	74.4
To an extent	48	7.6
Prefer <i>magwinya</i> with less oil		
Yes	475	74.9
No	47	7.4
Awareness of health implications of fried food consumption		
Yes	447	70.5
No	143	22.6
To an extent	44	6.9
Will purchase low-fat <i>magwinya</i>		
Yes	549	86.6
No	83	13.1

3.4. Conclusion

Magwinya is a popular African snack that has been in existence for a long time and is likely to continue for generations to come. *Magwinya* production and sales generate income for producers; while consumers get ready-to-eat food at convenience. Thus, knowledge on its processing, sales and consumption patterns is a viable information repository for future research. Technological challenges still exist in the production process due to lack of an automation system for mixing, proofing, frying and draining. From a health perspective, *magwinya* has low fibre content, and high in fat, sodium, and calories (which is an unhealthy food choice for consumers); but this has not stopped its frequent consumption, especially

during the winter season. Nutrient-rich and acceptable low-fat *magwinya* should be developed. If this can be achieved and the product commercialized, healthier *magwinya* options will be available for the consumers. Considering the fourth industrial revolution, the development of *magwinya*-production machine encompassing the unit operations is recommended.

CHAPTER 4: QUANTIFICATION OF OIL FRACTIONS OF A DEEP-FRIED WHEAT DOUGH AS AFFECTED BY BRAN AND MOISTURE LEVELS

Abstract

This chapter investigated oil distribution in two types of *magwinya* (fried batter and fried dough), enriched with two bran types (oat & wheat bran), each at six concentrations (1 – 20%). Total oil content of fried products was categorized as surface oil (SO), penetrated surface oil (PSO) and structural oil (STO) using spectrophotometric method. Moisture loss reduced ($P < 0.05$) from 23.35% in control to 15.19% fried batter (FB) with 20 g oat bran (OB), while a reverse trend was observed in fried dough. Reduction of total oil content from 0.43 g/g in control to 0.38 g/g at 20 g OB and 8 g WB was observed. At 15 g OB and 20 g WB, total fat reduced from 0.41 g/g in control to 0.26 g/g of FD. An $STO < SO < PSO$ trend was observed in FD while FB followed a $SO < STO < PSO$ trend. This investigation indicated that oil uptake reduction in fried dough products is achievable through ingredient modifications. The method of oil distribution measurement used herein can be applied to other thick deep-fried products.

Keywords: fibre, frying, moisture, oil fractions, penetrated surface oil, *magwinya*

4.1 Introduction

Fried foods are popular worldwide because of their palatability owing to the flavour imparted by fats and oils in foods. Deep-frying is a popular heat and mass transfer unit operation which entails immersion of food in hot oil at high temperatures (160 – 200°C) under atmospheric conditions, thus yielding processed foods with unique textures and rich flavours (Gamzuri and Bouchon, 2009). The transfer of heat from the oil to food gives rise to various chemical reactions which cause cellular and sub-cellular changes like starch gelatinisation, crust formation, protein denaturation, moisture loss, oil uptake and colour and flavour development. Mass transfer is explained as evaporation of moisture from the food during frying, which creates crevices or pathways in the food for oil uptake first at the crust, then gradually to the crumb. (Bouchon, 2009). This makes oil uptake reduction, modelling and distribution important quality parameters in fried foods. The reduction of lipid content in fried foods is required mainly because of its correlation to diabetes and coronary diseases (Suárez *et al.*, 2008).

Based on the different oil absorption mechanisms, oil fractions in fried foods can be categorised based on their location viz: surface, penetrated surface and structural oil. The surface oil (SO), which represents the oil fraction that remains on the food surface after removal from the frying medium is governed by the relationship between capillary forces and

interfacial tension which causes adsorption of oil to food (Pinthus *et al.*, 1993; Bouchon and Aguilera, 2001). Structural oil is absorbed into the core of the food through crevices caused by a continuous replacement of moisture by fat during frying (Lalam *et al.*, 2013). Penetrated surface oil (PSO), located in the crust / sub-surface of the food is the oil suctioned into the food at the cooling stage, through a vacuum effect caused by condensation of steam (Ufheil and Escher, 1996; Bouchon *et al.*, 2003). The PSO fraction is regarded as the most important in terms of oil reduction because it ranks highest of the three oil fractions. The amount of PSO is dependent on the structural integrity, surface roughness and permeability of the food crust. As frying commences, a rigid crust is formed due to rigorous water evaporation which leads to dehydration and creation of porous matrix at the food surface. The ability to control/influence the crust structure to make it less permeable is key to reducing oil uptake in fried foods.

An integral component of wheat flour dough is water; needed for hydration and activation of gluten network; to achieve this, the right amount of water is needed (Noort *et al.*, 2010). Besides, water also determines the texture and volume expansion of the product as a result of water vapour which is unable to escape from food core into the surrounding medium. As regards frying, one of the known mechanisms is a water-oil replacement which states that the amount of oil lost is directly proportional to the initial water content of the fried product (Ziaifar *et al.*, 2008). However, the process of moisture loss and oil uptake is asynchronous given that moisture loss occurs during the frying process and oil uptake during the cooling period (Gamzuri and Bouchon, 2009).

Wheat bran (WB) and oat bran (OB) are low-cost fibre source for food product development. Their benefits, attributed to insoluble and soluble fibre contents and nutrient load, include calorific reduction of foods, weight modulation, improvement of gut health, increased intestinal transit time (Xu, 2012; Onipe *et al.*, 2015) and oil reduction in fried dough (Yadav and Rajan, 2012; Kim *et al.*, 2012; Dueik *et al.*, 2014; Onipe *et al.*, 2019). The effect of bran inclusion on oil uptake reduction in fried dough foods is associated with ingredient formulations which follow the following mechanisms: (a) increased viscosity of the food matrix (Shih and Daigle, 2002), (b) water-retention augmentation (Yadav and Rajan, 2012) and (c) barrier-forming properties (Kim *et al.*, 2012; Yazdanseta *et al.*, 2015). Oil reduction effects of WB in fried foods is limited to < 20% substitution; while that of OB up to 11%. At higher amounts, WB increases oil absorption due to dilution of gluten network by coarse WB. Hence, it is recommended to reduce the particle size of bran to dimensions close to flour particles (Hemdane *et al.*, 2016). In this study, WB and OB were pulverized to 200 µm sizes.

In recent studies, oil uptake reduction attempts on *magwinya* was carried out in our research laboratory using mixtures of wheat flour and oat bran, psyllium husk fibre (Kwinda *et al.*, 2018), guar gum (unpublished) and WB of varying particle sizes (Onipe *et al.*, 2018, 2019). Most studies on oil distribution in fried foods is focused on potato products (Ufheil and Escher, 1996; Bouchon *et al.*, 2003; Durán *et al.*, 2007) and one study of surface oil of fried wheat dough model (Thanatuksorn *et al.*, 2005). To the best of my knowledge, this present work is the first study on oil distribution in a doughnut-like snack. Therefore, the objective of this study was to quantify the oil fractions (SO, PSO & STO) in cereal fried snacks with different moisture levels and cereal bran (OB and WB).

4.2. Materials and Methods

4.2.1 Materials

Jungle oat bran (OB), wheat bran (WB) and wheat flour (Sasko, South Africa) were all sourced from local grocery shops in South Africa. Sudan III (A18318 Alfa Aesar, England) and petroleum ether (40°C - 60°C) of analytical grade were used. Coarse wheat and oat bran were milled using an ultra-centrifugal mill (Retsch ZM 200, Haan Germany) fitted with a 200 µm sieve. Milled bran was transferred into an air-tight polyethylene bags and stored at 3°C until further use.

4.2.2 Experimental design

Experiments were constructed using a 2x2x7 factorial experimental design. Factors considered were two (2) bran types - wheat and oat bran; two (2) moisture levels and seven (7) bran amounts (0, 1, 5, 8, 10, 15 & 20 g/ 100 g wheat flour), respectively. Responses measured were fresh weight (g), moisture (%), surface oil (%), penetrated surface oil (g/g), structural oil (g/g) and total oil content (g/g).

4.2.3 *Magwinya* production

Magwinya was prepared according to the method described by Kwinda *et al.* (2018). All dry ingredients were weighed as follows: composite flour (100 g), sugar (15 g), salt (1 g), yeast (1 g), into a mixing bowl at ambient temperature (24°C). Lukewarm water - 65 ml and 100 ml water were added gradually until all ingredients were homogenously mixed to form a dough and batter, respectively (Figure 10). The dough was kneaded for 10 min in a mixer (Russell Hobbs RHSB237, South Africa) and cut into 50 g mass and formed into a ball. The dough and batter were proofed at 30°C in a T7 bread proofer (with temperature control limit of 46°C and 95% humidity) and fried at 180°C for 5 min in a deep fryer (Russell Hobbs RDF300, South Africa) with a built-in automatic temperature and time control system. Oil was pre-heated for 1 h before frying.

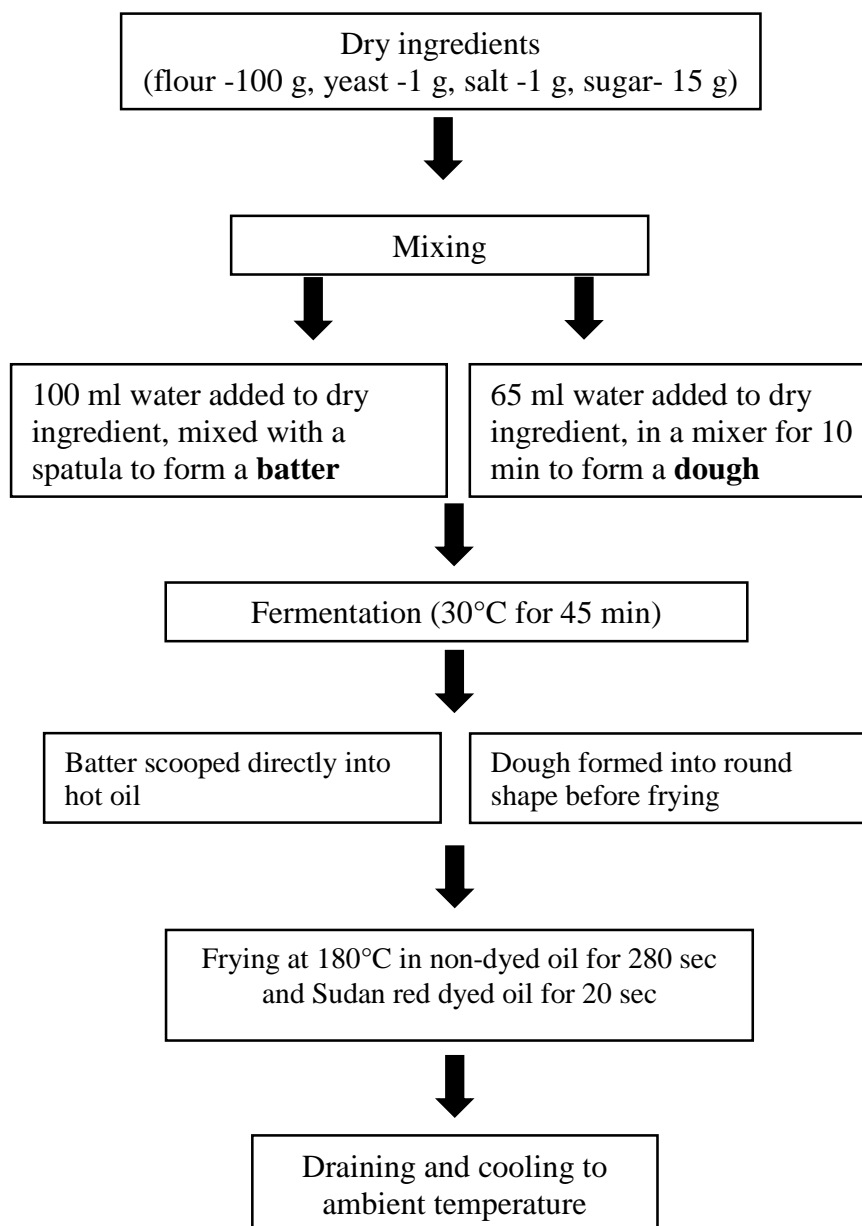


Figure 10. Flow chart for production of *magwinya*

4.2.3.1 Preparation of indicator oil bath dyed with Sudan III

Approximately 1 g Sudan III (A18318 Alfa Aesar, England) was weighed into a volumetric flask. About 100 ml sunflower oil was added and agitated to partially dissolve the dye and thereafter made up to 1 L mark. The volumetric flask containing the dye solution was transferred to a heating-stirrer plate at 30°C for 24 h until the dye was completely dissolved. Dyed oil bath was prepared by adding 233 ml of indicator oil to 3.5 L oil (Bouchon *et al.*, 2003).

4.2.3.2 Frying process

Frying was carried out in two stages viz according to the method of Bouchon *et al.* (2003) with slight modifications: (i) samples were fried in plain (non-dyed) oil for 4 min 40 sec, before transfer to dyed oil for the remaining 20 sec. Samples were removed from the fryer, drained, cooled for 30 min; and weighed (Ufheil & Escher, 1996; Bouchon *et al.*, 2003). Four samples were fried for each experimental run. Dough to oil ratio was kept at 1:23 by volume to avoid drastic fluctuation in frying temperature (Cruz *et al.*, 2018). Temperature gradients in the oil bath was maintained by stirring constantly while flipping the samples every 30 sec. Frying time and temperatures of 5 min and 180°C were chosen as optimum frying time and temperature as pre-determined from preliminary experiments (Appendix V). The choice of 20 sec in dyed oil was made for three reasons (i) this study was designed following the method of Bouchon *et al.* (2003) where potato cylinders were introduced to dyed oil at about 20 sec to the end of frying, (ii) We assumed that 20 sec will have the most significant effect based on the study of Ufheil & Escher (1996) where tubers were interchangeably fried in plain and dyed oil from 0 sec – 120 sec. (iii) At a total of 5 min (300 sec) frying time, the ratio of time spent in plain versus dyed oil in our study was 14:1 (sec) which is low compared to the time ratio in the study of Ufheil & Escher (1996); and similar to the time in the study of Bouchon *et al.* (2003). It is on the above premises that 20 sec was chosen. Since the purpose of this study was to quantify the various oil fractions in *magwinya*, we did not focus on optimisation of time in plain and dyed oil during frying. We, however, recommend further study on time variation in the frying of our products plain versus dyed oil.

4.2.3.3 Standard curve for determination of Sudan III concentration in sunflower oil

Solutions of different concentrations (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mg/ml) of Sudan III (A18318 Alfa Aesar, England) in sunflower oil were prepared as explained in section 4.2.3.1. Each solution was diluted 9 times by volume with petroleum ether 40°C to 60°C (Sigma-Aldrich), and the absorbance was measured at 509.6 nm (maximum absorption with a spectrophotometer (Biowave II, Biochrom Ltd., Cambridge, England) at room temperature. A linear plot (standard curve) of concentration versus absorbance was obtained from different concentrations (Appendix VI).

4.2.5 Fresh weight

Two sets of weights were collected using a digital weighing balance: (i) Fresh weights of cooled samples, and (ii) Weight after washing with petroleum ether – this was used in the calculation of surface oil (SO).

4.2.6 Moisture content and moisture loss determination

Moisture content was determined using approved AACC (2000) method 44-15. *Magwinya* with known weight (3.5.2) was thinly sliced into a pre-weighed moisture dish and dried in an OTE80 forced-air oven (Prolab Instruments, Reinach Switzerland) at 105°C for 24 h. Dried samples were transferred to desiccator to cool. Moisture content was calculated using equation 4.1. Dough/ batter (5 g) was weighed into moisture dishes and dried at 105°C for 24 h. Moisture loss was calculated using equation 4.2 (Zolfghari *et al.*, 2011).

$$\text{Moisture content (\%)} = \frac{\text{Wet weight} - \text{Dry weight}}{\text{Wet weight}} \times 100 \quad (4.1)$$

$$\text{Moisture loss (\%)} = \frac{\text{Moisture content of dough} - \text{Moisture content of fried sample}}{\text{Moisture content of dough}} \times 100 \quad (4.2)$$

4.2.7 Surface oil

Surface oil (SO) was determined using the method of Bouchon *et al.*, (2003) with modifications. SO was determined by washing each *magwinya* with 50 ml petroleum ether for 2 s into a clean pre-weighed 250-ml beaker. The extracted oil was collected by evaporating the petroleum ether. The flask containing the extracted oil was dried to a constant mass by heating at 105°C in an OTE80 forced-air oven (Prolab Instruments, Reinach Switzerland). Percentage SO was calculated using Equation 4.3:

$$\text{Surface oil (\%)} = \frac{W_i}{W_i + W_m} \quad (4.3)$$

W_i = Weight of oil (g) in the beaker

W_m = Weight of *magwinya* after washing with ether

4.2.8 Penetrated surface oil and structural oil

After SO extraction, *magwinya* were finely cut and dried at 60°C for 24 h. Subsequently, the dried *magwinya* were pulverized in a grinder. Approximately 5 g of ground *magwinya* was transferred into single-thickness cellulose extraction thimbles (Whatman Intl. Ltd., Maid stone, U.K.). Fat extraction was carried out using an automated Soxhlet machine based on AOAC (2000) method 960.39. The flasks containing the extracted oil were dried to constant mass by heating in an air-forced oven at 105°C for 1 h. The Soxhlet-extracted oil was diluted 9 times its volume with petroleum ether (40 – 60°C) and the absorbance measured at 509.6 nm using a UV-VIS spectrophotometer (Biowave II, Biochrom Ltd., Cambridge, England). The concentration of dyed oil was calculated using Equation 4.4. This fraction corresponds to the

oil picked up at the end of the frying process and had penetrated the structure of the product after cooling, that is, penetrated surface oil (PSO). The amount of non-dyed oil was calculated from the weight difference using Equation 4.5 (Bouchon *et al.*, 2003). Total oil content of *magwinya* was calculated using equation 4.6.

$$\text{PSO(g)} = \frac{\text{Soxhlet extracted oil (g)} \times \text{Dye concentration in extracted oil (g/l)}}{\text{Dye concentration in oil bath (g/l)}} \quad (4.4)$$

$$\text{STO (g)} = \text{Soxhlet-extracted oil (g)} - \text{PSO (g)} \quad (4.5)$$

$$\text{Total oil (g/g)} = \text{SO (g)} + \text{PSO (g)} + \text{STO (g)} \quad (4.6)$$

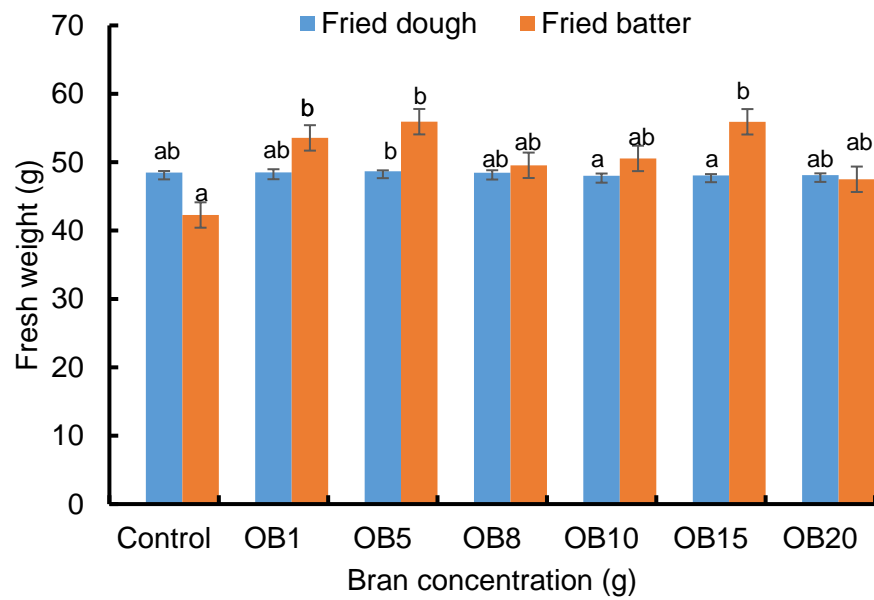
4.2.9 Statistical analysis

Frying experiment and analysis were conducted in four replicates. Multivariate linear regression was used to test the main and interaction effect of factors (amount of bran and water addition) on the dependent variables. Analysis of variance using Duncan's multiple range test was used to separate means ($P < 0.05$). Association between responses was determined using Pearson's correlation test (Onipe *et al.*, 2019).

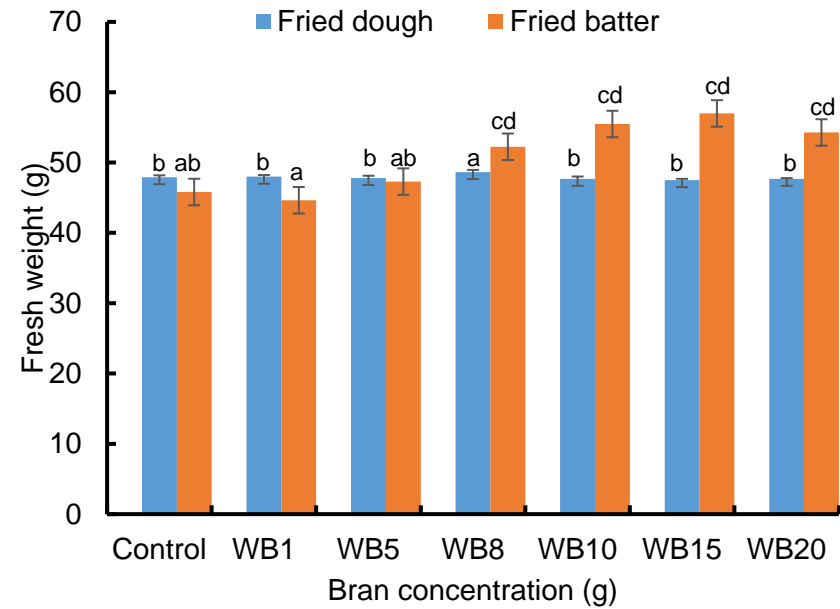
4.3. Results and Discussion

4.3.1 Weight of *magwinya*

Mean fresh weight of wheat bran *magwinya* samples ranged from 45.81 g to 56.98 g. Weights of oat bran fried dough (OBFD) ranged from 48.00 g to 48.66 g; while oat bran fried batter (OBFB) ranged from 42.28 g to 55.92 g. Mean weights of fried dough were all less than 50 g (Figure 11). Weights recorded for the samples were, largely dependent on the initial mass of the dough or batter. Dough mass was uniform at 50 ± 0.2 g. Fried batter samples, however, had varying weights because of unstandardised batter mass – resulting from manual extrusion/ piping of dough into frying oil. These variations were taken into consideration to assess how they affected oil uptake. The differences in weights could also be attributed to the dehydration effect of the frying oil relative to the mass of batter. To minimise temperature fluctuations, sample to oil ratio was kept at 1:23 (Cruz *et al.*, 2018), while frying time was kept constant throughout the experiment. There is a need for standardisation of dough weight in the traditional method of *magwinya* production. The relationship between weight and oil distribution is established in the latter part of this chapter using Pearson's correlation.



A



B

Figure 11. Fresh weight of oat and wheat bran *magwinya* – OB (A) and wheat bran – WB (B). Values are mean of four replications. Error bars are standard deviations. 1 – 20 represent the concentration of bran in *magwinya*. Control – 0 g bran in formulation.

4.3.2 Moisture content and moisture loss

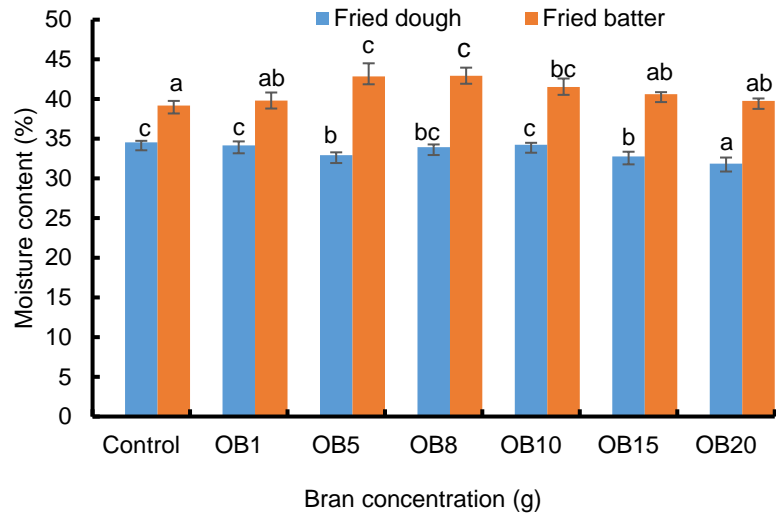
Moisture loss corresponds to the amount of water lost due to frying, while moisture content is the available moisture in the food which can be expressed on a wet or dry basis. In general, moisture values for fried batter were higher than fried dough because of lower initial water content in dough formulation. Moisture loss of OBFB samples ranged from 15.19 to 23.36% with the highest value in the control sample. This implies that OB significantly reduced the amount of moisture that was lost due to frying. There was no significant change in the moisture loss at 5 – 10% OB, but a significant increase was observed at 15 – 20% OB (Figure 12). Control had the least amount of moisture retained in the food at the end of the frying process. OB significantly enabled moisture retention in the food – which is a characteristic favoured by *magwinya* consumers. Interestingly, a different trend was observed for OBFD in terms of moisture retention and loss. The OBFD moisture loss values ranged from 8.13 to 17.66%. Control sample was significantly lower ($P < 0.05$) and OBD20 was markedly higher ($P < 0.05$) than the rest of the samples. In terms of moisture retained in the product, control had the highest moisture content of 34.54%, but this value was not significantly different from samples with 8 and 10% OB.

Moisture loss in wheat bran fried batter (WBFB) samples ranged from 18.69 - 23.36% and followed a significant ($P < 0.05$) upward trend from 5 to 20% WB addition (Figure 12) and no difference; meaning WB had a marked negative effect on moisture loss on fried samples. This may be attributed to the small particle size of wheat bran as water holding capacity of wheat bran has been previously reported to reduce with particle size reduction (Onipe *et al.*, 2017). Moisture loss in wheat bran fried dough (WBFD) samples ranged from 11.79 -14.21% and a downward significant trend ($P < 0.05$) was observed in moisture loss. This implies that WB reduced the amount of moisture lost during frying. Moisture content of the control sample was significantly ($P < 0.05$) higher than other samples; which indicates that WB allowed retention of moisture in the low-water dough system.

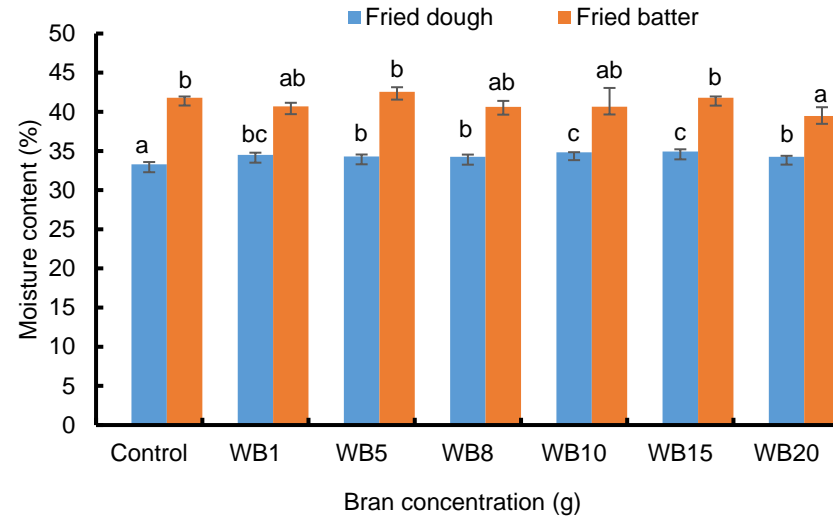
Water is an important ingredient to obtaining a desirable dough and/ or batter consistency because of the various flour components (starch and gluten) and other ingredients (salt and sugar) that compete for water. Moreover, water also directly impacts the texture of fried foods which is a direct effect of the amount of water used for dough/batter formation. Also, water influences volume expansion of the fried product through the process of water vaporisation, crust pore formation and structural integrity of the fried product (Chen *et al.*, 2001).

In a dough system, free water is lost first from the product in the first few seconds of frying. Crust formation commences, thus providing a diffusion gradient on the product surface. A

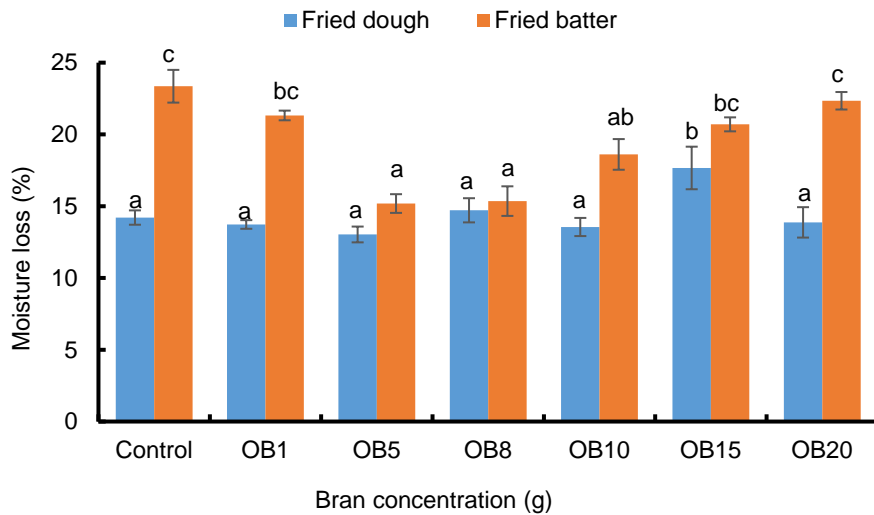
vapour pressure builds up at the core causes swelling (puffiness) of the dough during frying. As this pressure gradient is formed, moisture loss becomes inconsistent, as steam only escapes via the available minor sites in the crust (Ziaifar *et al.*, 2008). Thus, the extent and integrity of crust formation explains the variation in the moisture losses reported in this study. Moreover, moisture loss occurs as a result of partial vapour pressure difference between frying oil and product. Moisture loss in fried batter was higher than fried dough because of the high initial water content of the former.



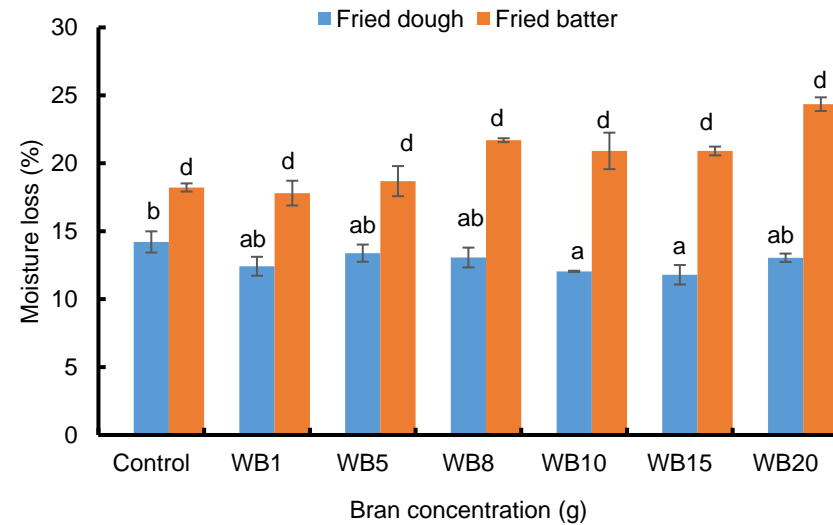
A



B



C



D

Figure 12. Moisture content and moisture loss of *magwinya* (A & B) and moisture loss (C & D) of oat bran (OB) and wheat bran (WB) *magwinya* samples. Values are mean of four replications. Error bars are standard deviations (≤ 0.05). 1 – 20 represent the concentration of bran in *magwinya*. Control – 0 g bran in formulation.

4.3.3 Total oil content

The total oil content of OBFB samples was in the range 0.37 – 0.43 g/g with significant reduction at 10 and 20% OB; and 0.26 – 0.41 g/g in OBFD with consistent reduction with increments to OB concentration (Figure 13A). The total oil content (g/ g) of fried products are presented in Tables 1 and 2. Total oil content of samples was in the range 0.38 – 0.50 g/g and 0.25 – 0.41 g/g in WBFB and WBFD (Figure 13B). Oil uptake reduction in WBFB was at 8 and 20% WB concentration, while in fried dough, there was no decrease from 0 – 8% WB, and a significant decrease ($P < 0.05$) at 10 – 20% WB. Oil uptake in fried batter was higher than fried dough for both OB and WB products. This effect can be linked to the difference in moisture contents of both products. Regression analysis showed that the linear effect of initial water content, bran addition and interaction effect of both factors had a significant effect ($P < 0.001$) in wheat and oat bran samples. In fried batter samples, there was significant oil uptake reduction in OB samples, while WB samples showed less variation in the values reported. In the fried batter, an initial increase (at 5 g OB) in total oil uptake followed by a decrease (from 8 g OB) was observed. A decrease in oil uptake from 0.43 (g/ g) in control to 0.38 (g/ g) at 20 g OB inclusion in fried dough was observed.

Previous authors have reported this moisture loss-oil uptake effect in fried products confirming that higher initial water content led to increased oil uptake due to formation of pores by moisture evaporation which in turn creates pathways for oil uptake. Yadav and Rajan (2012) attributed the effect of oat fibre on oil uptake reduction in *poori* (an Indian fried flatbread) as a function of its significant water retention capacity which impeded moisture loss. Furthermore, the chemical composition of the fibre is less responsible for its oil binding capacity than the porosity of the fibre that serves as storage space for water molecules when the fibre is hydrated thereby reducing oil binding property. Significant oil reduction effect of wheat and oat bran were observed in *poori* (an Indian deep-fried dough) at 3 g and 11 g, respectively. The higher the moisture loss, the higher the oil uptake. Moisture loss-oil uptake effect in fried potato products as adequately reviewed by Ziaifar *et al.* (2008) revealed that outflow of water from the food leaves cracks for oil to occupy. Factors that influence this mass transfer phenomenon in succession are (1) initial water content of the food which in turn determines the (2) rate of water vaporisation over the frying period which directly impacts (3) crust thermal conductivity and (3) crust texture and porosity.

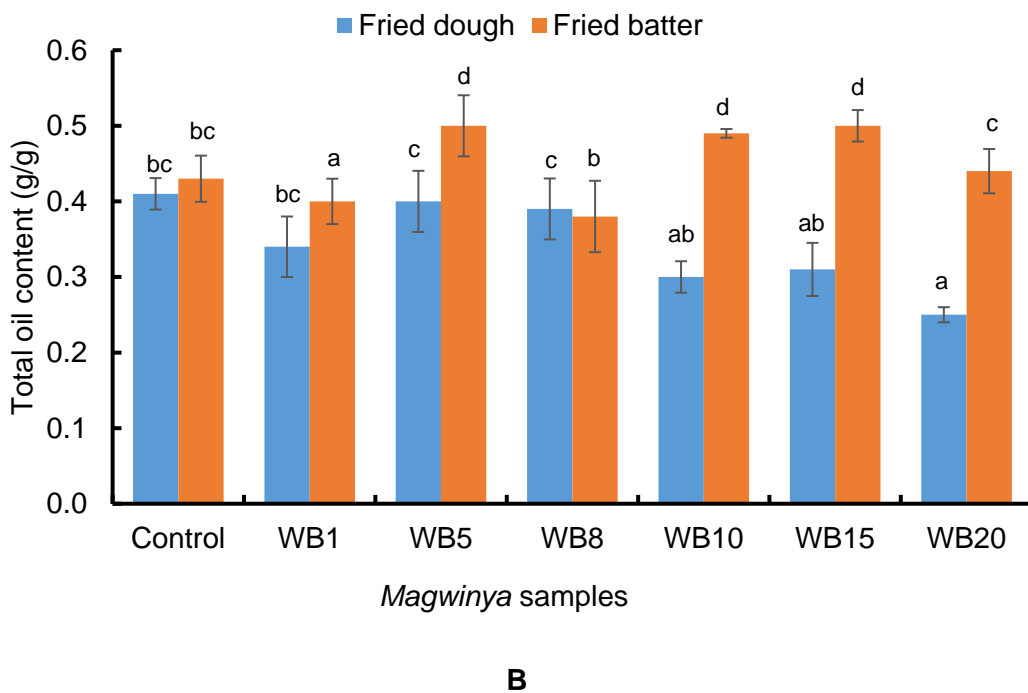
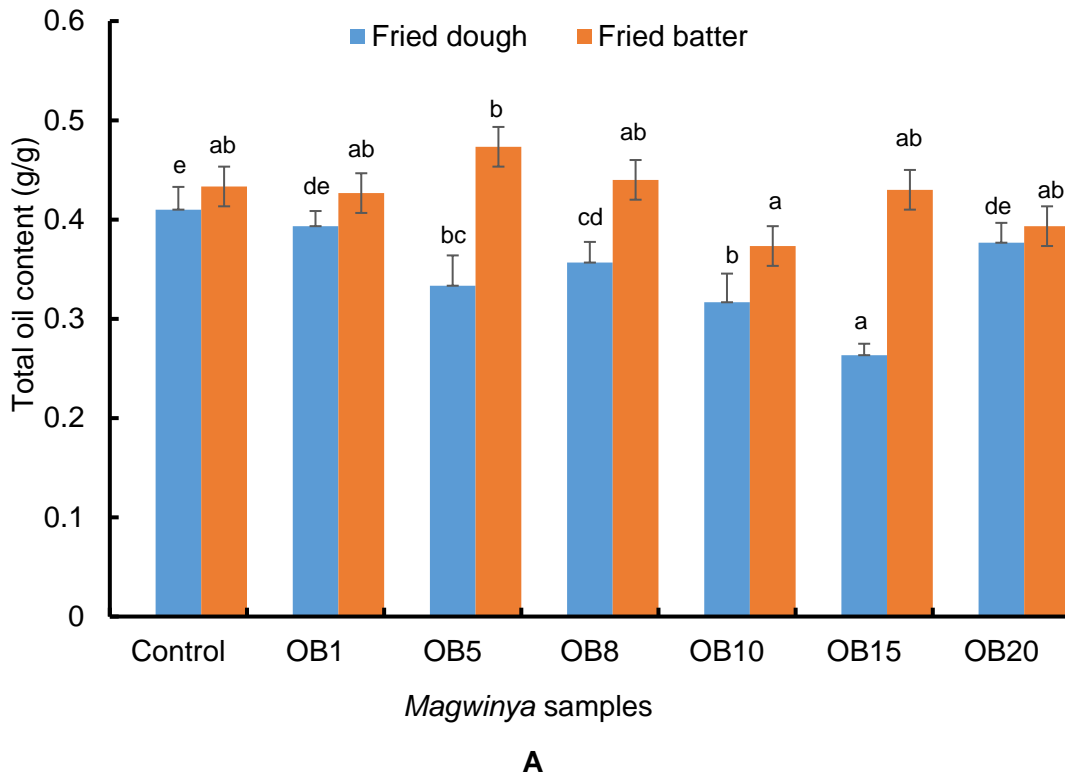


Figure 13. Total oil content (g/g) of *magwinya* samples. Values are mean of four replications. Error bars are standard deviations (≤ 0.05). OB – oat bran, WB – wheat bran. 1 – 20 represents the concentration of bran in *magwinya*, Control – 0 g bran in the formulation.

In terms of the effect of initial water content, Gazmuri and Bouchon (2009) reported no significant difference in oil uptake of fried dough matrix with 38 and 44% of water content but found a relevant difference when the amount of gluten in the products were considered. This 'no-significant' effect may be caused by the small difference (6%) in the water content of the compared dough matrices. In this study, a wide difference (35%) in water contents of dough and batter could account for the higher oil uptake in fried batter samples. Hence, to control crust and crumb texture of fried foods, the water-retention capacity of the dough, its initial water content and moisture content post frying are important.

Gluten protein in wheat is instrumental to the structural integrity of dough-based foods because of its viscoelastic properties which impart strength, rigidity and ability to sustain handling and gas and water retention during processing (Day *et al.*, 2006; Noort *et al.*, 2010). By the reason of the foregoing, gluten proteins are activated through hydration. Therefore, the amount of water available in a dough system is important for activation of gluten proteins and will affect its technological properties in food during and post-processing. On the one hand, reports of oil reduction effect of gluten have been reported in the formulated gluten-based product (Gamzuri and Bouchon, 2009) and battered squids (Fizman *et al.*, 2005). On the other hand, Moreno *et al.* (2010) reported increased oil uptake in gluten-based compared to potato-based formulated products. It is worthy to note that as an increase in gluten from 8 to 12% reduced oil uptake. Similarly, the highest oil uptake was reported in 100% wheat flour doughnut compared to the ones from rice flours (Shih and Daigle, 2002).

Normén *et al.* (1998) also reported an oil uptake increase in potato-gluten patties with increment in the gluten content of the product. Therefore, an increase in gluten content imparts oil-reduction properties in low-moisture dough system. The dry gluten content of WB and OB composite flour was correlated to the total oil content of samples. There was no correlation between the total oil content of fried batter samples and dry gluten content. However, negative significant correlation was found in WBFD ($P < 0.01$) and OBFD ($P < 0.05$) samples. Gluten increased with increase in oat and wheat bran addition which in turn reduced oil content of fried dough samples. These results agree with the report of Gamzuri and Bouchon (2009) that increase in gluten-reduced oil uptake in dough matrix of low-moisture content. The no-significant effect in fried batter may be as a result of moisture saturation in the batter compared to maximum water absorption in the dough samples (Onipe *et al.*, 2017).

Frying is a heat and mass transfer phenomenon. Heat transferred from the oil to the food culminates in mass transfer – moisture evaporation and oil influx into the food. Accompanied with this is phase and volume change. Volume expansion and heat transfer occurs through a

combination of convective (from oil to food) and conductive (within the food) forces. The rate of heat transfer during frying is affected by bubble movement that occurs but may be decreased when there is maximum or vigorous water loss which causes a resistance to heat transfer (Ziaifar *et al.*, 2008; Alvis *et al.*, 2009). In our study, the fried batter had higher bubble movement than fried dough which may be attributed to the high moisture content of fried batter; and high thermal conductivity coefficient of fried dough, as higher heat transfer coefficients have been reported in absence of vapour bubbling (Ziaifar *et al.*, 2010). Upon immersion in the hot oil, fried batter sank to the bottom of the oil and floated under 10 sec. Whereas, fried dough floated almost instantaneously. This variation in floatation time can be linked to water difference in the formulation and densities of the products (Ghaitaranpour *et al.*, 2013). As water is lost during frying, product volume increases, and density reduces which makes fried cereal snack (*magwinya*) float. Floatation results from heat conversion of water to steam which causes a pressure that pushes against the food crust and in turn, volume expansion puffiness occurs which causes the food to rise to the surface of the oil. Floatation may also be influenced by the fried food density. Floatation may also contribute to mass transfers differences in the products, thereby resulting in thermal conductivity variation at the top and bottom crust of the product. To combat this variation, the product was constantly turned every thirty seconds during frying. A cross-sectional figure of samples (Figure 14 and Figure 15) show the location of the various oil fractions of the samples as discussed in the subsequent sections.

Fried batter had higher bubble movement than fried dough which may be attributed to (i) high moisture content of fried batter; and (ii) high thermal conductivity coefficient of fried dough, as higher heat transfer coefficients have been reported in absence of vapour bubbling. Upon contact with the hot oil, fried batter sank to the bottom of the oil and floated under 10 sec. Whereas, fried dough floated almost instantaneously. This variation in floatation time can be linked to water difference in the formulation and densities of the products (Ghaitaranpour *et al.*, 2018). As water is lost during frying, product volume increases, and density reduces which makes *magwinya* float. Floatation results from heat conversion of water to steam which causes a pressure that pushes against the food crust and in turn, volume expansion puffiness occurs which causes the food to rise to the surface of the oil. Floatation may also be influenced by the fried food density.

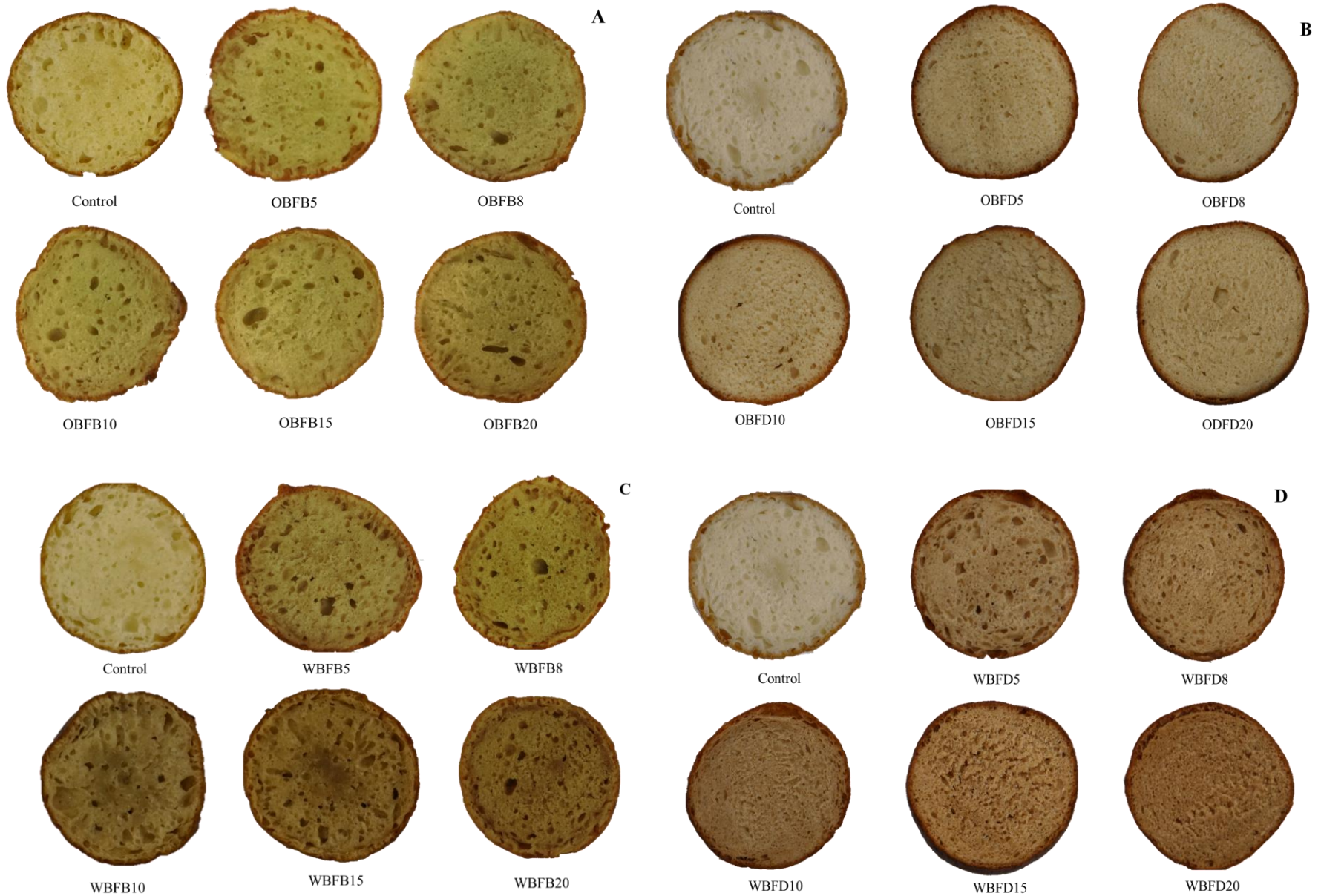


Figure 14. Samples of fried products: WBFD – Wheat bran fried dough, WBFB – WB fried batter, OBFD – oat bran fried dough, OBFB – OB fried batter.

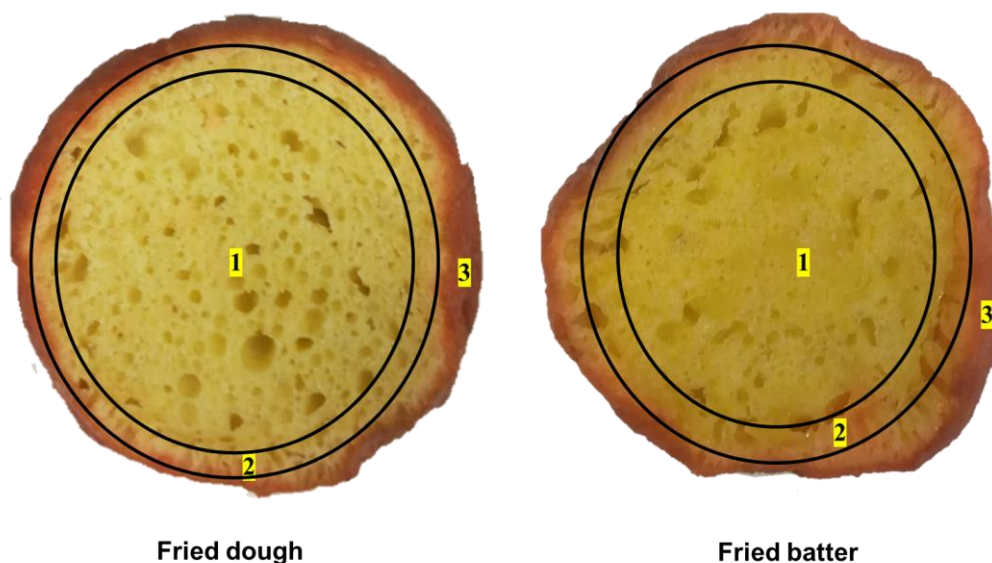


Figure 15. Stained fried dough and batter showing the oil fractions (1) Structural, (2) penetrated surface and (3) surface oil.

4.3.4 Surface oil of *magwinya*

Surface oil (SO) is the oil that adheres to the food surface after removal from the frying oil (Bouchon *et al.*, 2003). The amount of oil absorbed into the food during the cooling stage is largely dependent on the oil present on the food surface. Surface oil values of WB samples are presented in Figure 16. The SO of WBFB with 5 and 20% showed a significant increase ($P < 0.05$) from the control, and other samples showed no difference. Similarly, in WBFD samples, all samples showed a significant decrease ($P < 0.05$) from the control, except WBFB10. Duncan's mean separation test showed that there was no marked difference ($P < 0.05$) among the SO of control and OB samples, however, there were differences amongst the samples (Figure 16). However, the inclusion of OB in fried dough significantly reduced ($P < 0.05$) SO compared to control. Generally, fried dough showed higher SO than fried batter. Also, SO was higher in WB samples than OB samples.

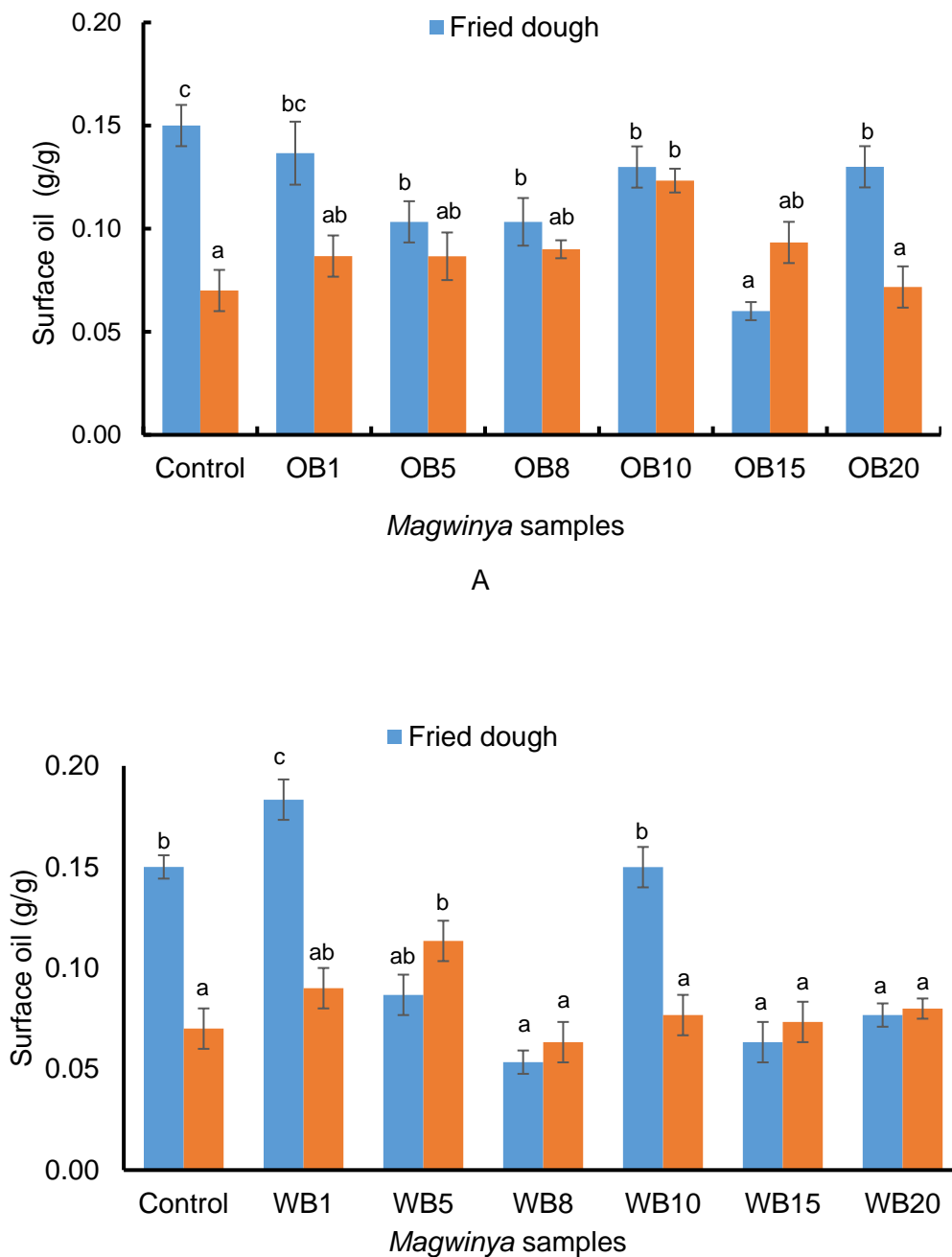


Figure 16. Surface oil of *magwinya* samples. Values are mean of four replications. Error bars are standard deviations (≤ 0.05). OB – oat bran, WB – wheat bran. 1 – 20 represent the concentration of bran in *magwinya*. Control – 0 g bran in formulation.

This may be as a result of the insoluble fibre present in WB which created a barrier on the food surface, thus slowing down the movement of oil into the product. Multivariate linear regression revealed that bran addition, product formulation and interaction effect of both factors all had a significant effect ($P < 0.05$) on surface oil of the products. The amount of oil absorbed into the food post frying is highly dependent on the surface oil. This is because

rigorous escape of steam at the food surface leads to the formation of crust which is characterised by tiny crevices that can allow permeation of oil into the food core. During cooling, as the pressure gradient in the core of the food drops, surface oil is absorbed through capillary forces into the food through cracks in the crust. Therefore, it is recommended that appropriate draining technique be applied to remove the surface oil, thus reducing oil uptake.

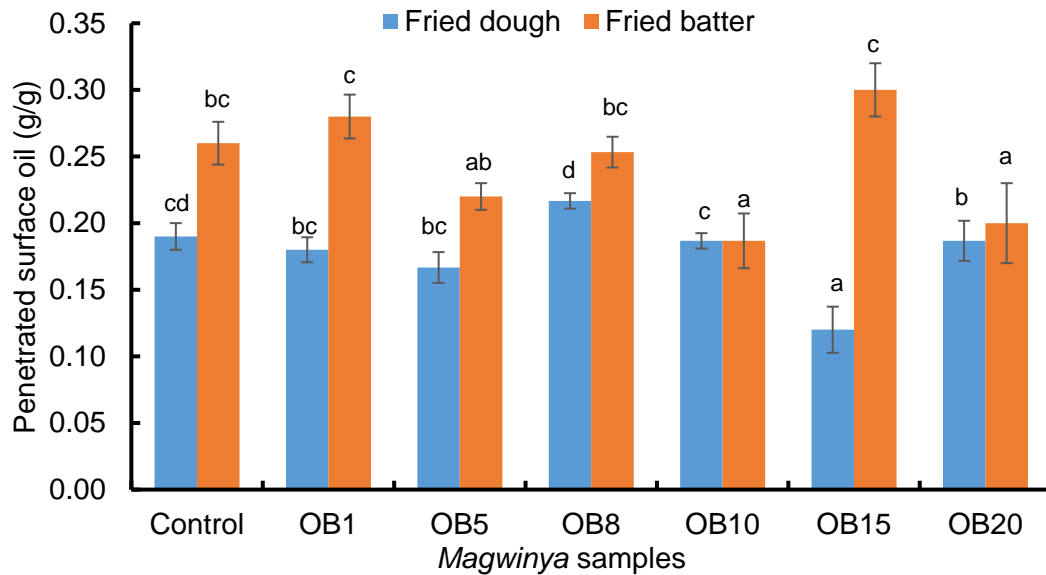
Thanatuksorn *et al.* (2005) reported an increase in surface oil of fried batter with increase in initial water content of a fried wheat dough matrix. Contrary to this, we observed a higher SO content in fried dough (low moisture *magwinya*). This contradiction in results could be due to (i) difference in surface oil collection method. Where Thanatuksorn *et al.* (2005) used the dipping method our product surface was washed to collect adhered oil. Dipping the sample could have caused extraction of the sub-surface oil alongside adhered oil. (ii) Besides, small sample size (<10 g) coupled with prolonged frying time of 7 min increased crust-crumbs ratio which meant more surface oil. From this study, it was observed that SO of fried dough was higher than fried batter. This can be explained thus: (i) the dough had a better structural integrity due to good pliability during kneading which led to gluten formation and gas retention (ii) the consistency of batter caused more bubble formation which made the fried batter spongier (than its dough counterpart), and as a result, could not retain oil on the food surface.

4.3.5 Penetrated surface oil of *magwinya*

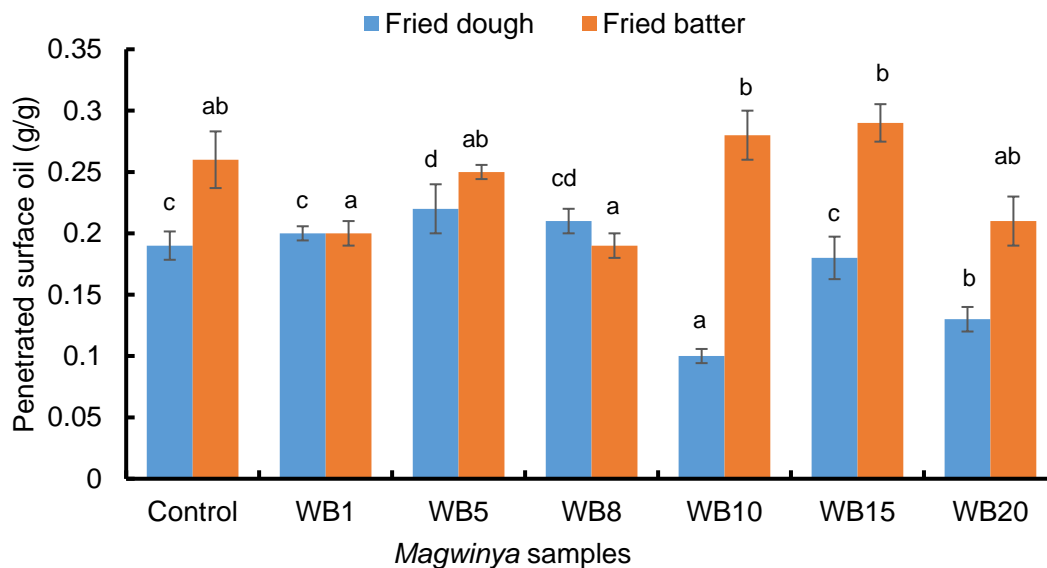
Penetrated surface oil (PSO) was significantly higher in fried batter samples. The PSO were in the range 0.19 – 0.29 g/g and 0.08 – 0.22 g/g in WBFB and Wbfd respectively (Figure 17), while OB samples ranged from 0.19 – 0.30 g/g and 0.12 – 0.22 g/g in OBFB and OBFD respectively (Figure 17). A significant decrease ($P < 0.05$) in PSO was observed at 5, 8 & 20% WB concentration, while in fried dough, the reduction was only reported at 10 & 20% WB concentration. For OBFB samples, a significant reduction from control was found at 10 & 20% OB concentration, while in OBFD samples, the reduction was seen at 8 & 15% OB. (Figure 17). Linear effect of the formulation and bran addition had a significant effect ($P < 0.05$) on the PSO of *magwinya*. PSO has been considered the most significant oil fraction because it accounts for the higher percentage of the total oil content of fried foods (Bouchon *et al.*, 2003; Pedreschi *et al.* (2008). PSO is the amount of oil absorbed in the crust microstructure and this occurs toward the end of the frying (10 – 20 sec) and cooling stage due to change in temperature and core pressure gradient (Pedreschi *et al.*, 2008; Zhang *et al.*, 2016).

Dehydration in *magwinya* is most intense at the surface because of the high temperature (180°C), which in turn leads to the formation of pores in crust microstructure which are subsequently filled with oil and/ air as the core temperature of the product decreases. Oil

uptake in fried foods is a surface-related phenomenon which starts at the onset of cooling due to competition between drainage and suction forces through which oil moves into the crust substructure.



A



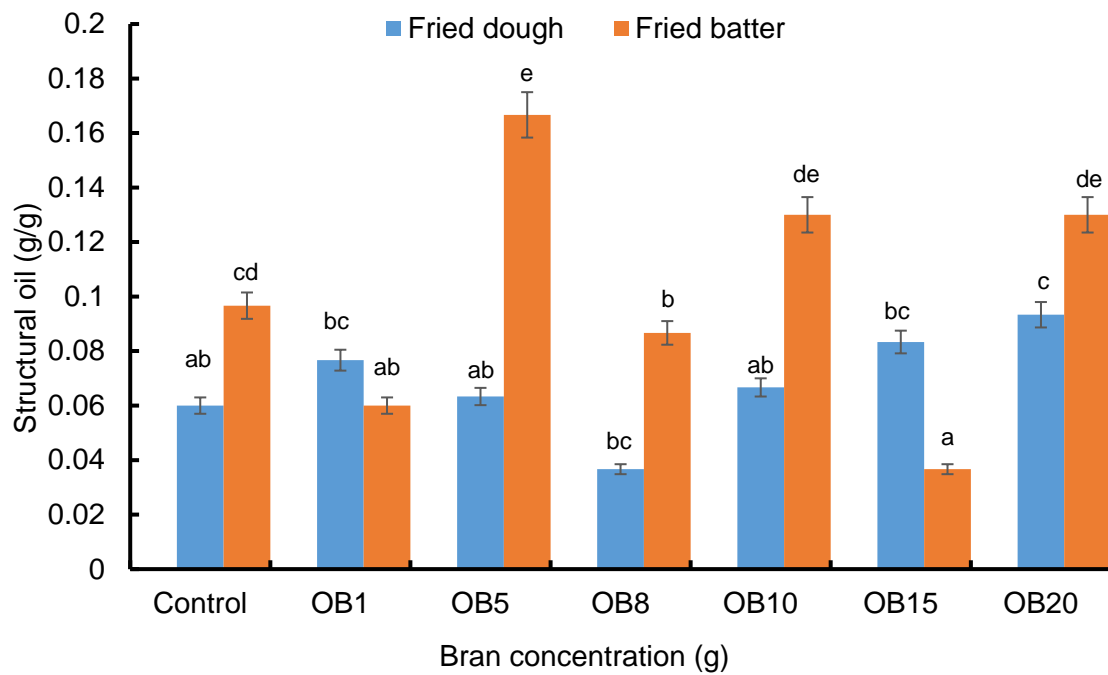
B

Figure 17. Penetrated surface oil of *magwinya* samples. Values are mean of four replications. Error bars are standard deviations (≤ 0.05). OB – oat bran, WB – wheat bran. 1 – 20 represents the concentration of bran in *magwinya*, Control – 0 g bran in *magwinya* without bran.

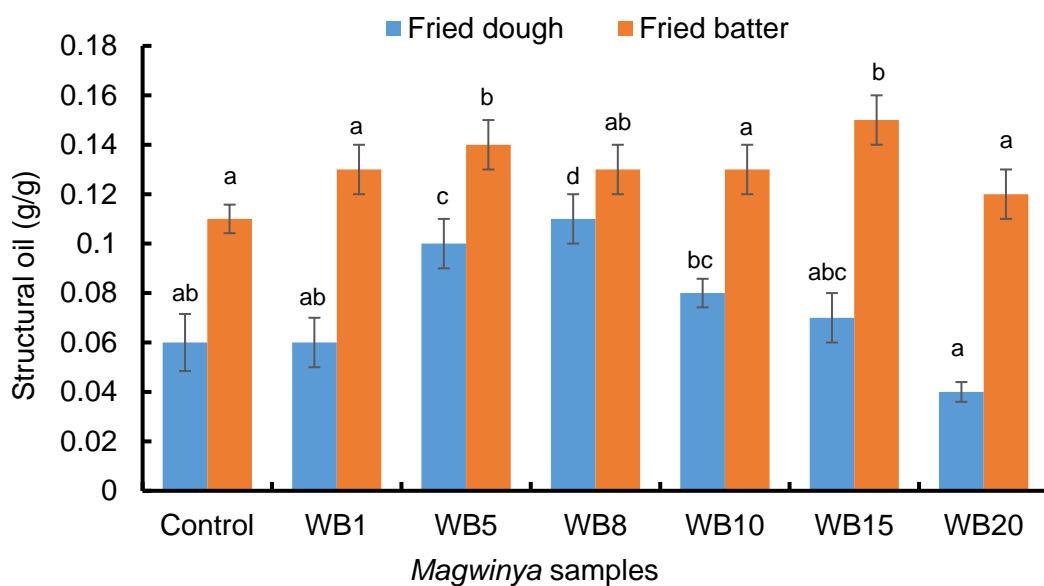
In this study, percentage of PSO was highest in all the samples as also confirmed from previous studies (Ufheil and Escher, 1996; Bouchon *et al.*, 2003; Pedreschi *et al.*, 2008). Another factor that could potentially contribute to increased PSO is the food surface roughness of food increased the cracks in the crust which increases the uptake of surface oil (Thanatuksorn *et al.*, 2005; Moreno *et al.*, 2010).

4.3.6 Structural oil of *magwinya*

Structural oil (STO) corresponds to the amount of oil that is embedded in the core of the food. The STO values were found in the range 0.04 – 0.11 g/g in WBFD and 0.12 – 0.15 g/g in WBFB; while STO for OBFD ranged from 0.04 to 0.09 g/g and OBFB ranged from 0.04 to 0.17 g/g (Figure 18). While the values of WB fried batter were close, that of OBFB were spread over a wide range, which could be attributed to batter mass which was not as precise as dough mass before frying. There was no significant difference ($P < 0/05$) among WBFB samples, while STO showed a significant increase in WBFD samples at 5 – 15% WB concentration and a decrease at WB20 (Figure 18). Control STO of OBFB decreased from 0.11 g/g to 0.09 g/g and 0.04 g/g at 8 and 15% OB concentrations (Figure 18). Generally, STO values were significantly higher in fried batter than fried dough samples. Structural oil (STO) corresponds to the amount of oil that is embedded in the core of the food. In the fried batter, STO was significantly higher compared to fried dough samples (Figure 18). This may be a function of higher porosity in the fried batter which allowed a free flow of oil into the food core. Out of the three oil fractions, STO was the lowest. The case is contrary to the potato product in which SO was the lowest oil fraction (Bouchon *et al.*, 2003; Pedreschi *et al.*, 2008; Zhang *et al.*, 2016). This reveals that oil distribution in fried foods differ from one product to the other. Unlike thin products like potato chips that absorb most of the oil during frying; *magwinya* is a high-moisture and thick food and thus, the less oil is absorbed during frying, and more during cooling (Pedreschi *et al.*, 2008; Zhang *et al.*, 2016).



A



B

Figure 18. Structural oil of *magwinya* samples. Values are mean of four replications. Error bars are standard deviations (≤ 0.05). OB – oat bran, WB – wheat bran. 1 – 20 represents the concentration of bran in *magwinya*, Control – 0 g bran in the formulation.

4.3.7 Correlation of oil fractions and other properties of fried products

Pearson's correlation examined the relationship among the properties of fried snack as presented in Table 10. In OB fried batter, weight correlated negatively to moisture loss; meaning that the higher the weight, the lesser the moisture loss at the specific frying time (5 min.); while weight correlated positively to total moisture content. There was a negative significant correlation ($P < 0.01$) between PSO and STO implying that higher PSO led to lower STO and vice versa. Similarly, SO correlated positively to total oil uptake. This buttresses the point that oil absorbed into the sample during cooling is dependent on the oil on the product surface. There was no significant correlation between weight and *magwinya* oil properties because the weights of fried dough were uniform. Moisture loss correlated negatively to the various oil fractions except STO (Table 10). Surface oil positively influenced PSO and total oil content; while PSO negatively correlated to STO. This implies that these oil fractions are not uniformly distributed in *magwinya*.

Table 10. Pearson's correlation for oat bran enriched fried snack

Oat bran fried batter					
	Weight	Moisture loss	Surface oil	Penetrated SO	Structural oil
Moisture loss	-.530*	1			
Surface oil (SO)	.062	-.257	1		
Penetrated SO	.177	.149	.367	1	
Structural oil	-.072	-.239	-.205	-.824**	1
Total oil content	.392	-.325	.651**	.402	.108
Oat bran fried dough					
Moisture loss	-.205	1			
Surface oil	.099	-.817**	1		
Penetrated SO	.346	-.443*	.512*	1	
Structural oil	-.212	.169	-.094	-.728**	1
Total oil content	.424	-.679**	.789**	.538*	-.079

** . Correlation is significant at the 0.01 level. * . Correlation is significant at the 0.05 level.

Weight of WB fried batter correlated positively to moisture loss and PSO. The three oil fractions all correlated positively ($P < 0.01$) to total oil content (Table 11). In fried dough, weight correlated positively ($P < 0.01$) to STO and total oil content while only PSO. Moisture loss correlated positively only to PSO. Structural oil correlated positively to PSO; implying that

increase in one led to an increase in the other. Total oil content had a positive significant relationship with weight, PSO and STO.

Table 11. Pearson's correlation for wheat bran enriched fried snack

Wheat bran fried batter					
	Weight	Moisture loss	Surface oil	Penetrated SO	Structural oil
Moisture loss	.496*				
Surface oil (SO)	0.04	0.129			
Penetrated SO	.521*	0.278	-0.044		
Structural oil	0.35	0.264	.604**	.698**	
Total oil content	0.35	0.264	.614**	.624**	1.000**
Wheat bran fried dough					
Moisture loss	0.335				
Surface oil (SO)	-0.021	-0.287			
Penetrated SO	0.263	.520*	-.730**		
Structural oil	.599**	0.065	-0.235	.438*	
Total oil content	.560**	0.266	0.107	.529*	.713**

** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level.

4.4 Conclusion

Inclusion of OB in fried dough from 5 to 20% and fried batter at 10% reduced total oil content. Significant oil uptake reduction in WB fried dough occurred at 10 - 20%. Oil distribution in *magwinya* revealed interesting results as follows: The oil fraction PSO contributed mostly to the total oil content of all the samples at 46% for WBFD, 53% and 57% for WBFB and OBFB respectively. Also, oil distribution varied based on the type of formulation (low or high moisture). In fried batter (high moisture), the ranking is as follows: PSO>STO>SO while in the fried dough (low moisture) samples, the rank is PSO>SO>STO. Results confirm that oil uptake in *magwinya* is a surface-related phenomenon. However, the distribution of the oil fractions varied from one snack-type to the other. Given that PSO is the highest oil fractions, strategies to reduce this fraction will ultimately reduce oil uptake. The following post-frying recommendations are suggested: (i) surface modification through coating systems using food hydrocolloids; (ii) effective draining system (such as centrifugation) upon removal from the hot oil.

CHAPTER 5: CONFOCAL LASER SCANNING MICROSCOPY AND IMAGE ANALYSIS FOR ELUCIDATING OIL UPTAKE, CRUMB AND CRUST MICROSTRUCTURE OF BRAN-ENRICHED SOUTH AFRICAN FRIED DOUGH AND BATTER

Abstract

A double-staining protocol for image acquisition using confocal microscopy (CLSM) technique coupled with image analysis were employed to elucidate crust and cross-sectional properties of fried dough. Penetrated oil by image analysis (POia), porosity and pore features were quantified from the cross-section micrographs; while crust surface roughness was measured using fractal metrics. Crumb porosity ranged between 54.94 – 81.84% and reduced ($P < 0.05$) with bran addition. Crumb pore sizes ranged from 0 – 475 μm with <1 circularity, indicating elliptical shape. POia values were notably higher ($P < 0.05$) than PO by soxhlet extraction (POsox) except for wheat bran (WB) fried dough where the values of POia and POsox were closely ranked. Linear effect of initial moisture content and bran concentration showed significance on image properties. Mean fractal dimension (FD) decreased as initial moisture increased. Addition of WB caused a significant reduction of FD of fried dough while an opposite effect was noted for its oat bran counterpart. Due to non-collinearity of image properties (FD, POia & porosity), data were fitted to cubic polynomial regression with R^2 values >0.70 . Confocal microscopy and image analysis were effective in measuring oil absorption and interpreting crumb properties of fried dough. The protocol used in this study can be applied to other thick deep-fried foods for qualitative observation and quantitative measurement of specific physical or chemical property.

Keywords: *magwinya*, image analysis, confocal micrograph, fried dough, bran

5.1. Introduction

The transformation of dough and batter into *magwinya* encompasses vital microstructural changes highly dependent on processing conditions such as mixing, and dough development, fermentation (bubble formation as a result of CO_2 release in the dough/ batter), moulding of dough and/or scooping of batter and thermal treatment (frying). The features of bread crumb such as cell wall thickness, cell size, void fraction, porosity and shape have been quantified using image analysis as reviewed by Pérez-Nieto *et al.* (2010). The application of confocal laser scanning microscopy (CLSM) for qualitative analysis of food microstructure offers visualisation of changes to food products, characterisation of complex food systems, and the distinction between food components (Dürrenberger *et al.*, 2001). Confocal microscopy use in

the study of fried foods offers a non-invasive approach to qualitative and measurable evaluation of oil uptake in fried chicken nugget (Adedeji *et al.*, 2011), gluten-based and potato-based food matrices (Moreno and Bouchon, 2013). In addition to oil uptake, quality changes to the texture of the foods such as crust formation, pore size distribution and porosity measurement have all been estimated using image analysis (Ghaitaranpour *et al.*, 2018a).

The CLSM choice in the visualisation of changes in fried foods is due to its ability to produce images with clear contrast differentiating one food component (fat, protein or carbohydrates) from the other and the empty pores. This is achieved using dyes specific to a food component and simultaneously viewing these components. Staining post-frying has been used, but this could yield unreliable results due to the timing of staining which does not reveal oil uptake relative to frying unit operation. Moreover, the formation of artefacts occurs, leading to error in data analysis from the resulting image. Instead, staining the food and oil before frying is a better step in sample preparation which cuts out post-processing staining (Bouchon *et al.*, 2003). Choice of fluorophore depends on its affinity to the food component of interest, emission spectra, and behaviour in the food matrix (Pedreschi *et al.*, 2008; Moreno and Bouchon, 2016)

Quantitative analysis of food products observed microscopically has become a vital tool to evaluate the quality of products. In the same way, food is processed through application of different unit operations, significant information can be extracted from an image by subjecting it to processing through application of mathematical operations on the raw image (Mandelbrot, 1983; Jekle and Becker, 2011; Cález Ramírez *et al.*, 2017; Wasnik *et al.*, 2017). Raw and processed foods have irregular and complex geometry and as such can be quantified using fractal metrics - a useful tool to measure the surface topography of various objects including food (Moreno *et al.*, 2010; Rahimi and Ngadi, 2016). In fried foods research, fractal analysis has been used to measure surface roughness (Ghaitaranpour *et al.*, 2018a) and correlated to oil absorption (Thanatuksorn *et al.*, 2005; Moreno *et al.*, 2010; Rahimi and Ngadi, 2016). Surface roughness of foods is quite sensitive because what looks smooth to the human eye may be jagged when examined microscopically. FD measures how much space is filled in an image using thresholding and edge detection tools (Jekle and Becker, 2011).

Application of CLSM to study the microstructure and surface topography of fried dough (*magwinya*) has not been carried out for quantitative evaluation. Therefore, this chapter aimed to extract meaningful empirical information from the crust and cross-section confocal micrographs of *magwinya* to (1) observe and enumerate penetrated oil, porosity and pore properties of crumb; (ii) determine the surface roughness of crust as affected by bran levels

in *magwinya* formulation; using fractal metrics; and (iii) evaluate the effect of surface roughness on surface and absorbed oil contents, and to assess the relationship between surface roughness and crust texture.

5.2. Materials and method

5.2.1 Materials

For dry ingredients – refer to chapter 5. Sunflower oil (Spar, South Africa) was used as a frying medium. Nile Red - 72485, Sigma Aldrich, USA) was used to stain the frying oil and fluorescein-5-isothiocyanate (Fluka, Switzerland) was used to stain the dough matrices.

5.2.2 *Magwinya* production and frying process

The depth of oil in the fried products were observed using confocal laser scanning microscopy (CLSM), using the non-invasive double staining method of Moreno and Bouchon (2013). To enable proper differentiation between oil and other components of the samples, two types of dyes were used – FITC (for batter and dough staining) and Nile red (oil staining). Fluorescein-5-isothiocyanate (FITC) was chosen for dough staining because it stains starch and gluten well. It works on the principles of hydrophobicity, that is, its ability to gather in hydrophobic regions, such as gluten rich portion of the dough (Moreno and Bouchon, 2013). Nile red was chosen for oil staining for its fat-soluble, thermo-resistance, and emission spectra which is distinct from FITC. Staining procedure was done in two phases according to the method of Moreno and Bouchon (2013).

Phase one: Dry ingredients (flour – 100 g, sugar – 15 g, salt – 1 g, yeast – 1 g) were weighed and mixed thoroughly. About 0.01% w/w FITC was dissolved in distilled water at 20°C for 1 h by agitation using a magnetic stirrer. The FITC-stained water - 65 ml and 100 ml were added to the dry ingredients for dough and batter preparation respectively for identification of the solid matrix during CLSM observation, without further staining post frying.

Phase 2: The second staining was carried out by dissolving Nile Red at 0.05 g/L in the frying oil to identify oil distribution in the *magwinya* during CLSM observation. The FITC-stained dough and/ or batter was deep-fried in the Nile red-stained oil at 180°C for 5 min. The fried *magwinya* was cooled to ambient temperature in the desiccator until imaging.

5.2.3 Confocal imaging of *magwinya*

Cross-section and crust of samples were observed with an LSM780 confocal microscope (Zeiss, Oberkochen, Germany) equipped with Argon multiline laser at 200 µm depth. Thin cross-section slices (0.5 x 0.5 x 0.5 cm) were cut from each fried product and directly viewed

under the lenses of the microscope, with two channels of observation of the confocal microscope in fluorescent mode. The FITC-stained solid matrix (dough and batter) was observed in channel one, while the Nile-Red-stained oil showing fat distribution was viewed in channel two after exciting at 488 nm and 543 nm, respectively with the Argon laser. Images were acquired by setting the objective lens at 10x magnification with a numerical aperture of 0.3 over regions of 512 x 512 pixels. The 2-D stack of images was collected to produce 8-bits 2-D images in CZI format at 2.768 μm / pixel in x and y directions. Images were collected to a computer using the ZEN 2.3 SP1 software (Carl Zeiss, Oberkochen, Germany 2012), and were enhanced using the maximum intensity projection and best fit function. Images were exported to ImageJ version 1.52q (National Institute of Health, USA) for further analysis.

5.2.4 Image segmentation and analysis

Image segmentation was done using ImageJ software (Abramoff *et al.*, 2004; Schneider *et al.*, 2012). The stacked composite was opened in software using the split channel function in the hyper stack plugin. The red channel was analysed for fat distribution, while the green channel for the solid matrix. Automatic thresholding was done in ImageJ using the Otsu's algorithm, followed by two steps of erosion and dilation to remove noise (Otsu, 1979; Adedeji *et al.*, 2011). Demarcation between the pore and rest of the image was done in one of two ways: (1) TIF version of the composite image was loaded onto ImageJ software, the pore area (in black) were manually selected by holding down the SHIFT key and the lines were traced around the boundaries of the pores; and/ or (2) using the wand tool (at 4-connectedness and 1 - 5 tolerance level) to automatically select the pore areas. The Wand tool creates a selection by tracing objects of uniform colour or similar pixels. All the selected areas were subsequently filled with alternate colour (blue) before thresholding (Appendix VII) with the Triangle algorithm in ImageJ software. Triangle algorithm was used because it gave better pore segmentation from the solid matrix with minimal noise. The segmented images were analysed for pore circularity (Equation 5.1), particle count (ΣP), total area (ΣA), average size ($\bar{\phi}$), solidity and perimeter (P) using the analyse particle function. Porosity and penetrated oil by image analysis (POia) were calculated using equations 5.2 & 5.3 (Adedeji *et al.*, 2011; Moreno and Bouchon, 2013).

$$\text{Circularity (C)} = 4\pi \frac{\text{Area}}{\text{Perimeter}^2} \quad (1)$$

$$\text{Porosity (\%)} = \frac{\text{Area of empty pores} + \text{Areas of pores filled with oil}}{\text{Total image area}} \times 100 \quad (2)$$

$$POia (\%) = \frac{\text{Area of pores filled with oil}}{\text{Total image area}} \times 100 \quad (3)$$

5.2.5 Quantification of surface roughness of *magwinya* using fractal analysis

Fractal dimension was used to quantify surface roughness of the crust of *magwinya* using the box-counting method through the fractal box count algorithm of ImageJ software. The crust images were loaded onto the software and were binarised. Erosion and dilation operations were applied to remove noises from the images. Thereafter, a Sobel edge detector was used to find the edges of the images. This operation distinguished the edge from the background. The fractal dimension (FD) of the edged image was calculated by applying the 'fractal box-count plugin' which counts the number of boxes of increasing sizes (2, 3, 4, 6, 8, 12, 16, 32 & 64) needed to cover the boundary of the binary object per pixel and applies the method (Smith *et al.*, 1996). A graph of the logarithmic values of fractal box size versus count was plotted and fitted linearly. The slope of the graph taken as fractal dimension (Equation 5.4). The value of the slope is explained as the surface roughness index for the actual surface.

$$D = - \frac{\log(N)}{\log(r)} \quad (5.4)$$

Where N is the number of boxes, and r is the size (length) of the boxes and D (slope of the graph) is the fractal dimension. As proposed by Rahimi and Ngadi (2016) because FD was quantified from a 2-D space, the addition of an extra one dimension is necessary to fully capture the three-dimensional features of images of batter surfaces. Therefore, FD was recalculated as per Equation 5.5.

$$FD=1+D \quad (5.5)$$

5.2.6 Statistical analysis

All images were acquired in a replicate of four per frying time. Analysis of variance was carried out to determine the effect of bran concentration on the porosity, POia and fractal dimension (FD). Means were separated using Tukey's HSD test at $P < 0.05$ where the effect of the independent variable was significant. Test of significance for FD of fried batter and dough was carried out using an independent T-test, at 95% confidence level (Moreno and Bouchon, 2013). Multivariate regression analysis was carried out to determine the linear and interaction effect of bran addition, initial moisture content and bran type on dependent variables. The relationships between crust parameters and FD of the fried products were fitted through a regression model using the curve estimation function of SPSS software (SPSS statistics version 26, IBM Co. USA).

5.3. Results and Discussion

This section presents qualitative observations and quantitative data extracted from cross-section micrographs of *magwinya*. Crumb properties (pore size, solidity, circularity and total particle count) of *magwinya* were quantified and crust micrographs were analysed for surface roughness and how it relates to hardness and oil uptake.

5.3.1 Qualitative analysis of microstructure

During preliminary experiments, the samples were stained with Nile red after frying and this led to the formation of artefacts. Oil and water droplets moved from samples to the microscope slide, thus yielding unreliable results. Therefore, the double staining protocol of dough and oil staining was used because it yielded a satisfactory contrast between the oil (red), empty pore (black) and the solid matrix (green) as shown in Figure 19. Enhancement of good image contrast offers good benefits in terms of the information that can be extracted from such an image (Scalon and Zghal, 2001).

5.3.1.1 Oat bran (OB) *magwinya*

Cross-sectional micrographs of OB fried dough are presented in Figure 19. As noticed in all samples, there is intensity of red colour (oil) clusters at the crust section and subsequent penetration at different depths into the solid matrix - indicated by the diminishing intensity of red dye (or blue arrow) from the crust to the crumb in the solid matrix. Thus, confirming that oil uptake in *magwinya* is a surface-related phenomenon following a crust to crumb path flow. Depth of oil penetration show similar proximity to the crust at 5 and 8% and further from the crust at 10 – 20% OB. It is worthy to note that the sample size used for microscopy may not reflect the whole sample compared to conventional techniques. Pores in the fried dough (Figure 19) were small and almost evenly distributed while in fried batter, the pores were mostly large (Figure 20). Large pores are indicative of weak gluten-starch matrix and thin liquid lamellae caused by reactions between surface-active agents of dough and gluten network which collapses upon heating, leading to the formation of large coalesced gas cells in the crumb (Mills *et al.*, 2003). This consecutively causing large pathways through for oil penetration. Hence the increased depth of penetration compared to fried dough samples which had stronger structural integrity. These qualitative observations are supported by the soxhlet extraction results where fried batter absorbed more oil than fried dough samples.

5.3.1.2 Wheat bran *magwinya*

The depth of oil penetration was less in the fried dough (Figure 21) than fried batter (Figure 22). Comparing both cereal brans, least oil penetration was observed in wheat bran (WB)

enriched *magwinya*. This implies that WB reduced oil uptake better than oat bran (OB). Generally, the fried batter had larger geometric features than fried dough. The differential water contents and viscosity of dough and batter (liquid dough) explains the reason for the structural difference among the samples. Like its OB counterpart, large pores were also observed in WB fried batter. Pores of *magwinya* developed and increased as steam is generated in the crumb. Subsequently, crumb pressure and temperature drop during cooling and pores are stabilised as they assume their final shape. In addition to these visual observations, measurable comparisons made using quantitative image analysis is presented in the subsequent section. Quantifiable data (porosity, pore size, penetrated oil) were extracted from the micrographs using Image J software.

As the onset of fermentation, gas cells expand, and their stability is hinged on the viscoelastic gluten starch matrix. However, later in the fermentation process, liquid lamellae are formed from flour surface active components like lipids, polysaccharides and proteins. The liquid lamellae act as dual protection on each side of the cell wall to prevent rupturing of the gas cells (Mills *et al.*, 2003). In batter products with high moisture content, the gas cells become highly discontinuous, thus leading to the formation of large holes in the final product as seen in fried batter products in this study. However, Bran addition in batter formulation reduced large pores in *magwinya* crumb at 8 – 20% OB (Figure 20) and 15 – 20% WB (Figure 22). This is because soluble fibres have been reported to strengthen dough structure. In this case, β -glucans in OB a gel-like structure which contributed to the improvement of *magwinya* crumb. On the other hand, insoluble fibre in WB have been reported to lower gas cell stability through gluten reaggregation thus impacting the dough negatively (Bonnand-Ducasse *et al.*, 2010; Noort *et al.*, 2010). The large crumb pores in WB fried batter suggests that the negative effect is related to the increased surface area of fine WB which increases chemical interactions gluten. Components like phytates, glutathione and monomers of conjugated ferulic acid which binds to the cell wall of the insoluble fibre, thus altering the functionality of gluten network to stabilise the gas cells (Noort *et al.*, 2010).

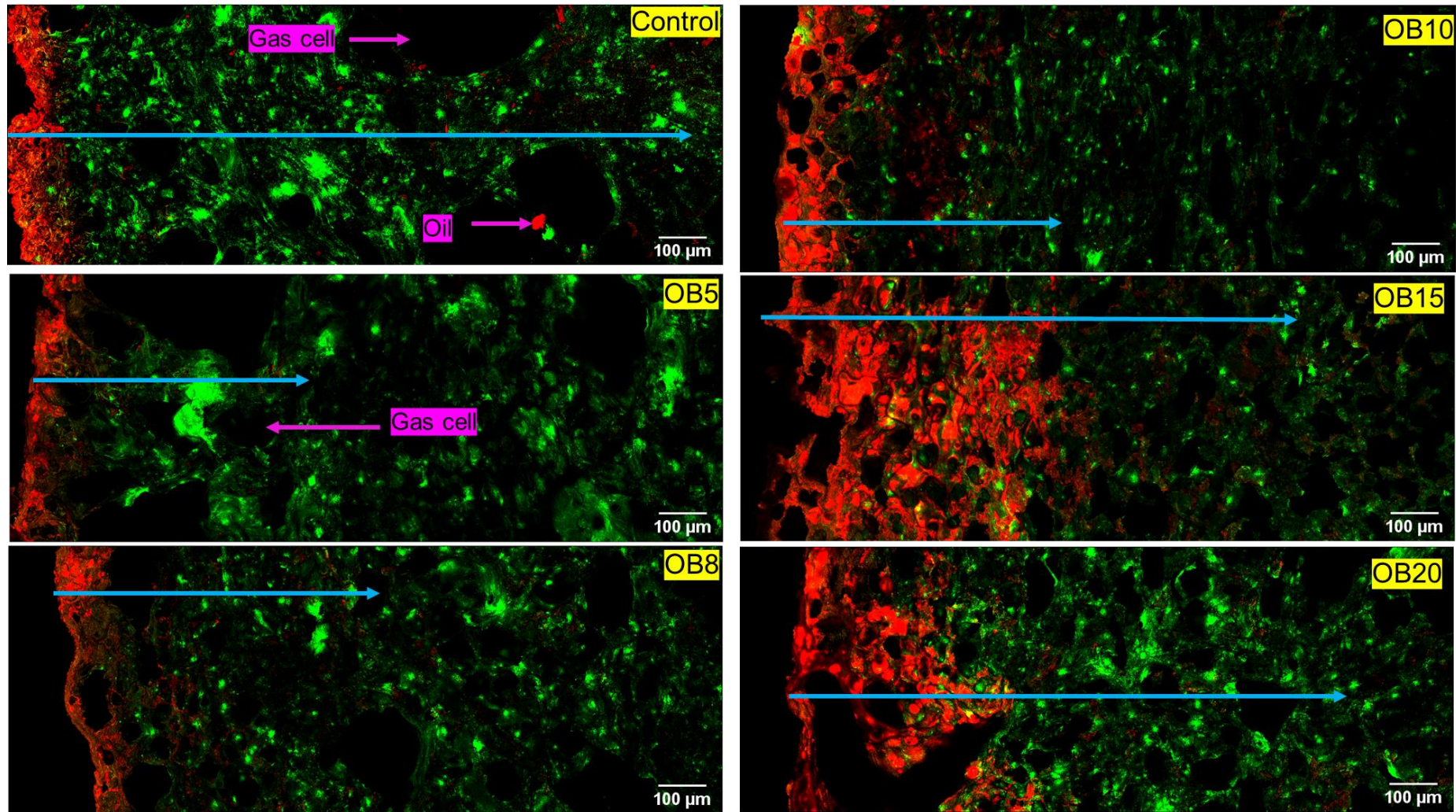


Figure 19. Cross-section micrographs of fried dough enriched with oat bran (OB). Red = oil, green = dough matrix. Blue arrows indicate the depth of oil penetration in the solid matrix

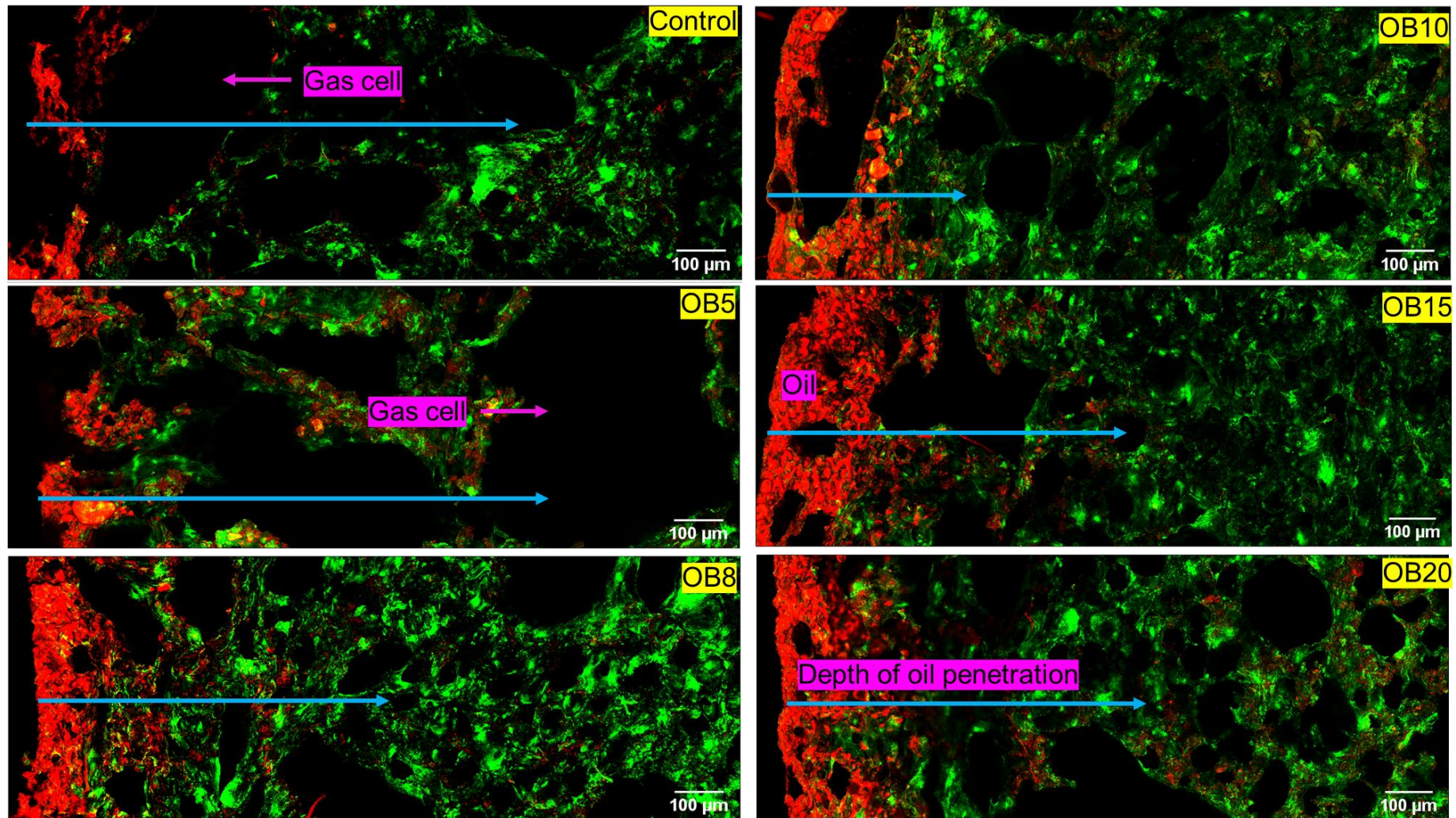


Figure 20. Cross-section micrographs of oat bran fried batter enriched with oat bran (OB). Red = oil, green = batter matrix. Blue arrows indicate the depth of oil penetration in the solid matrix

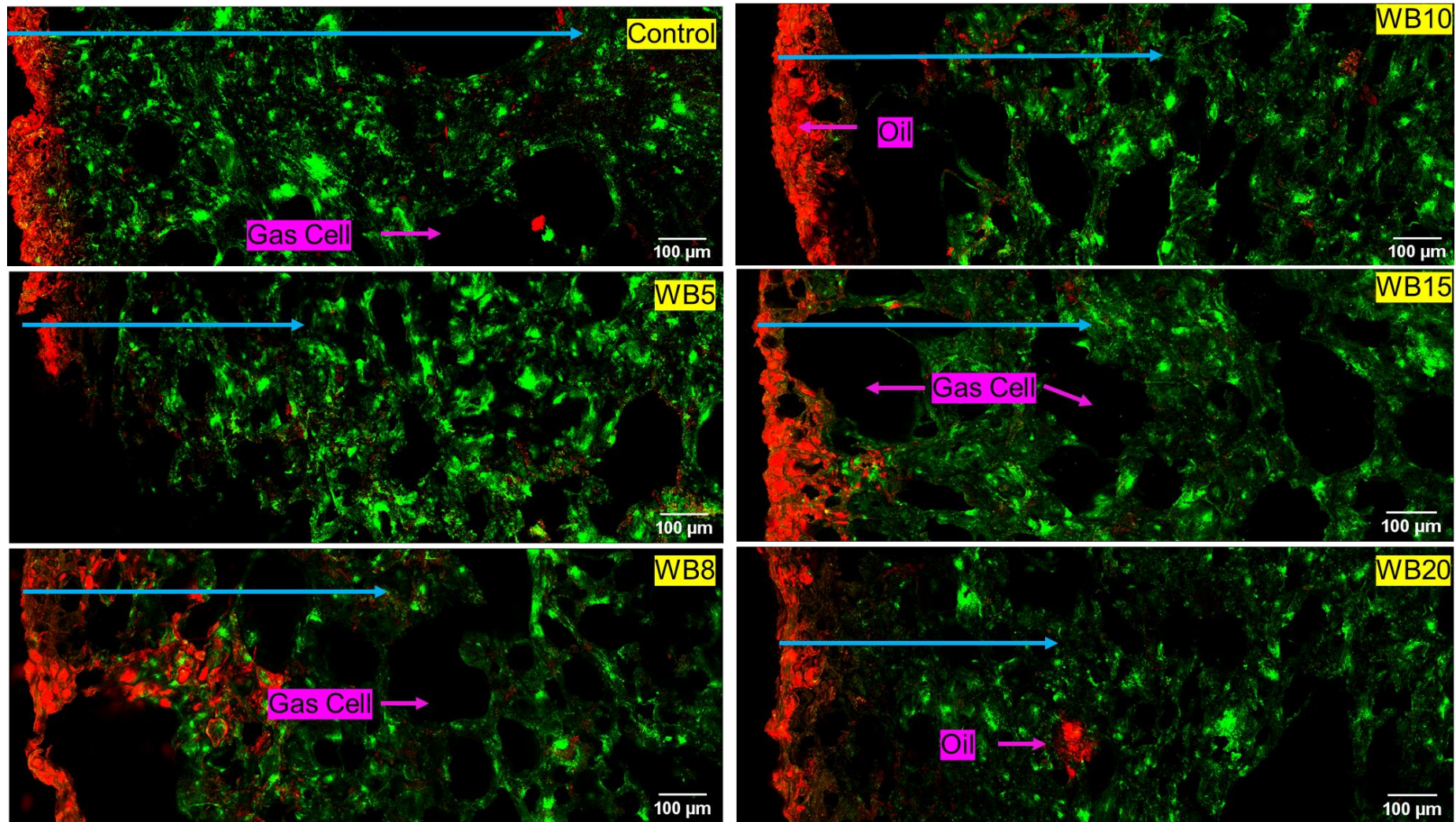


Figure 21. Cross-section micrographs of fried dough enriched with wheat bran (WB). Red = oil, green = dough matrix. Blue arrows indicate the depth of oil penetration in the solid matrix

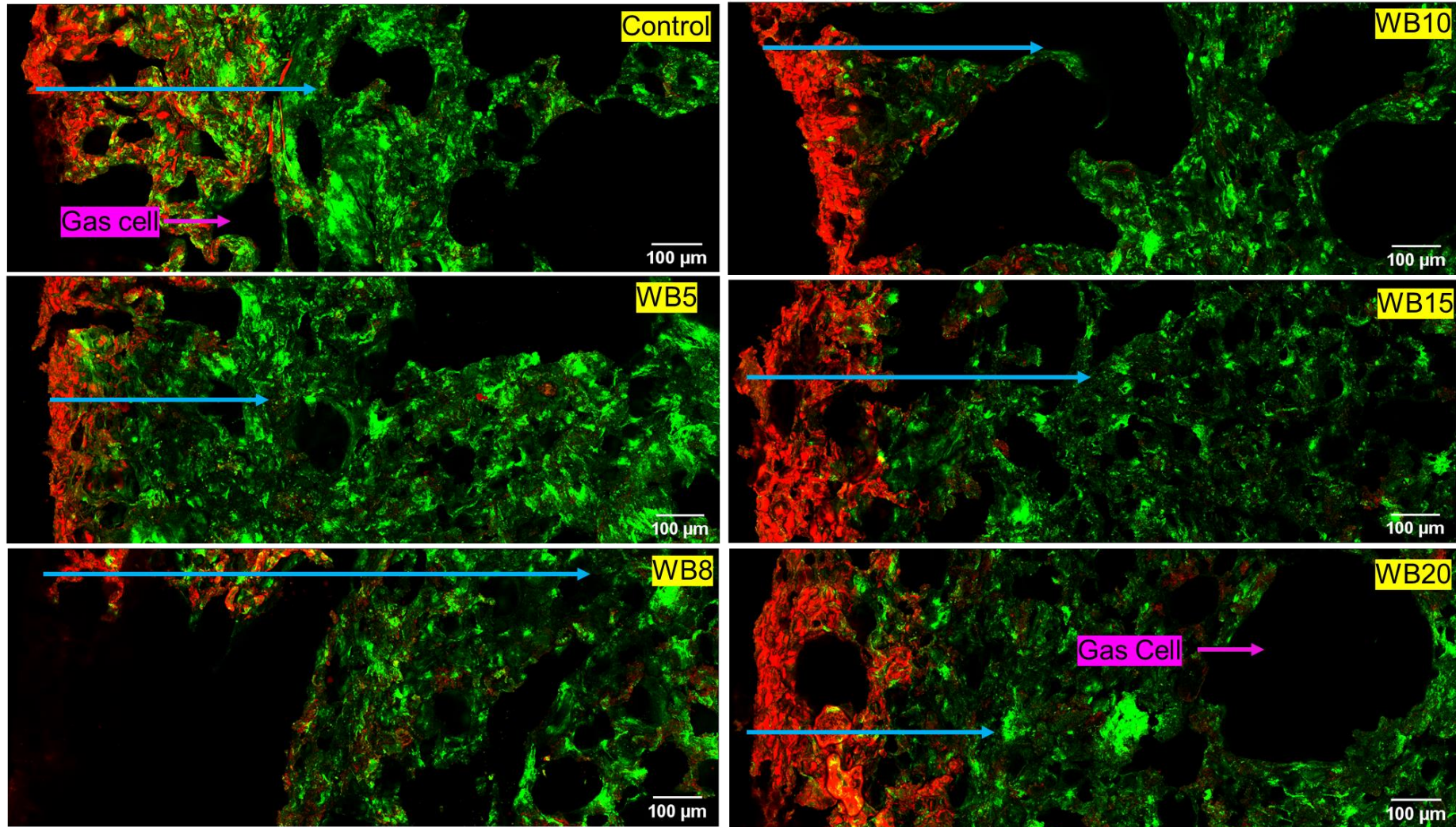


Figure 22. Cross-section micrographs of fried batter enriched with wheat bran (WB). Red = oil, green = batter matrix. Blue arrows indicate the depth of oil penetration in the solid matrix

5.3.2. Penetrated oil content by image analysis

Quantitative data were extracted from the micrographs as presented in Table 12 for porosity, penetrated oil by Soxhlet extraction (POsox) and image analysis (POia). Fried batter showed significantly higher POia values ($P < 0.05$) than fried dough for WB and OB *magwinya*.

5.3.2.1 Oat bran *magwinya*

The POia values for OB-enriched fried batter and fried dough were in the ranges 14.52 – 18.22% and 5.56 - 18.92%, respectively. The POia values for fried batter were significantly lower than the control (19.66%) except for OB15 (18.22%) while the opposite trend was observed for the fried dough. The POia was significantly higher ($P < 0.05$) than POsox for all samples except for control and OB5 fried dough, possibly due to the small amount of bran, thus showing no significance from control. Increase in bran concentration (control to 20 g) caused a decrease in POia of fried batter. In the fried batter, OB5 – 10 and OB20 samples were not significantly different ($P < 0.05$) from each other but were markedly lower than control and OB15. In OB fried dough, a reverse trend was observed. Similar trend was observed in POsox results (Table 12). POia values OB8 – 20 for fried dough samples were significantly higher than control.

5.3.2.2 Wheat bran *magwinya*

The POia of WB fried dough and batter ranged from 4.57 – 9.29% and 13.18 – 16.00%, respectively. On the one hand, results of oil content were comparable in WB fried dough for both image analysis (4.57 – 9.29%) and Soxhlet extraction (5 – 8%). Moreno and Bouchon (2013) reported a similar trend where oil content was ranked similarly by CLSM and Soxhlet in gluten fried matrices. However, in fried batter, POia was markedly ($P < 0.05$) higher than POsox. A significant reduction was noted in POia from WB0 – WB10 fried batter and increase beyond. This implies that WB reduced oil penetration in fried batter up till 10% substitution. In fried dough, oil uptake reduction was noticed at WB5 and WB10 – 20. While Soxhlet is a conventional, destructive and invasive method, image analysis is a non-destructive technique, where the samples can be imaged in whole or sections. However, the information collected was restricted to the image resolution which accounted for differences in results.

The effect of frying time and temperature on oil location in potato chips using CLSM was studied by Pedreschi and Aguilera (2000). Although only a visual observation was made, crucial information regarding the mechanical effect of cutting potato and its implication on oil uptake was reported. As previously stated, that oil absorption is predominantly a surface-associated phenomenon. Bouchon *et al.* (2003) demonstrated this phenomenon by observing a fried-potato slice under a CLSM, and it was noticed that oil absorbed post frying was in the

crust microstructure, as observed in this study. Moreno and Bouchon (2016) developed the double-staining procedure where dough matrix and oil were both stained before frying. A relationship was established between the microstructure of the products and oil absorption by comparing the values of fat content from soxhlet extraction and image analysis. A positive correlation was established between the results. Also, porosity, and pore size distribution were estimated from the confocal images and a directly proportional relationship was established between oil absorption and porosity of a gluten-based product; revealing that higher porosity, led to higher oil absorption. Fat distribution, pore sizes, surface topography and porosity of fried chicken nuggets were also estimated using CLSM. However, the samples were stained with Nile A and images were collected in the reflective and fluorescent mode for determination of surface topography and fat distribution in the chicken nuggets. There was a positive correlation between the results from CLSM image analysis and conventional Soxhlet fat extraction (Adedeji *et al.*, 2011).

Table 12. Porosity & penetrated oil (PO) content determined by image analysis (POia) and PO by soxhlet

Bran concentration (g)	Fried batter			Fried dough		
	(%)			(%)		
	POia	POSox	Porosity	POia	POSox	Porosity
Control	19.66 ^b ± 0.92	8.67 ^b ± 0.08	81.84 ^b ± 1.24	8.29 ^b ± 0.74	8.20 ^e ± 0.05	80.03 ^d ± 4.31
Oat bran 5	16.55 ^{ab} ± 0.47	9.47 ^c ± 0.08	67.10 ^a ± 2.62	5.56 ^a ± 0.34	6.67 ^{bc} ± 0.16	69.21 ^c ± 1.35
Oat bran 8	16.03 ^{ab} ± 1.21	8.80 ^b ± 0.14	73.66 ^{ab} ± 0.84	14.28 ^d ± 0.37	7.13 ^{cd} ± 0.11	54.94 ^a ± 1.53
Oat bran10	14.52 ^a ± 0.61	7.47 ^a ± 0.08	76.77 ^b ± 1.79	11.31 ^c ± 0.20	6.33 ^b ± 0.15	58.58 ^{ab} ± 1.41
Oat bran15	18.22 ^{ab} ± 0.60	8.60 ^b ± 0.30	80.68 ^b ± 1.05	18.92 ^e ± 0.48	5.27 ^a ± 0.06	65.93 ^{bc} ± 2.79
Oat bran20	14.97 ^a ± 1.63	7.87 ^a ± 0.11	65.91 ^a ± 1.68	11.73 ^c ± 0.42	7.53 ^d ± 0.08	60.76 ^{ab} ± 2.68
Control	19.66 ^c ± 0.74	8.53 ^b ± 0.46	81.84 ^c ± 1.24	8.29 ^{bc} ± 0.16	7.07 ^c ± 0.31	80.03 ^d ± 4.31
Wheat bran5	13.18 ^a ± 0.47	9.93 ^c ± 0.61	55.71 ^{ab} ± 3.92	4.57 ^a ± 0.22	8.00 ^d ± 0.26	52.15 ^a ± 0.59
Wheat bran8	13.81 ^{ab} ± 0.30	7.67 ^a ± 0.08	53.12 ^a ± 2.69	9.29 ^c ± 0.03	7.73 ^d ± 0.57	73.08 ^{cd} ± 1.22
Wheat bran10	16.00 ^b ± 0.53	9.87 ^c ± 0.09	70.56 ^{bc} ± 2.10	6.43 ^b ± 0.03	6.07 ^b ± 0.31	54.68 ^{ab} ± 2.69
Wheat bran15	13.52 ^a ± 0.31	10.13 ^c ± 0.31	75.69 ^c ± 2.57	7.58 ^{bc} ± 0.11	6.20 ^b ± 0.40	56.88 ^{ab} ± 2.33
Wheatbran20	15.31 ^b ± 0.30	8.73 ^b ± 0.98	76.05 ^c ± 2.23	7.94 ^{bc} ± 0.17	5.00 ^a ± 0.15	65.15 ^{bc} ± 2.66

Results are mean ± standard deviation (n = 4). Values in the same column with different alphabets for each bran type are significantly different from each other (P ≤ 0.05) using Tukey's HSD test. OB – oat bran and WB – wheat bran. 5 – 20 represent bran concentration in product formulation. Control – 0 g bran in formulation.

5.3.3 Porosity and pore distribution

Porosity is a measure of the void fractions in a material (Lawrence and Jiang, 2017). These voids can be closed or open, connected or disconnected as shown in Figure 23. Fried products were highly porous with values of OB fried dough and batter ranging from 54.94 to 80.03% and 65.91 to 81.84% respectively while WB fried dough and batter ranged from 52.15% to 80.03% (Table 12). Main effect of bran type had no significant effect ($P > 0.05$) on porosity while the linear effect of initial moisture content and bran concentration showed a significant effect ($P < 0.05$) on porosity. Besides, the interaction effect of all independent variables showed a significant effect ($P < 0.05$) on the porosity of the samples. Porosity reduced significantly in fried dough compared to fried batter.

The higher range of porosity in fried batter may be due to higher coalescence of gas cell sizes. These values fall within the range reported in the literature for similar foods such as bread (Wang *et al.*, 2011) and doughnut (Ghaitaranpour *et al.*, 2018b). However, the variations in these values and may be attributed to different image acquisition methods and scales used in the analysis. The use of X-ray microtomography by Wang *et al.* (2011) showed bread crumb porosity value (79 – 84%) in the range of values in this study. Use of microscopic image acquisition may account for this similarity. Meanwhile, the use of digital camera imaging in the study of Ghaitaranpour *et al.* (2018b) may have accounted for the lower porosity profile of deep-fried doughnut crumb in the range of 54 – 66%. Moreover, ingredients in their doughnut formulation like eggs, gluten, xanthan gum, milk powder, vegetable oil accounted for the difference in dough rheology which impacted aeration and stability cell sizes of the doughnut crumb.

The mechanism of pore development in *magwinya* follows water movement from the core to the evaporation zone at the crust followed by its dissipation from the product as vapour. However, remnant vapour left within the pores becomes superheated and expands distorting pore walls (Ziiaifar *et al.*, 2010) hence contributing to porosity development of *magwinya*. Regression analysis revealed that the linear effect of independent variables (bran type, initial moisture content and bran concentration) showed significant effect on PO_{ia} of fried products. Interaction effect of the independent variables showed significance ($P < 0.05$) on PO_{ia} (Table 13).

Table 13. Regression analysis showing the main and interaction effects of independent variables on porosity (%) and penetrated oil by image analysis

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig. ^c
Corrected Model	Porosity	29879 ^a	23	1299.09	20.04	< 0.001
	POia	4661 ^b	23	202.67	55.83	< 0.001
Intercept	Porosity	825377	1	825377	12732.53	< 0.001
	POia	27124	1	27124.72	7471.72	< 0.001
X	Porosity	88	1	88.43	1.36	0.244
	POia	1000	1	1000.28	275.54	< 0.001
Y	Porosity	6938	1	6938.65	107.04	< 0.001
	POia	1104	1	1104.91	304.36	< 0.001
Z	Porosity	13384	5	2676.86	41.29	< 0.001
	POia	715	5	143.03	39.4	< 0.001
X * Y	Porosity	2256	1	2256.54	34.81	< 0.001
	POia	1.56	1	1.56	0.43	0.513
X * Z	Porosity	1005	5	201.12	3.10	0.01
	POia	533	5	106.52	29.34	< 0.001
Y * Z	Porosity	2927	5	585.48	9.03	< 0.001
	POia	1047	5	209.32	57.66	< 0.001
X * Y * Z	Porosity	3278	5	655.62	10.11	< 0.001
	POia	261	5	52.05	14.34	< 0.001
Error	Porosity	10891	168	64.82		
	POia	610	168	3.63		
Total	Porosity	866147	192			
	POia	32396	192			
Corrected Total	Porosity	40769	191			
	POia	5271	191			

Df: Degree of freedom, X = bran type, Y = initial moisture content, Z = Bran concentration. POia – penetrated oil by image analysis (%). (a) $R^2 = .733$ (b) $R^2 = .884$ (c) Computed using alpha = .05

Similar to bread, pore development in *magwinya* is influenced by product ingredients and processing unit operations as follows: yeast which causes bubble formation in the dough; complexation of food molecules such as gluten-starch matrix which are hydrated to a network of pores fully developed during mixing, moulding and fermentation unit operations (Scalon and Zghal, 2001). Onset of gas cells begins during mixing of ingredient through aeration or incorporation of air into the dough matrix. As heat is applied during frying, the porous structure

becomes stabilised which causes modification of the molecular arrangement of the polymers in the cell wall.

5.3.4 Pore distribution in *magwinya* crumb

Three types of pores which may influence oil penetration have been identified (Ziaifar *et al.*, 2010) in fried products, and viz: (a) Interconnected pores – these are accessible from various points and greatly influence the flow of oil due to the continuous paths formed by the interconnection of the pores. (b) Non-connected pores are inaccessible and do not influence the flow of oil through the food matrix. (c) Isolated pores: which are accessible from just one direction and have limited influence on oil flow. Non-connected and inter-connected pores are open while blind pores are closed pores (Figure 23). These pores were identified in our products.

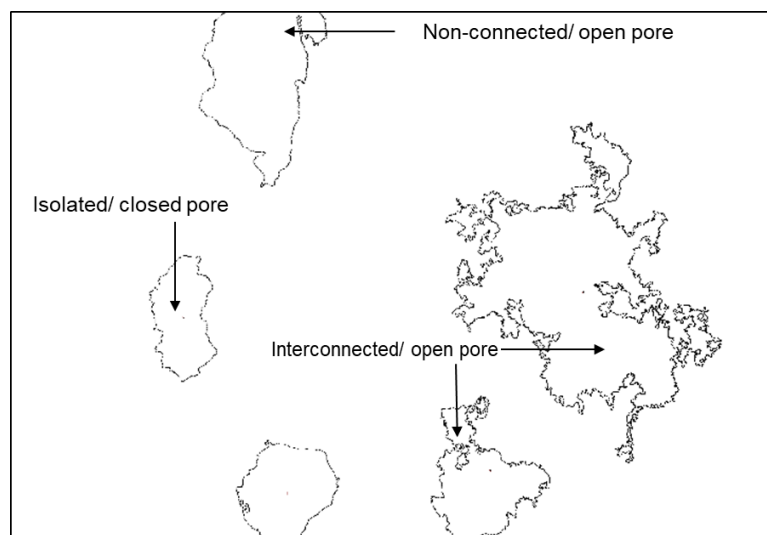


Figure 23. Types of pores identified in *magwinya*

Pore sizes of *magwinya* obtained from cross-section micrographs were found in a range of 0 – 475 μm (Figure 24). In fried dough samples, the control pore sizes between 0 – 25 μm had the highest frequency (80%), while in fried batter samples, control pore sizes of 0 – 25 μm peaked at 35% and 50% for OB and WB fried batter, respectively. For the rest of the samples at 5 – 20% bran addition pore sizes, 0 – 175 μm peaked at 52%, 40%, 60% & 47% in WB & OB fried dough, WB and OB fried batter, respectively, then dipped at > 125 μm size. Moreno and Bouchon (2013) reported a pore size range of 0 – 85 μm for gluten-based fried matrices. These differences in pore sizes could be accounted for by the following variations: (i) Sample weight and size variation – thick products (at least 50 g weight and > 50 mm diameter) in this study and their thin product (4 g weight, 2 mm thickness). (ii) Extent of sample dehydration/moisture loss which contributes to enlargement and shrinkage of pores. At a final

moisture content of 2% in the report of Moreno and Bouchon (2013), extreme shrinkage of pores associated could be explained by maximum moisture loss, total starch gelatinisation which led to alteration of gas cell size formed during mixing and kneading unit operations.

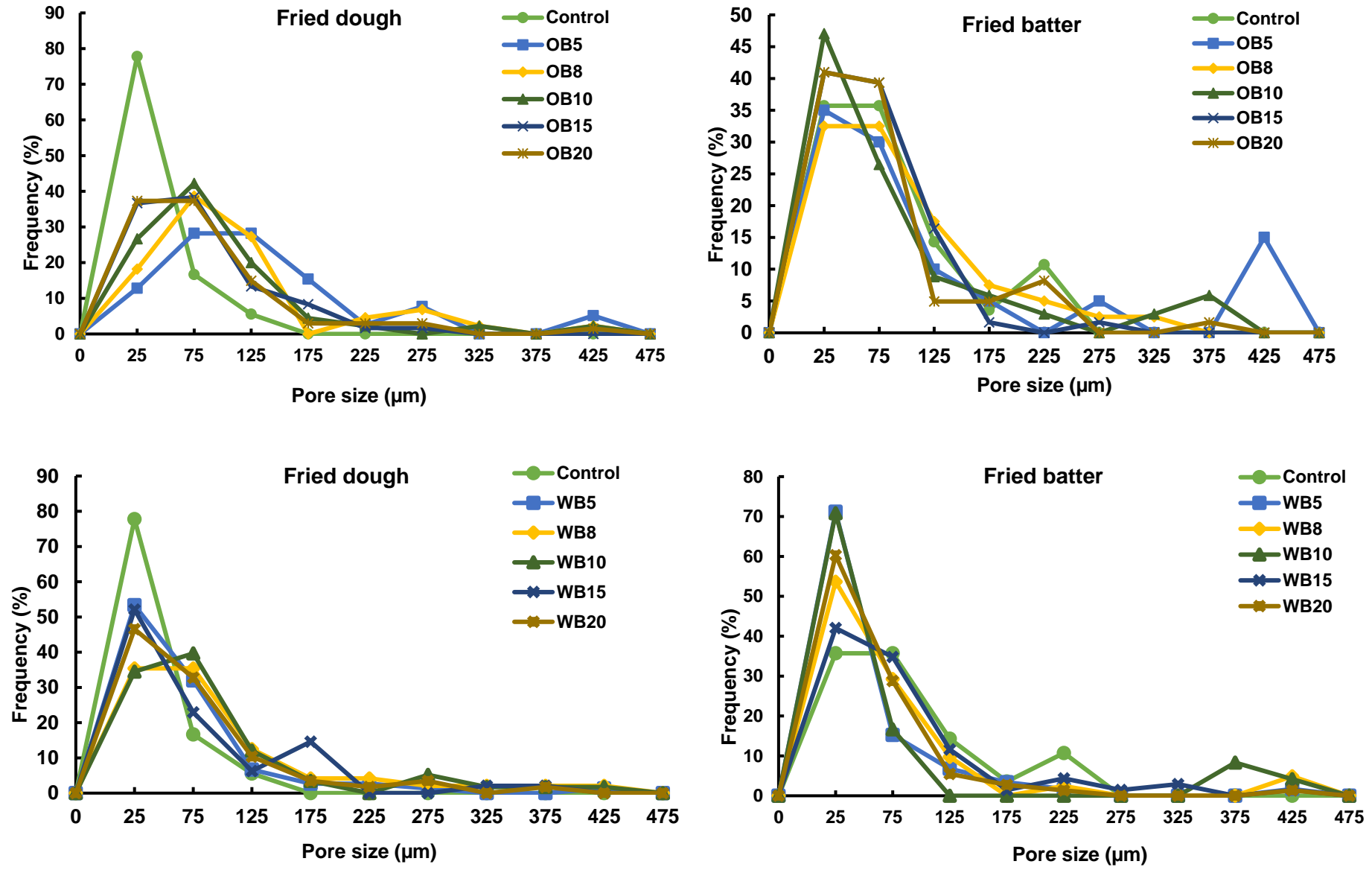


Figure 24. Pore size distribution in cross-section micrographs of fried dough and batter samples. OB & WB - oat and wheat bran; 5 - 20 represent concentration (g) of bran in sample formulation. Control – 0 g bran in formulation.

Magwinya consists of a solid medium of an interconnected web of pores, filled with either oil or air. Properties of empty pores of *magwinya* are presented in Table 14. After segmentation of the image using the wand tool in the HSB colour space, the number of pores in each image was estimated with the analyse particle function.

Table 14. Pore properties of *magwinya* samples as influenced by the addition of water and bran variation

Bran concentration	Fried dough					
	Particle count	Total area	Average size	Perimeter	Circularity	Solidity
	(ΣP)	($\times 10^4 \mu\text{m}^2$)	(μm^2)	(μm)	(C)	(S)
Control	18	12	728	150	0.29	0.73
OB5	44	27	6120	948	0.10	0.64
OB8	44	23	5216	1119	0.15	0.67
OB10	45	12	2746	696	0.13	0.65
OB15	60	15	2503	435	0.18	0.73
OB20	67	22	3303	551	0.15	0.69
WB5	76	23	3085	611	0.14	0.64
WB8	48	29	6036	566	0.19	0.77
WB10	59	28	4756	545	0.16	0.70
WB15	49	26	5237	389	0.24	0.75
WB20	59	22	3703	606	0.14	0.66
	Fried batter					
Control	28	25	3470	343	0.42	0.81
OB5	20	42	2102	861	0.15	0.72
OB8	40	17	4207	415	0.34	0.84
OB10	34	20	5846	508	0.19	0.77
OB15	62	17	2692	430	0.16	0.67
OB20	62	23	3690	495	0.18	0.72
WB5	59	17	2836	319	0.16	0.66
WB8	41	43	10288	564	0.20	0.74
WB10	24	38	15662	544	0.19	0.73
WB15	69	27	3890	558	0.14	0.66
WB20	73	25	3361	311	0.22	0.72

OB &WB - oat and wheat bran; 5 - 20 represent concentration (g) in sample formulation.

Control – 0 g bran in formulation.

Fried batter was characterised by large pores evidenced by the cell size (Table 14). Circularity is a shape descriptor defined as the ratio of the area of an object to the area of a circle with the same perimeter and it is also known as the compactness of an object/shape (Wirth, 2001; Olson, 2011). It describes how perfect to a circle an object is because a circle is a compact shape. A value of 1 describes a perfect circle, while zero (0) describes an elliptical/ irregular shape. A pore with a perfect circle with no connectedness to other pores is an isolated pore and will not aid oil flow; whereas irregularity of the pores may aid in oil flow. Solidity is another shape descriptor that measures the density of a particle which is derived from the ratio of the area to the convex hull area of a particle. A solidity value of 1 means the particle is a solid object and value less than 1 represent object with irregular boundary (Wirth, 2001).

5.3.4.1 Pore distribution in fried dough

The empty pores of *magwinya* were found in a broad range of sizes (0 – 475 μm), as gas cells in an uninterrupted foam-like structure. Particle counts of the empty pores of fried dough samples are shown in Table 14. Pore particle count increased with increase in bran concentration (Table 14). Bran incorporation facilitated even distribution of gas cells in the samples. Particle count in the fried dough was higher than fried batter which implies even distribution of pores in the former due to incorporation of air during kneading. Solidity values of 0.64 – 0.77 in fried dough samples falls into the category of a star-shaped particle described by Wirth (2001); depicting while circularity ranged from 0.13 to 0.39. The total area ($11.98 - 28.97 \times 10^4 \mu\text{m}^2$), the average size of the gas cells ($727.79 - 6120.47 \mu\text{m}^2$) and perimeter ($150.14 - 1119.44 \mu\text{m}$) are presented in a wide range of sizes in the samples (Table 14). Circularity of fried dough samples (0.10 – 0.29) were all less than 1 which denotes elliptical shape for *magwinya* pores.

5.3.4.2 Pore distribution in fried batter

Particle count of pores increased with bran increase in fried batter samples especially at 15 and 20% (Table 14). Circularity was highest in control at 0.42 meaning pores in the control sample were less elongated compared to the rest of the samples. Similarly, solidity was highest in sample OBB8 at 0.84 meaning the pores have boundaries close to a perfect solid object (circle). Solidity was higher in OB samples at 0.72 – 0.81 than WB samples (0.66 – 0.74). Other properties like total area ($17.69 - 42.03 \times 10^4 \mu\text{m}^2$), average size ($2691 - 21016 \mu\text{m}^2$), and perimeter ($310.75 - 860.90 \mu\text{m}$) were found in a wide range of sizes across WB and OB concentrations. Fried batter was characterised by large pores linked to rupture of the gas cell as a result of weak gluten network in the batter because stability against coalescence is maintained when the gluten film expands biaxially without rupturing (Sroan and MacRitchie, 2008). The extent of expansion of gas cells at the frying stage determines the final volume

and crumb structure of the fried products. Shearing/ disruption of the pore network could also occur as a result of mechanical stress induced during moulding of dough and cutting of the fried sample for imaging. These pores are an interconnected network which can be empty or filled with oil.

Unlike bread crumb made up of two phases (air and cell wall), *magwinya* crumb is made up of three phases – a fluid (oil), a solid (cell wall material) and gas (air). Aeration of the *magwinya* dough/batter matrix may occur through one or both of two ways: (i) physical aeration as a result of air entrapment in the dough during mixing of the batter and kneading of the dough and (ii) biological aeration of the gas cells due to the action of baker's yeast *Saccharomyces cerevisiae* which releases CO₂ in the dough and cause almost ten-fold increase of the air incorporated at the mixing stage (Verheyen, 2016). Air is entrapped into the porous structure during mixing (Shimiya and Nakamura, 1997). The nuclei for gas cells found in bread crumb is majorly formed at this stage is reliant on mixer type used (Scalon and Zghal, 2001). The air entrapped during mixing forms the basis of the cell size in the crumb, especially kneading which promotes an increase in gas volume fraction. Also, an increase in gas cell size as fermentation commenced in bread dough has been reported (Shimiya and Nakamura, 1997; van Duynhoven *et al.*, 2003). Considering this and the type of mixing in this study – manual mixing (use of spatula) for the batter and mechanised mixing (dough mixer) for dough; air incorporation and occlusion differed in both dough types and the final crumb structure of *magwinya*.

Homogeneity of foam structure during dough development is influenced by gravitational force and pressure difference exerted by CO₂ during gas cell expansion at various points in the dough mass. Where gravitational force is negligible or insignificant, gas cell sizes attain homogeneity, but when gravity is significant, distribution of gas cells at the top, centre and bottom will differ from each other (Sroan *et al.*, 2009). During proofing, as the gas cells expand under CO₂ gas released by leavening agent, coalescence and liquid drainage/ separation from dough may occur. However, stability and coalescence of gas cells in crumb is set during thermal treatment (baking, frying or steaming). As dehydration occurs during frying, a liquid-to-solid phase conversion, gluten protein reaggregates conferring a rigid structure to the gas cells; thus, terminating coalescence (Turbin-Orger *et al.*, 2012).

5.3.5 Relationship between microstructural properties against Soxhlet fat extraction

To categorise the relationship between microstructure and oil absorption, PO_{sox} data was plotted against PO_{ia} and porosity. The data presented non-linear relationships and were thus fitted to a cubic polynomial model as established in the regression equations (Table 15).

Furthermore, the effectiveness of the image analysis technique for oil absorption measurement was tested by plotting PO_{ia} values against and against PO_{sox} results. As established in Table 12, fried batter products were more porous, hence retained more oil than fried dough.

5.3.5.1 Oat bran fried batter

The strongest relationship was found in PO_{sox} vs PO_{ia} where $R^2 = 0.9051$ (Table 15) – also corroborating a strong positive Pearson's correlation found among both parameters ($P < 0.001$). Porosity vs PO_{sox} ($R^2 = 0.7298$) follows and could be as a result of differences in conversion parameters at the macroscopic and microscopic level of analysis while Porosity vs PO_{ia} ($R^2 = 0.7252$) showed the weakest relationship which could be attributed to product type and variation in oil penetration of fried batter samples.

5.3.5.2 Oat bran fried dough

The relationship between PO_{ia}, PO_{sox} and porosity of OB fried dough is shown in Table 15. The data was fitted to a 3rd order (cubic) polynomial regression. The strongest relationship was found in porosity vs PO_{ia} ($R^2 = 0.8432$) attributable to the similarity of scale used in image analysis, hence the higher fitness of data. Porosity vs PO_{sox} had $R^2 = 0.7243$ and weakest was PO_{ia} vs PO_{sox} ($R^2 = 0.7011$). Moreno and Bouchon (2013) reported $R^2 = 0.8372$ for a linear relationship between PO_{ia} and PO_{sox} in gluten-based fried matrix.

5.3.5.3 Wheat bran fried dough

Although both methods ranked oil content of WB fried dough in similar ranges, the relationship between PO_{ia} and PO_{sox} in WBFD products was the weakest ($R^2 = 0.7283$). This weakness may be accounted for by the difference in micron-range scale in image analysis compared to macro-scale factors in soxhlet extraction. This contradicts the report of Moreno and Bouchon (2013) where PO_{ia} and PO_{sox} ranked gluten-based fried product in the same manner with a strong linear relationship. Porosity and PO_{sox} had the strongest regression coefficient ($R^2 = 0.9885$). Whereas porosity vs PO_{ia} had $R^2 = 0.7555$ (Table 15)

5.3.5.4 Wheat bran fried batter

In WB fried batter, the strongest to the weakest relationship was PO_{ia} and porosity ($R^2 = 0.9521$), PO_{ia} and PO_{sox} ($R^2 = 0.8420$) and PO_{sox} and porosity ($R^2 = 0.7077$) as shown in Table 15. Pearson's correlation showed that an increase in porosity led to an increase in oil penetration ($P < 0.01$). It is worthy to note that the relationships of fried batter ranked higher than fried dough and this may be because PO_{ia} of fried batter ranked higher than Soxhlet data for the samples.

Table 15. Cubic polynomial regression equation for plots of image properties and soxhlet extraction

Fried dough	Porosity vs POia	POia vs POsoxhlet	POsoxhlet vs Porosity
Oat bran	$y = 0.0976x^3 - 3.3741x^2 + 34.041x - 30.943$	$y = -1.4146x^3 + 32.453x^2 - 242.33x + 601.78$	$y = 5.757x^3 - 110.56x^2 + 698.29x - 1386.4$
R ²	0.84	0.70	0.72
Wheat bran	$y = -1.1344x^3 + 24.837x^2 - 169.76x + 417.88$	$y = 0.0276x^3 - 0.2017x^2 - 1.3834x + 15.904$	$y = -16.047x^3 + 309.49x^2 - 1957.3x + 4120.2$
R ²	0.76	0.73	0.99
<hr/>			
Fried batter			
Oat bran	$y = -0.6708x^3 + 35.179x^2 - 608.8x + 3551$	$y = 0.0603x^3 - 3.2654x^2 + 58.596x - 339.77$	$y = -22.873x^3 + 577.36x^2 - 4839.6x + 13544$
R ²	0.73	0.91	0.71
Wheat bran	$y = 0.0001x^3 + 0.0038x^2 - 2.3198x + 105.18$	$y = 6.1133x^3 - 164.02x^2 + 1457.5x - 4274.8$	$y = 0.0009x^3 - 0.2033x^2 + 14.426x - 325.69$
R ²	0.95	0.84	0.71

POia & POsoxhlet - penetrated oil by image analysis and Soxhlet extraction. OB &WB - oat and wheat bran.

5.3.6 Crust surface roughness of *magwinya*

Crust micrographs and the grey level intensity maps of the crust micrographs of WB fried batter and fried dough is presented in Figure 25, while that of OB fried batter and dough is shown in Figure 26. The crust micrographs of fried products showed slight variation in terms of intensity. Fried dough had lower intensity than fried batter. Structurally, the geometric

appearance of fried batter showed closely knit cells while that of fried dough was slightly larger, accounting for the lower intensity in the former. Grey-level intensity maps of a product can reveal the nature of its surface. The surface plot maps were all jagged and closely related, and have a similar range of pixel intensity variation except for a few differences seen in WBFB8 and WBFD8. The similarities may also be attributed to the close range of FD values in this study were within a narrow range which could be indicative of similarity in product formulation with minimal difference. In a comparison of the surface roughness of pumpkin and chocolate, Quevedo *et al.* (2002) observed that pumpkin had a more jagged surface intensity than pumpkin than chocolate. Rahimi and Ngadi (2016) also reported strong similarities in the intensity of the surface plots of fried batters made from wheat and rice flour. Use of fractal analysis has been applied to in quantifying the surface roughness of fried potato, chocolate, pumpkin (van Duynhoven *et al.*, 2003), fried batter (Thanatuksorn *et al.*, 2005; Rahimi and Ngadi, 2016), bread (Pedreschi *et al.*, 2000).

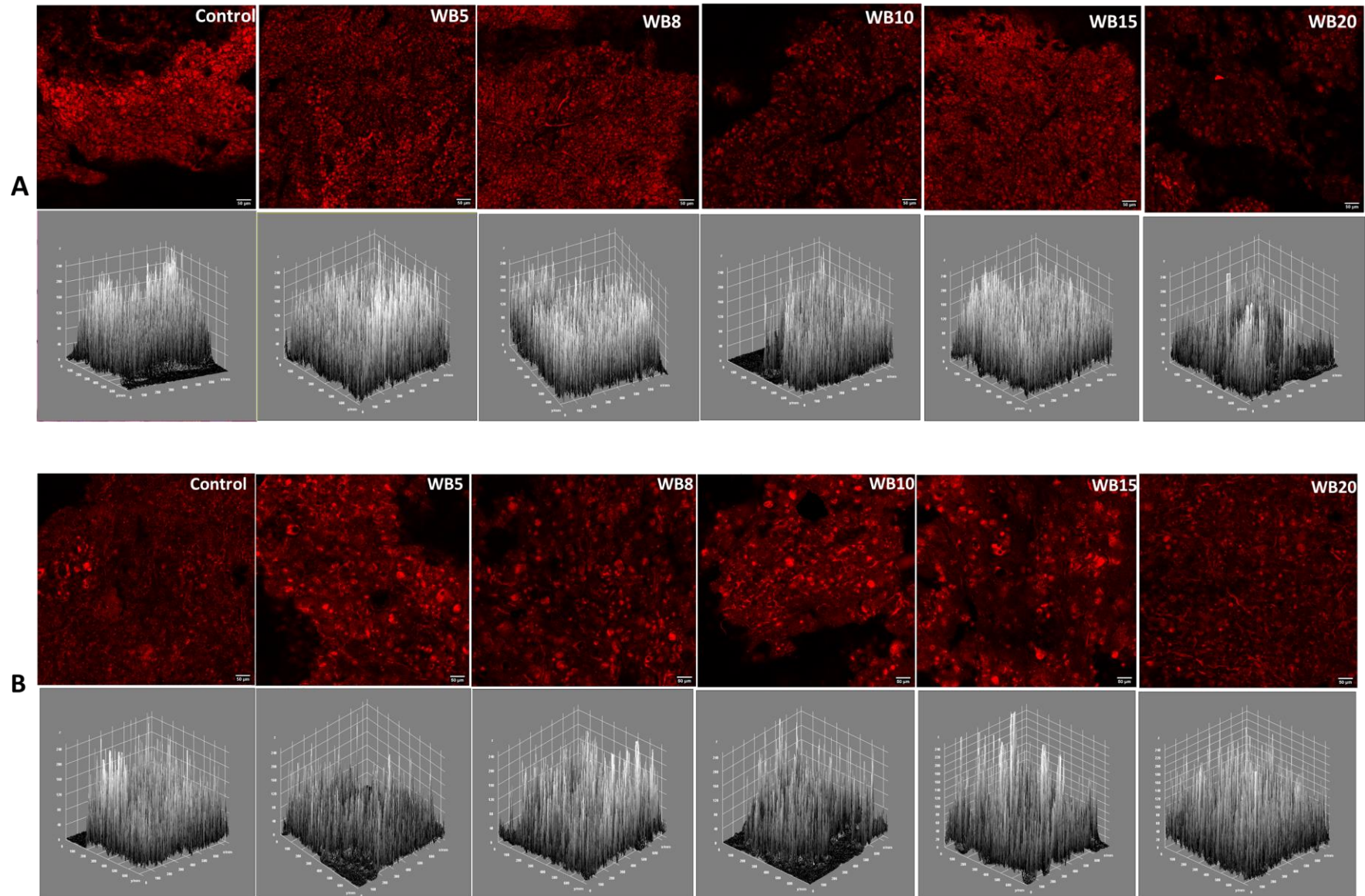


Figure 25. Crust confocal micrographs of wheat bran fried batter (A) and fried dough (B) and their respective grey level intensity plots (bottom).
 WB - wheat bran; 5 - 20 represent the concentration of bran in sample formulation. Control – 0 g bran in formulation.

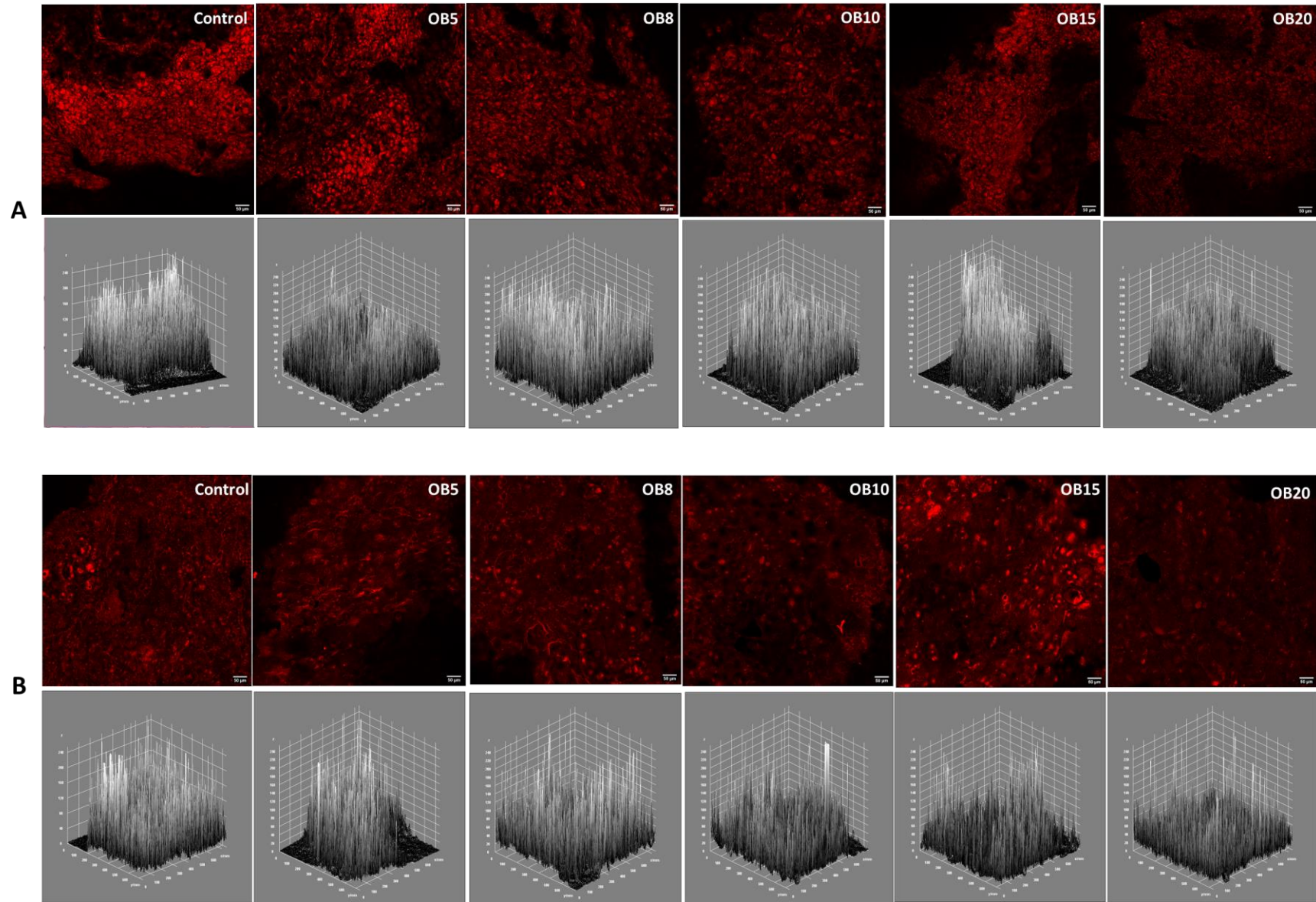


Figure 26. Crust confocal micrographs of oat bran fried batter(A) and fried dough (B) and their respective grey level intensity plots (bottom). OB - oat bran; 5 - 20 represent the concentration of bran in sample formulation. Control – 0 g bran in formulation.

5.3.7 Surface roughness using fractal metrics

Fractal dimension is ranked on a value scale of 1 – 3 depending on the extent of deviation from regularity or as occupied in a Euclidean space (Cáez Ramírez *et al.*, 2017). Fractal dimension (FD) of fried dough and fried batter ranged from 2.56 to 2.76 and 2.55 to 2.67 across WB and OB samples respectively (Table 16). These values were in a similar range to the report of Rahimi and Ngadi (2016). In WB samples, FD of fried batter was higher than that of fried dough although there was no statistical significance indicating the former had higher surface roughness than the latter. It appears WB had a similar effect on surface roughness of the two types of products. However, in OB samples, fried dough had higher FD ($P < 0.05$) indicative of rougher surface compared to the fried batter. Thanatuksorn *et al.* (2005) reported an increase in fractal dimension of fried wheat dough model with an increase in the initial moisture content of fried batter. Compared to their study, a reverse trend was observed in the control sample where the fried dough had a rougher surface than fried batter. Moreover, Thanatuksorn *et al.* (2005) only determined surface edge contour of the samples whereas, the entire crust surface was taken into consideration in this study and not the contours, hence the possible difference in results.

Table 16. Fractal dimension (FD) values of fried products

Bran concentration (g)	Fried dough	Fried batter	Significance
Control	2.69 ^b ± 0.13	2.58 ^{ab} ± 0.04	0.02
Oat bran 5	2.73 ^b ± 0.05	2.55 ^a ± 0.04	0.001
Oat bran8	2.74 ^b ± 0.02	2.62 ^{bc} ± 0.05	0.01
Oat bran10	2.76 ^b ± 0.08	2.61 ^{abc} ± 0.05	0.01
Oat bran15	2.57 ^a ± 0.08	2.58 ^{ab} ± 0.03	0.95
Oat bran20	2.73 ^b ± 0.04	2.66 ^c ± 0.04	0.05
Control	2.69 ^c ± 0.13	2.58 ^a ± 0.04	0.02
Wheat bran 5	2.67 ^{bc} ± 0.03	2.64 ^a ± 0.09	0.53
Wheat bran8	2.57 ^{ab} ± 0.09	2.64 ^a ± 0.06	0.30
Wheat bran10	2.66 ^{abc} ± 0.02	2.65 ^a ± 0.07	0.95
Wheat bran15	2.56 ^a ± 0.07	2.67 ^a ± 0.09	0.12
Wheat bran20	2.63 ^{abc} ± 0.06	2.64 ^a ± 0.05	0.60

Mean values with the same superscripts down the column for each bran type show significance ($P < 0.05$) using Duncan's multiple range test. P values in the third column show a significant difference between fried batter and dough using T-test. OB &WB - oat and wheat bran; 5 - 20 represent concentration (g) of bran in sample formulation. Control – 0 g bran in formulation.

Measurement of surface roughness is crucial because it displays the irregularities of the crust which may negatively impact oil absorption and consumer acceptance. Ghaitaranpour *et al.* (2018a) reported maximum FD value of 1.97 for deep-fried doughnut. This value was lower than the results in this study because of the extra 1 dimension added to FD values in this study. We hypothesized that surface roughness of fried batter will supersede that of fried dough due to higher moisture loss in fried batter. The hypothesis can, however, be rejected based on the results obtained and sample type and shape – *magwinya* being a thick and round fried food had its development of surface texture at different rates due to the nature of the shape. The effect WB and OB had on surface roughness differed based on the initial moisture content, chemical composition of the fibres, the difference in solubility of the bran which imparted surface texture during frying. Besides, higher surface roughness of fried dough could be attributed to the drier surface as a result of lower initial moisture content.

Multivariate regression analysis showed that the linear effect of bran concentration had a significant effect ($P < 0.001$) on FD while bran type had no significant effect ($P = 0.104$). Main effect of initial moisture content and bran type were significant ($P < 0.05$) on fractal dimension values of the products. Interaction effect of bran type and initial moisture content ($P < 0.001$), bran type and bran concentration ($P = 0.03$) significantly affected FD. The interaction effect of the three independent variables (bran type * bran concentration * initial moisture content) all had no significant effect ($P = 0.243$) on FD. (Appendix VIII)

5.3.8 Correlation of fractal dimension to surface oil and texture of fried products

Scatterplots revealed that the relationships between FD and crust properties (surface oil and hardness) are non-linear, regression models using a curve estimation function was adopted to observe and quantify the model. Some studies have shown increased surface roughness had an impact on oil uptake through a positive linear correlation between surface oil and fractal dimension (Thanatuksorn *et al.*, 2005; Rahimi and Ngadi, 2016). In this study, the data between FD and crust features were fitted to a polynomial cubic model as shown by the regression equations and coefficient of determination (R^2) in Table 17.

5.3.8.1 Fractal dimension versus surface oil

Surface oil was plotted against FD values for each product type and the cubic polynomial model with the R^2 values in the following order in Table 17: fried dough (WB: 0.98, OB: 0.97) and fried batter (WB: 0.96, OB: 0.88). Fried dough samples ranked higher in terms of fitness of the data. Surface oil of fried dough were higher than fried batter and this could be responsible for the higher R^2 values in the former.

Table 17. Cubic polynomial regression equation for plots of fractal dimension versus surface oil and crust hardness

Sample	Cubic polynomial regression model equations	R ²	Sig
WB fried dough	A = 67.39B ³ - 51.454B ² + 12.752B + 1.6384	0.9822	0.030
	Y = -3E+06X ³ + 2E+07X ² - 5E+07X + 4E+07	0.5615	0.388
WB fried batter	A = 653.52B ³ - 335.94B ² + 54.869B - 0.2328	0.9568	0.031
	Y = -3E-10X ³ + 1E-06X ² - 0.0008X + 2.5568	0.8451	0.043
OB fried dough	A = 7.3707B ³ - 14.254B ² + 5.9505B + 2.0324	0.9736	0.05
	Y = 1E-09X ³ - 7E-06X ² + 0.0155X - 8.7206	0.8072	0.01
OB fried batter	A = -291.74B ³ + 158.88B ² - 28.375B + 4.2429	0.8771	0.032
	Y = 465122X ³ - 4E+06X ² + 9E+06X - 8E+06	0.9853	0.001

Where A & Y = fractal dimension, B = surface oil, X = crust hardness. Sig – significance at P < 0.05. OB & WB - oat and wheat bran

5.3.8.2 Fractal dimension versus crust hardness

Fractal dimension values were also plotted against crust hardness and the relationship also fitted a cubic polynomial model with R² values thus: fried dough (WB: 0.56, OBM: 0.81) and fried batter (WB: 0.85, & OB: 0.99) as shown in Table 17. Significant correlation was only noticed in fried batter samples compared to fried dough. Fried batter ranked higher than fried dough in terms of surface roughness and crust hardness. Factors like frying time, temperature, product composition and initial moisture content, affect crust formation which directly impacts hardness of the food (van Koerten *et al.*, 2015). Fried batter was softer than fried dough because of the higher initial moisture content.

5.4. Conclusion

This chapter presents the first study on quantitative analysis of *magwinya* crumb and crust properties as determined from confocal micrographs. The use of distinct fluorescent dyes in sample preparation proved adequate for characterisation of oil penetration and structural changes in the samples using image analysis technique; which revealed important knowledge on the relationship between oil uptake and microstructure of *magwinya*. Furthermore, the results obtained from image analysis were correlated to results from conventional soxhlet extraction and the former could be used as an alternative analysis choice for future studies.

Cross-section micrographs of *magwinya* revealed notable differences in terms of oil distribution, depth of oil penetration, structure and pore properties of the fried products, thus emphasising the impact of ingredient formulation (water and bran variation) on oil penetration. Incorporation of oat and wheat bran in *magwinya* formulation reduced porosity and oil penetration. Fried batter was more porous than fried dough owing to the higher initial moisture content of the former which led to increased evaporation of water causing disproportionate heterogeneous pore sizes consisting mainly of larger ones. In comparison to the control sample, a reduction in porosity was observed for all samples. Cubic polynomial relationship was established between PO_{ia} , porosity and PO_{sox} for *magwinya* crumb, as well as between FD and crust hardness and surface oil for the crust. Penetration of oil into the crumb reduced and varied among the samples with reduction of initial moisture content and increase in bran concentration ($P < 0.05$), although minimal effect was observed for bran type. Utilisation of the double fluorescent staining protocol and multichannel CSLM observation was effective for observation and quantitative analysis of *magwinya* crumb and crust microstructure. Considering the information provided in this, there exist rich prospects for future studies and improvements to the work done.

CHAPTER 6: *IN VITRO* STARCH DIGESTIBILITY OF *MAGWINYA* ENRICHED WITH OAT AND WHEAT BRAN

Abstract

Digestibility of a starchy meal depends on processing conditions that alter its microstructure and physical properties. One important change that occurs during frying is starch gelatinisation which is affected by fibre and moisture levels of the food during processing, and these have a direct impact on the digestion of starchy foods. In this study, Bran concentration and moisture levels were varied for determination of rapidly, slowly and unavailable glucose, digestible and resistant starch (RS) contents and estimated glycemic index (eGI) of *magwinya*. Rapidly available glucose (RAG) of control fried dough (60.31 g/ 100 g) product was 33% less than fried batter (90.07 g/ 100 g) and more unavailable glucose (UG) less than fried batter. Oat (OB) and wheat bran (WB) significantly reduced RAG and increased UG of fried products. Oat bran showed about 9% more reduction than wheat bran. A similar trend was observed for rapidly digestible starch and resistant starch of fried products. The eGI of products indicated control fried batter to be a high GI food (eGI = 80.02) and control fried dough to be medium GI food (eGI = 58.11). WB fried dough, fried batter and OB fried dough can be categorised as medium GI foods at eGI of 56.46 – 58.39, 65.93 – 68.84 & 56.34 – 57.27 respectively; while OB fried batter at 73.57 – 80.03 as high GI foods. RS showed negative significant correlation with eGI ($r = -0.866, -0.932, P < 0.01$) and fat content ($r = -0.618, -0.671, P < 0.01$) for OB and WB fried products. These results reveal that ingredient modification and processing is effective for regulation of starch digestion and related eGI of deep-fried dough/ batter foods.

Keywords: *In vitro* assay, eGI, bran, glucose, starch, fried dough

6.1 Introduction

Starch is the major carbohydrates found in human food sources such as maize, wheat, potato and rice (Contardo *et al.*, 2016). However, its excessive consumption is of health concern, as it is a predisposing factor to other metabolic-related diseases like obesity, cardiovascular and diabetes – a projected leading cause of death by the year 2030 according to the World Health Organisation (Gourineni *et al.*, 2017; Torres *et al.*, 2019). Moreover, the ability to predict and regulate postprandial glucose absorption of starchy food is vital to the global epidemic called diabetes (Parada & Santos, 2016; Kumar *et al.*, 2018). Starch digestion (SD) is characterized by the rate and the duration of postprandial glycemic response. Starch can be undigested, rapidly or slowly digested. Starch granule characteristics, state, size, processing methods and presence of other ingredients all influence SD (Singh *et al.*, 2010).

Understanding the relationship between the physicochemical and physiological properties of food is a vital way to explain the value of carbohydrate in human nutrition. The glycaemic glucose fraction of a food, excluding lactose, is a summation of glucose in its glycaemic carbohydrate fraction (Englyst *et al.*, 2003). Glucose content of a carbohydrate-rich food is categorised as either rapidly available glucose (RAG) and slowly available glucose (SAG), to signify its potential rate of release and absorption or non-absorption for unavailable glucose (UG). The rate of postprandial glucose release can be described by its *in vitro* RAG and SAG values which are the main determinants of glycemic index of cereal-based foods (Singh *et al.*, 2010). Glycemic index (GI) helps classify carbohydrate foods based on how they influence postprandial plasma glucose response (Borczak *et al.*, 2018). GI is expressed as the percentage increase of the glucose area under the curve of a test food against a standard food such as glucose or white bread (Englyst *et al.*, 2003; Wolever *et al.*, 2003). GI is usually measured *in vivo*, however, because of the time, resources it requires, *in vitro* methods have been adopted over the years as a relevant nutromic analysis tool to measure the rate of hydrolysis and estimate a glycemic index of foods (Wolever *et al.*, 2003; Odenigbo *et al.*, 2012).

Previous works present persuasive evidence that dietary fibres noticeably reduced GI of foods, although the magnitude of GI reduction is dependent on the food type and processing involved (Brennan, 2005; Taye *et al.*, 2016). Due to the resistance of dietary fibres to amylolytic enzymes in the small intestine, no digestion/ absorption occurs. The fibres are thus moved to the colon where they transform to short-chain fatty acids via fermentation. These processes result in physiological benefits such as blood glucose regulation, laxative and prebiotic properties (Brennan, 2005; Onipe *et al.*, 2015).

Magwinya is made from refined wheat flour and may not be the ideal food for individuals with diabetes. However, its glycemic index has not been investigated or reported. Wheat and oat bran are fibre-rich food additives – which when incorporated into *magwinya* formulation may reduce its GI. *Magwinya* can be categorised as a high-carb high-fat food because its major ingredient – refined wheat flour contains about 70 g/100 g carbohydrates (Kumar *et al.*, 2011; Kim *et al.*, 2012). However, its starch digestion has not been studied. Therefore, the objectives of this chapter are:

- (i) To quantify available glucose and digestible starch fractions of *magwinya* via *in vitro* assay;
- (ii) To assess the effect of bran concentration and initial moisture content on available glucose and digestible starch content of *magwinya*; and
- (iii) To calculate the estimated glycemic index of *magwinya*

6.2. Materials and methods

6.2.1. Materials

Wheat flour (carbohydrates – 71%, protein – 10.2%, moisture – 12%, fibre – 3.7%, fat – 0.9%) wheat bran (carbohydrates – 22%, protein – 15.5%, fat -4.5%, fibre – 42.5% , moisture -), oat bran (fibre – 20%, protein – 13%, carbohydrate – 50%, fat – 5.5%), sugar, salt, yeast, sunflower oil were used for production of fried products (Section 3.2.2). Guar gum (G4129), amyloglucosidase (A7095), pepsin (P7000), invertase (I4504) and pancreatin (P7545), sulfuric acid (25810-5), glucose oxidase peroxidase kit (GAGO 20) for *in-vitro* digestion assays were sourced from Sigma–Aldrich, St Louis, MO, USA. Potassium hydroxide, acetic acid, hydrochloric acid and other chemicals used were of analytical grade. **Amyloglucosidase A7095** (also known as glucoamylase) from *Aspergillus niger* is capable of hydrolysing the α -D-(1-4), the α -D-(1-6), and the α -D-(1-3) glucosidic bonds of oligosaccharides. Amyloglucosidase is an extracellular enzyme that converts starch to dextrans and glucose. The enzyme is used in the starch-processing industry for the commercial production of D-glucose from corn syrups. Glucoamylase (EC 3.2.1.3) 10,000 U/ml, from *Aspergillus niger* (1U releases 1 μ mol glucose/min from starch, at 55°C, pH 4.5). **Pancreatin P7545** contains trypsin, amylase and lipase produced from exocrine cells of the porcine pancreas. Pancreatin hydrolyses proteins, starch and fats. This combination of enzymes allows it to hydrolyse proteins, starch and fats. **Invertase I4505** Invertase hydrolyses sucrose into glucose and fructose yielding a colourless product.

6.2.2. Sample preparation

The method of Kwindu *et al.* (2018) was used for the production of fried products. Wheat and oat bran were substituted in wheat flour at 1, 5, 8, 10, 15 and 20%. Other dry ingredients were added per 100 g flour as follows: salt – 1 g, sugar – 15 g, yeast – 1 g. Water was added to dry ingredients at 65 ml and 100 ml for dough and batter respectively which were deep-fried at 180°C for 5 min. Various sample crushing methods have been reported such as milling/grinding, mincing and chewing. Mincing was selected because it gave consistent results close to chewing in grain foods (Germaine *et al.*, 2008). To keep analysis as close as possible to real human experience, samples were analysed fresh as opposed to some analysis where samples were dried before digestion. For each sample, one whole freshly fried *magwinya* was minced (Figure 27) using an electronic meat grinder (Brabantia BBEK1092V, Brabant, Netherlands) equipped with 0.9 cm diameter hole (Englyst *et al.*, 1999).



Figure 27. Whole (A) and minced (B) *magwinya*

6.2.3. Starch digestibility protocol

In vitro starch digestibility of *magwinya* was determined by the method of Englyst *et al.* (1999; 2018) and Cortado *et al.* (2016) with modifications in the following three steps:

Step 1: Incubation of magwinya with pepsin for proteolysis

Approximately 1.5 g of minced sample was transferred to 50 ml centrifuge tubes where 5 ml of 50% (w/v) saturated benzoic acid solution and 10 ml of pepsin guar-gum solution (5 g each per litre of 0.05 M HCl). Guar gum was added to standardise the solution viscosity. To initiate proteolysis, each tube was covered, vortex-mixed, and transferred to a water bath for 30 min at 37°C. TO each tube, 5 ml sodium acetate buffer solution (0.5 M) at pH 5.2 and 15 glass beads of 5 mm diameter were added, shaken gently and equilibrated for 3 min in the water bath.

Step 2: Starch hydrolysis of magwinya

Five ml of fresh enzyme cocktail of amyloglucosidase–invertase–pancreatin (at 4:6:18 per 100 ml enzyme mixture) was added to each tube and agitated at 137 rpm in a 37°C shaking water bath at time zero. Using a stopwatch, each tube was timed and removed from the water bath at exactly 20 and 120 min after addition of the enzyme cocktail. Approximately 0.2 ml of the digest was pipetted into 4 ml absolute ethanol and vortex-mixed to end enzymatic hydrolysis (Figure 28). These were G20 and G120 fractions (glucose concentration at 20 min and 120 min).

Step 3: Extreme digestion for hydrolysis of starch to glucose

The tubes were covered, mixed vigorously on a vortex mixer, and cooled for 5 min in an ice water bath. Subsequently, 7 M KOH solution (10 ml) was added and mixed. The tubes were agitated horizontally for 30 min in an ice water bath. A 0.2 ml portion of the content was pipetted into 1 ml acetic acid 1 M solution after which 40 μ l of amyloglucosidase solution was added. The tubes were vortex mixed and transferred to a water bath for 30 min at 70°C. The tubes were cooled for 15 min in an ice bath and subsequently allowed to reach ambient temperature before adding 12 ml of absolute ethanol. This tube corresponded to the TG (total glucose) portion. Glucose oxidase and peroxidase assay kit (Appendix IX) was used to estimate glucose concentration of G20, G120, and TG portions.

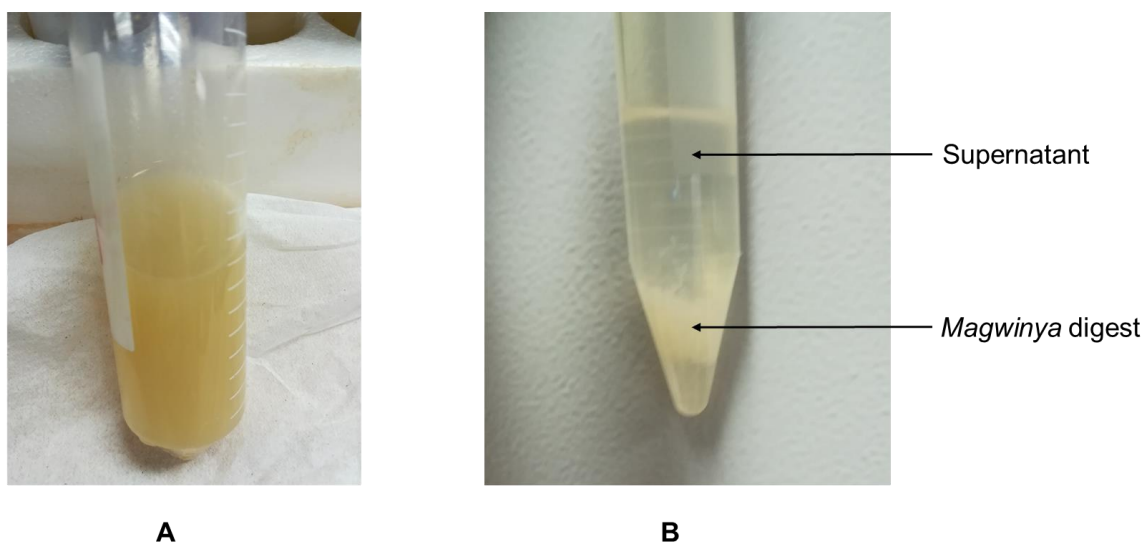


Figure 28. Digested *magwinya*(A) and digest in ethanol (B)

6.2.4. Glucose measurement in *magwinya* digest

The contents of G20, G120, TG and FSG suspended in ethanol were centrifuged at 1786 rpm for 5 min. Fifty μ l was drawn from the supernatant into a 15 ml centrifuge tube and 1 ml deionised water was added. At zero-time, 2 ml of assay reagent was added to the first tube and mixed. Exactly 30-sec intervals were maintained between additions of assay reagent to subsequent tubes. The tubes were incubated for 30 min at 37°C. Two ml of 12 N H₂SO₄ was added to each tube after thirty-minute mark at 30-sec intervals. The tubes were thoroughly mixed, and absorbance was read against the blank at 540 nm. Standard solutions were prepared from the glucose standard (G3285) provided in the kit and the concentration of glucose in each sample was read against a standard curve.

Rapidly available glucose (RAG), slowly available glucose (SAG), and unavailable glucose (UG) fractions were estimated using equations 6.1 – 6.3 as described by Contardo *et al.* (2016).

$$\text{RAG (g/100 g)} = G_{20}/\text{TG} \times 100 \quad (6.1)$$

$$\text{SAG (g/100 g)} = (G_{120} - G_{20})/\text{TG} \times 100 \quad (6.2)$$

$$\text{UG (g/100 g)} = (\text{TG} - G_{120})/\text{TG} \times 100 \quad (6.3)$$

6.2.5 Free sugar glucose content of *magwinya*

Free sugar glucose was determined for calculation of digestible starch portions of the samples using the methods of Englyst *et al.* (1999) and Englyst *et al.* (2018). Approximately 1 g of minced *magwinya* was weighed into 50 ml centrifuge tubes together with 15 glass beads. Next, 25 ml of 0.1 M acetate buffer was added to each tube and tubes were vortex-mixed vigorously and placed in a boiling water bath for 30 min. The tubes were cooled to 37°C and 0.2 ml invertase was added and incubated horizontally in a shaking water bath at 37°C for 30 min. The mixture was vortex-mixed, and 0.2 ml of the digest was transferred into 4 ml of ethanol, vortex-mixed and centrifuged for 5 min at 1786 rpm. This portion corresponded to the FSG portion which was measured with GAGO20 kit.

6.2.6 Digestible and total starch of *magwinya*

The digestible starch fractions of samples were calculated using equations 6.4 – 6.7

$$\text{Rapidly digestible starch (RDS)} = (G_{20} - \text{FSG}) \times 0.9 \quad (6.4)$$

$$\text{Slowly digestible starch (SDS)} = (G_{120} - G_{20}) \times 0.9 \quad (6.5)$$

$$\text{Resistant starch (RS)} = (\text{TG} - G_{120}) \times 0.9 \quad (6.6)$$

$$\text{Total starch (TS)} = (\text{TG} - \text{FSG}) \times 0.9 \quad (6.7)$$

6.2.7 Expected glycemic index of *magwinya*

The kinetics of starch hydrolysis was described by the non-linear first-order equation 6.8 established by Goñi *et al.* (1997). Estimated glycemic index of *magwinya* was calculated from the equation 6.9.

$$C = C_{\infty} (1 - e^{-kt}) \quad (6.8)$$

$$eGI = 39.71 + 0.548^* HI \quad (6.9)$$

The hydrolysis index (HI) was obtained from a division of area under hydrolysis curve (AUC) of *magwinya* sample and AUC of the reference sample (Whitebread) as presented in the equations 6.10 and 6.11 (Goñi *et al.*, 1997; Annor *et al.*, 2015).

$$HI = \frac{AUC_{magwinya}}{AUC_{white\ bread}} \quad (6.10)$$

$$AUC = C_{\infty}(t_f - t_0) - (C_{\infty}/k) [1 - e^{-k(t_f - t_0)}] \quad (6.11)$$

Where; C_{∞} is the equilibrium concentration at the final time (120 min) and k is the kinetic constant. t_f is final time t_0 is the initial time, C is the starch hydrolysed at a chosen time t .

6.2.8 Statistical analysis

Results were presented as the mean of triplicate determinations. Analysis of variance was carried out to determine the effect of bran concentration on the glucose fractions. Duncan's multiple range test was used to separate means ($P < 0.05$) where the effect of the independent variable was significant. Multivariate linear regression was carried out to determine the test of between-subject effect, that is, linear and interaction effect of bran addition, initial moisture content and bran type on dependent variables. The association between independent and dependent variables was tested using Pearson's correlation. All statistical tests were done using SPSS software (SPSS statistics version 26, IBM Co. USA).

6.3. Results and discussion

This section presents the results of the glucose content (RAG, SAG, UG), digestible starch content (RDS, SDS, RS) and glycemic index of *magwinya* as a function of increasing bran concentration in the *magwinya* formulation.

6.3.1 Rapidly and slowly available glucose content of *magwinya*

The glucose fractions of *magwinya* have their source linked to starch and sugar components of *magwinya*. Rapidly available glucose (RAG) and rapidly digestible starch (RDS) are both significant determinants of glycemic response. Where RAG highlights rapid absorption and the RDS emphasize rapid digestion (Annor *et al.*, 2015). The RAG of OB fried dough significantly ranged from 41.47 – 60.31 g/100 g while that of fried batter ranged from 71.84 –

90.07 g/ 100 g (Table 18). Significant progressive reduction was observed up till 10 g OB in both sets of *magwinya*. In fried dough, the increase beyond 10% OB was not significant ($P > 0.05$), while in fried batter it was significant. Initial moisture content and bran concentration was positively correlated to RAG content of OB (0.942) and WB *magwinya* (0.931) at $P < 0.01$. Higher RAG values in fried batter depicts higher gelatinisation degree of fried batter compared to fried dough. Gelatinised starch is easily hydrolysed because of the loss of amylopectin crystallinity which makes the granules porous and accessible to digestive enzymes (Contardo *et al.*, 2016). Control sample had the highest RAG values; which implies that inclusion of OB reduced RAG values in both fried products. Human/animal subjects were not used for the study. However, this result implies that incorporation of OB in *magwinya* formulation will delay the release of glucose in the bloodstream at 20 min post-ingestion. RAG values of WB fried batter ranged from 72.33 – 90.07 g/ 100 g while that of fried dough ranged from 47.11 – 60.31 g/ 100 g. The RAG values of deep-fried dough matrix in the report of Contardo *et al.* (2016) was about 40 g / 100 g.

Like OB samples, a significant decrease was noted up till 10% WB supplementation, followed by an increase thereafter in WB fried dough and batter. As initial moisture content increased, RAG values also increased ($r = 0.931$, $P < 0.01$) denoting that consumption of WB fried batter will cause rapid release of glucose in the bloodstream. Slowly available glucose (SAG) in both fried dough and batter were < 20 g/100 g in OB and WB *magwinya* (Table 18). Of the three glucose contents, SAG ranked lowest. Slowly available glucose in both sets of samples were also below 20 g/100 g and increased significantly with increments to bran concentrations in all samples. Pearson's correlation showed that increase in initial moisture content increased SAG of WB ($r = 0.596$) and OB *magwinya* ($r = 0.557$) at $P < 0.01$.

6.3.2 Unavailable glucose content of *magwinya*

Unavailable glucose (UG) values of OB fried batter increased significantly ($P < 0.05$) at higher substitution levels of OB. The UG content corresponds to the amount of glucose not released within 120 min ingestion and it is mainly from resistant starch. Results showed UG values were significantly high in fried dough than fried batter. This can be attributed to less water in the former which led to incomplete starch gelatinisation, and as such, digestive enzymes could not hydrolyse the starch at final digestion time. The UG values of fried batter for OB and WB *magwinya* were < 20 g/ 100g; while those of OB and WB fried dough ranged from 37.07 – 54.74 g/ 100g and 35.27 – 45.44 g/ 100 g respectively. Values reported in this study were close to those of Contardo *et al.* (2016) where UG of dough matrix fried under atmospheric conditions was 46%. A negative correlation was established for initial moisture content and UG of WB *magwinya* ($r = -0.985$) and OB *magwinya* ($r = -0.935$) at $P < 0.01$. High UG values

in WB fried dough may be accounted for by incomplete gelatinisation as a result of competition for water by fibre, salt, and sugar molecules causing less water for starch granules to attain full gelatinisation upon heating (Singh *et al.*, 2010; Contardo *et al.*, 2016).

Table 18. Available glucose content (g/ 100 g) of *magwinya* enriched with oat and wheat bran

<i>Magwinya</i> samples	Fried batter			Fried dough		
	RAG	SAG	UG	RAG	SAG	UG
Control	90.07 ^d ± 1.80	6.88 ^{ab} ± 0.78	3.05 ^a ± 0.61	60.31 ^c ± 1.51	2.62 ^a ± 0.03	37.07 ^a ± 0.24
OB1	82.25 ^c ± 0.28	14.07 ^c ± 0.78	3.68 ^a ± 0.59	45.70 ^b ± 1.77	2.48 ^a ± 0.48	51.82 ^c ± 1.30
OB5	76.46 ^{ab} ± 0.72	9.72 ^c ± 0.59	13.82 ^c ± 0.13	44.27 ^b ± 0.11	4.35 ^a ± 0.21	51.38 ^c ± 0.11
OB8	75.41 ^{ab} ± 0.91	13.49 ^c ± 0.76	11.10 ^b ± 0.15	43.71 ^{ab} ± 1.03	8.45 ^b ± 0.98	47.84 ^b ± 0.21
OB10	71.84 ^a ± 0.75	11.30 ^{bc} ± 0.71	16.86 ^d ± 0.96	41.47 ^a ± 0.25	3.79 ^a ± 0.10	54.74 ^d ± 1.15
OB15	79.82 ^{bc} ± 0.57	4.62 ^a ± 0.57	15.57 ^{cd} ± 0.14	43.25 ^{ab} ± 0.40	10.18 ^b ± 0.41	46.57 ^b ± 0.21
OB20	80.31 ^{bc} ± 0.87	8.98 ^{abc} ± 0.17	10.71 ^b ± 0.70	43.58 ^{ab} ± 0.22	5.00 ^a ± 0.85	51.43 ^c ± 0.37
Control	90.07 ^e ± 1.80	6.88 ^a ± 0.78	3.05 ^a ± 0.61	60.31 ^e ± 1.51	2.62 ^a ± 0.03	37.07 ^{ab} ± 0.24
WB1	83.17 ^d ± 1.13	9.45 ^b ± 0.43	7.39 ^c ± 0.30	56.81 ^d ± 0.12	4.69 ^b ± 0.20	38.50 ^b ± 1.08
WB5	80.14 ^c ± 0.25	10.93 ^b ± 0.62	8.93 ^d ± 0.36	53.67 ^c ± 1.31	11.06 ^{de} ± 0.42	35.27 ^a ± 0.11
WB8	76.09 ^b ± 0.05	18.76 ^d ± 0.05	5.15 ^b ± 0.01	48.10 ^a ± 0.20	10.45 ^d ± 0.05	41.46 ^c ± 0.15
WB10	73.38 ^a ± 0.58	17.99 ^d ± 0.76	8.63 ^d ± 0.18	49.18 ^{ab} ± 0.85	12.56 ^e ± 0.12	38.26 ^b ± 0.27
WB15	78.58 ^c ± 0.18	14.77 ^c ± 0.68	6.65 ^c ± 0.51	51.12 ^b ± 0.33	5.67 ^{bc} ± 0.17	43.21 ^c ± 1.63
WB20	72.33 ^a ± 1.78	18.11 ^d ± 0.85	9.57 ^d ± 0.07	47.11 ^a ± 0.25	7.45 ^c ± 0.50	45.44 ^d ± 1.25

Values are presented as mean ± standard deviation (n = 3). Superscripts with different alphabets show significance (P < 0.05) using Duncan's multiple range test. RAG- rapidly available glucose, SAG – slowly available glucose, UG – unavailable glucose. OB – oat bran, WB – wheat bran. 1 – 20 represent concentration (g) of bran in *magwinya* formulation. Control – 0 g bran in formulation.

Multivariate linear regression revealed that linear effect of the independent variables (bran type, initial moisture content and bran concentration) showed significance ($P < 0.05$) on RAG, SAG and UG contents of *magwinya* (Table 19).

6.3.3. Digestible starch content of *magwinya*

Starch content of a food can be rapidly digested, slowly digested or undigested after 2-hour mark post-ingestion (Englyst *et al.*, 1999; Singh *et al.*, 2010). In humans, the starch fraction that causes a rapid rise in postprandial blood glucose concentration after ingestion of a meal is categorised as rapidly digestible starch (RDS); while the fraction that is slowly digested is labelled slowly digestible starch (SDS). However, in an *in vitro* system, RDS is categorised as the starch fraction digested within 20 min after adequate digestive enzymes are introduced into the digestion reaction chamber at the appropriate temperature and pH conditions; while SDS is the starch fraction completely digested within 120 min (Dona *et al.*, 2010). The potential of SDS in human health is in its slow and stable release of glucose in the bloodstream which is particularly beneficial to healthy and diabetic individuals.

Table 19. Multivariate linear regression for main and interaction effects of independent variables on glucose content of *magwinya*

Source	Glucose	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	RAG	21891.934 ^a	27	810.812	247.460	< 0.001	0.992
	SAG	1850.413 ^b	27	68.534	22.608	< 0.001	0.916
	UG	28774.288 ^c	27	1065.714	707.408	< 0.001	0.997
Intercept	RAG	346564	1	346564.693	105771.474	< 0.001	0.999
	SAG	7093	1	7093.400	2340.005	< 0.001	0.977
	UG	59338	1	59338.699	39388.274	< 0.001	0.999
X	RAG	185	1	185.275	56.546	< 0.001	0.502
	SAG	221	1	221.474	73.061	< 0.001	0.566
	UG	811	1	811.884	538.918	< 0.001	0.906
Y	RAG	19016	1	19016.533	5803.842	< 0.001	0.990
	SAG	596	1	596.338	196.723	< 0.001	0.778
	UG	26347	1	26347.936	17489.425	< 0.001	0.997
Z	RAG	2159	6	359.961	109.860	< 0.001	0.922
	SAG	486	6	81.084	26.748	< 0.001	0.741
	UG	759	6	126.532	83.990	< 0.001	0.900
X * Y	RAG	230	1	230.980	70.495	< 0.001	0.557
	SAG	11	1	11.148	3.678	0.060	0.062
	UG	140	1	140.641	93.355	< 0.001	0.625
X * Z	RAG	182	6	30.393	9.276	< 0.001	0.498
	SAG	173	6	28.866	9.523	< 0.001	0.505
	UG	311	6	51.852	34.419	< 0.001	0.787
Y * Z	RAG	44	6	7.481	2.283	0.048	0.197
	SAG	108	6	18.162	5.991	< 0.001	0.391
	UG	140	6	23.340	15.493	< 0.001	0.624
X * Y * Z	RAG	72	6	12.023	3.669	0.004	0.282
	SAG	252	6	42.130	13.898	< 0.001	0.598
	UG	263	6	43.915	29.150	< 0.001	0.757
Error	RAG	183	56	3.277			
	SAG	169	56	3.031			
	UG	84	56	1.507			
Total	RAG	368640	84				
	SAG	9113.	84				
	UG	88197	84				
Corrected Total	RAG	22075	83				
	SAG	2020	83				
	UG	28858	83				

X: bran type, Y: initial moisture content, Z: bran concentration a. R Squared = .992 (Adjusted R Squared = .988), b. R Squared = .916 (Adjusted R Squared = .875), c. R Squared = .997 (Adjusted R Squared = .996) RAG- rapidly available glucose, SAG – slowly available glucose, UG – unavailable glucose

6.3.3.1 Digestible starch content of wheat bran *magwinya*

Digestible starch content of WB *magwinya* is presented in Table 20. Total starch content of *magwinya* ranged from 47.15 – 66.81% and 49.14 – 58.27% with the highest values in control samples and lowest in WB1 and WB5 in fried batter and fried dough respectively. Total starch content of 66.81% (control fried batter) in this study is close to 67% starch content reported for South African bread (Gibson *et al.*, 2011). Various values have been reported in the literature for total starch of white bread such as 51% (Scazzina *et al.*, 2009), 69% (Reshmi *et al.*, 2017) and 74% (Goñi *et al.*, 1997), to name a few.

Table 20. Digestible starch content of wheat bran *magwinya* (% wet weight)

<i>Magwinya</i> samples	RDS	SDS	RS	TS	K	HI
Fried dough						
Control	38.79 ^d ± 0.65	1.61 ^a ± 0.36	17.87 ^a ± 1.08	58.27 ^e ± 0.07	0.045 ^e ± 0.00	33.52 ^{de} ± 1.00
WB1	28.29 ^c ± 0.18	2.40 ^a ± 0.06	19.67 ^b ± 0.55	50.36 ^b ± 0.24	0.041 ^d ± 0.00	34.03 ^e ± 0.24
WB5	25.91 ^b ± 0.79	5.55 ^{de} ± 0.71	17.68 ^a ± 0.06	49.14 ^a ± 0.13	0.036 ^c ± 0.00	33.27 ^{cd} ± 0.38
WB8	23.95 ^a ± 0.06	5.34 ^d ± 0.03	21.18 ^c ± 0.07	50.47 ^b ± 0.04	0.032 ^a ± 0.00	30.51 ^a ± 0.10
WB10	24.54 ^a ± 0.66	6.54 ^e ± 0.58	19.91 ^b ± 0.14	50.99 ^c ± 0.21	0.033 ^{ab} ± 0.00	32.28 ^{bc} ± 0.20
WB15	25.65 ^b ± 1.01	2.96 ^{ab} ± 0.37	22.57 ^d ± 0.85	51.18 ^c ± 0.21	0.034 ^b ± 0.00	31.69 ^b ± 0.19
WB20	26.56 ^b ± 0.13	4.26 ^c ± 0.06	25.98 ^e ± 0.71	56.80 ^d ± 0.01	0.032 ^a ± 0.00	32.63 ^{bc} ± 0.29
Fried batter						
Control	60.93 ^d ± 0.44	3.16 ^a ± 0.25	2.72 ^a ± 0.47	66.81 ^e ± 0.22	0.122 ^e ± 0.00	74.51 ^f ± 0.13
WB1	39.02 ^{ab} ± 0.42	4.58 ^b ± 0.75	3.56 ^c ± 0.10	47.15 ^a ± 0.23	0.088 ^d ± 0.00	47.75 ^a ± 0.34
WB5	39.79 ^{abc} ± 0.10	5.65 ^b ± 0.35	4.59 ^d ± 0.16	50.03 ^b ± 0.09	0.079 ^c ± 0.00	49.61 ^b ± 0.33
WB8	40.31 ^c ± 1.05	10.19 ^d ± 0.57	2.78 ^{ab} ± 0.55	53.29 ^c ± 0.06	0.071 ^b ± 0.00	51.90 ^e ± 0.97
WB10	38.81 ^a ± 0.67	9.92 ^d ± 0.97	4.74 ^{de} ± 0.65	53.47 ^c ± 0.35	0.065 ^a ± 0.00	50.78 ^{cd} ± 0.14
WB15	39.23 ^{abc} ± 0.22	7.68 ^c ± 0.35	3.44 ^{bc} ± 0.26	50.36 ^b ± 0.32	0.076 ^c ± 0.00	50.10 ^{bc} ± 0.08
WB20	40.10 ^{bc} ± 1.01	10.20 ^d ± 0.04	5.37 ^e ± 0.04	55.67 ^d ± 0.01	0.064 ^a ± 0.00	51.24 ^{de} ± 0.60

Results are presented as mean ± standard deviation (n = 3). Mean values with different superscript down the row for each product type (batter/ dough) show significance (P < 0.05). RDS – rapidly digestible starch, SDS – slowly digestible starch, RS- resistant starch, TS – total starch, K - constant (dimensionless), HI – hydrolysis index, WB – wheat bran. 1 – 20 represent concentration (g) of bran in *magwinya* formulation. Control – 0 g bran in formulation.

Fried batter had significantly higher values of RDS than fried dough. Incorporation of WB markedly reduced RDS of *magwinya*. Incorporation of fibre-rich additives such as wheat bran to starchy foods slows down starch digestion because digestive enzymes have less effect of fibre during digestion (Singh *et al.*, 2010). About 90% of total starch in control fried batter (traditional *magwinya*) was digested in 120 min, while only about 66% of fried dough (modified *magwinya*) was digested in the same amount of time. The abundance of moisture in traditional *magwinya* coupled with thermal processing at high temperature (180°C) allowed for complete

starch gelatinisation which in turn aided easy accessibility to enzymatic degradation. This is also supported by the highest hydrolysis index (HI) value of 74.51% in control fried batter. Due to the many complexities of using human subjects, *in vitro*, starch hydrolysis tests are much less complicated and inexpensive empirical method of estimating the glycemic response of carbohydrate foods (Barine & Yorte, 2016; Magallanes-Cruz *et al.*, 2017).

Resistant starch is the starch portion not susceptible to enzyme action in the small intestine, which is transferred to the colon where it is fermented into short-chain fatty acids (Parada & Santos, 2016). Incorporation of WB coupled with low moisture content caused an increase in RS in the fried dough (ranging from 17.68 – 25.98%) compared to the fried batter (2.72 – 5.37%). SDS of WB fried dough and batter samples were $\leq 10.2\%$; lowest in control sample at 1.61% and highest in WB10 at 6.54% for fried dough, and 3.16 in fried batter control to 10.20% in WB20. Hydrolysis index of WB *magwinya* was highest in fried batter control (74.51%) and reduced with WB addition in fried batter and dough. The HI values of fried dough (30.51 – 34.03) were significantly lower than fried batter samples. Sozer *et al.* (2014) reported HI of 29 for biscuit enriched with 15 g fine WB. At higher WB concentration, HI values of fried dough were significantly lower ($P < 0.05$). Similarly, kinetic constant (K) was highest in fried batter control (0.122); indicating less resistance hydrolytic enzymes (Deepa *et al.*, 2010).

6.3.3.2 Digestible starch content of oat bran *magwinya*

Digestible starch and kinetics of starch hydrolysis is presented in Table 21. Total starch of OB *magwinya* was slightly higher than WB *magwinya* and ranged from 56.14 – 62.12% in fried dough 64.77 – 73.17% in fried batter. Oat bran also contains a significant amount of starch which caused the increase in total starch content. Total starch of brown bread (74%) was higher than white bread (69%) as reported by Reshmi *et al.* (2017). The RDS of OB fried dough were within the same range as WB fried dough while that of fried batter was higher (47.96 – 60.93%). However, OB addition also significantly ($P < 0.05$) reduced RDS values in fried dough and batter. Kumar & Prabhasankar (2018) reported a 40% decrease in RDS of noodles made with oat flour relative to noodles from 100% refined wheat flour. Like WB *magwinya*, SDS values of OB *magwinya* were less than 10%. Resistant starch increased with increments to OB concentration in fried dough. Considering this, OB fried dough will reduce postprandial blood glucose because higher RS values showed that most of the food matrix were not digestible within 120 min of ingestion. The solubility of β -glucans made less water available for starch hydration (Kumar & Prabhasankar 2018), thus causing incomplete gelatinisation - which has been positively correlated to high RS content (Englyst *et al.*, 2003; Contardo *et al.*, 2016). The HI values were below 35 compared to fried batter with higher RDS, and HI values above 60. Moreover, kinetic constant in fried batter (0.062 – 0.122) were higher

than fried dough (0.0.26 – 0.045) and both reduced significantly with OB addition in these products. HI, values of fried batter (61.67 - 74.51) corresponded well to HI of 63 - 73 for South African white bread (Gibson *et al.*, 2011). The results showed that the more the bran concentration in the product formulations, the lower the HI and consequently reduced eGI.

Table 21. Digestible starch content of oat bran *magwinya* (% wet weight)

<i>Magwinya</i> samples	RDS	SDS	RS	TS	K	HI
Fried dough						
Control	38.79 ^c ± 0.65	1.61 ^a ± 0.36	17.87 ^a ± 1.08	58.27 ^b ± 0.07	0.045 ^c ± 0.00	33.52 ^b ± 1.00
OB1	26.17 ^b ± 1.82b	1.47 ^a ± 0.08	30.76 ^c ± 0.77	58.40 ^b ± 0.77	0.030 ^c ± 0.00	31.42 ^a ± 1.04
OB5	25.26 ^{ab} ± 0.24	2.52 ^a ± 0.02	29.79 ^c ± 0.25	57.57 ^b ± 0.17	0.029 ^c ± 0.00b	30.30 ^a ± 1.20
OB8	23.82 ^a ± 0.32a	4.85 ^{bc} ± 0.57	27.46 ^b ± 0.15	56.14 ^a ± 0.27	0.028 ^{abc} ± 0.00	30.84 ^a ± 0.96
OB10	25.11 ^{ab} ± 0.17	2.40 ^a ± 0.06	34.61 ^d ± 0.73	62.12 ^d ± 0.38	0.026 ^a ± 0.00	30.86 ^a ± 0.86
OB15	23.62 ^a ± 0.02	5.92 ^c ± 0.98	27.06 ^b ± 0.17	56.60 ^a ± 0.20	0.027 ^{ab} ± 0.00	31.45 ^a ± 0.10
OB20	25.15 ^{ab} ± 0.05	3.09 ^{ab} ± 0.53	31.76 ^c ± 0.23	59.99 ^c ± 0.80	0.027 ^{ab} ± 0.00	31.99 ^{ab} ± 0.58
Fried batter						
Control	60.93 ^d ± 0.44	3.16 ^a ± 0.25	2.72 ^a ± 0.47	66.81 ^{ab} ± 0.22	0.122 ^d ± 0.00d	74.51 ^f ± 0.13
OB1	57.61 ^c ± 0.11	10.62 ^b ± 0.47	2.55 ^a ± 0.34	70.78 ^{bc} ± 0.92	0.085 ^c ± 0.01c	73.44 ^{ef} ± 0.76
OB5	57.00 ^c ± 0.37	7.54 ^b ± 0.49	10.67 ^{cd} ± 0.25	75.21 ^c ± 0.37	0.071 ^{ab} ± 0.01	70.40 ^{cd} ± 0.77
OB8	47.96 ^a ± 0.28	9.32 ^b ± 0.91	7.50 ^b ± 0.36	64.77 ^c ± 0.99	0.069 ^{ab} ± 0.01	61.67 ^a ± 0.01
OB10	50.57 ^b ± 0.72	8.27 ^b ± 0.66	12.34 ^d ± 0.52	71.18 ^{bc} ± 0.58	0.062 ^a ± 0.00a	63.72 ^b ± 0.49
OB15	57.16 ^c ± 1.76	3.40 ^a ± 0.05	11.65 ^d ± 0.99	72.22 ^c ± 0.71	0.078 ^{bc} ± 0.00	68.99 ^c ± 0.32
OB20	58.32 ^c ± 0.20	6.79 ^{ab} ± 0.04	8.06 ^{bc} ± 0.71	73.17 ^c ± 0.55	0.080 ^{bc} ± 0.00	71.81 ^{de} ± 0.54

Results are presented as mean ± standard deviation (n = 3). Mean values with different superscripts down the row for each product type (batter/ dough) show significance (P < 0.05). RDS – rapidly digestible starch, SDS – slowly digestible starch, RS- resistant starch, TS – total starch, K - constant (dimensionless), HI – hydrolysis index, OB – oat bran. 1 – 20 represent concentration (g) of bran in *magwinya* formulation. Control – 0 g bran in formulation.

6.3.4 Estimated glycemic index (eGI) of *magwinya*

6.3.4.1 Estimated glycemic index of fried batter

Estimated glycemic index of *magwinya* is shown in Table 22. Fried batter exhibited high eGI viz: 65.93 – 80.62 in WB samples and 73.75 – 80.62 in OB samples. Linear effect of initial moisture content and its interaction effect with other independent variables showed significance (P < 0.05) on eGI of OB and WB *magwinya*. There was a significant reduction in eGI of *magwinya* with increments to OB and WB concentrations in *magwinya* formulation. In the control sample, eGI of fried batter at 80.62 revealed traditional *magwinya* to be a high GI food, while that of fried dough at 58.11 can be classified as intermediate GI food.

Carbohydrate-rich foods can either be labelled low GI (<55), medium (55 - 70) and/or high GI (> 70) (Foster-Powell *et al.*, 2002; Brennan, 2005; Lanzerstorfer *et al.*, 2018). The eGI of OB fried batter samples were all above 70 indicating that traditional *magwinya*, despite OB addition are high GI foods. WB fried batter samples, on the other hand, were in the 65 – 68 range and were significantly ($P < 0.05$) lower than their OB counterpart. In the glycemic index table collated by Foster-Powell *et al.* (2002), GI of doughnut from an *in vivo* study was reported as 76 and 108 when glucose and white bread were used as reference food respectively. Fried dough samples exhibited lower eGI than fried batter samples.

6.3.4.2 Estimated glycemic index of fried dough

The eGI values of WB fried dough were in the range of 56.46 – 58.39 and were significantly lower than control (58.11) except WB1 (Table 22). The eGI of OB fried dough ranged from 56.34 – 57.27 and were significantly lower than control (58.11), but no significance was observed among the samples. This implies that *magwinya* with low initial moisture content (fried dough) are medium GI food. This reduction in GI of fried dough samples can be attributed to the combined effect of dietary fibre and limited water in the food matrix, which would have caused incomplete starch gelatinisation, and of a consequence resistance to amyolytic enzymes in the digestion time frame (Torres *et al.*, 2019). In a food matrix such as *magwinya* which has other components such as sugar (sucrose), salt, protein (gluten) and fibre, coupled with low initial water in the dough; these components compete with the starch granules for water. For complete gelatinisation to occur, heat and water are two crucial factors viz: water must enter the crystalline region (amylopectin) where swelling occurs (upon heating) and the cell ruptures, causing the amylose to leach out. It is easier for digestive enzymes to hydrolyse the exposed amylopectin and amylose into glucose monomers for absorption in the body. However, when there is incomplete gelatinisation, the foregoing does not occur, and this reduces the GI of the food (Parada & Aguilera, 2011).

Table 22. Estimated glycemic index of *magwinya*

<i>Magwinya</i> samples	Wheat bran <i>magwinya</i>	
	Fried dough	Fried batter
Control	58.11 ^f ± 0.55	80.62 ^f ± 0.07
Wheat bran1	58.39 ^{ef} ± 0.13	65.93 ^a ± 0.19
Wheat bran5	57.97 ^{cde} ± 0.21	66.95 ^b ± 0.18
Wheat bran8	56.46 ^a ± 0.05	68.21 ^e ± 0.53
Wheat bran10	57.43 ^{bc} ± 0.11	67.59 ^{cd} ± 0.07
Wheat bran15	57.11 ^b ± 0.65	67.22 ^{bc} ± 0.04
Wheat bran20	57.62 ^{bcd} ± 0.15	67.84 ^{de} ± 0.33
	Oat bran <i>magwinya</i>	
	Fried dough	Fried batter
Control	58.11 ^b ± 0.55	80.62 ^f ± 0.07
Oat bran1	56.96 ^a ± 0.57	80.03 ^{ef} ± 0.42
Oat bran5	56.34 ^a ± 0.66	78.36 ^{cd} ± 0.42
Oat bran8	56.64 ^a ± 0.52	73.57 ^a ± 0.10
Oat bran10	56.65 ^a ± 0.47	74.69 ^b ± 0.27
Oat bran15	56.98 ^a ± 0.05	77.58 ^c ± 0.72
Oat bran20	57.27 ^{ab} ± 0.32	79.13 ^{de} ± 0.29

Mean values with different superscript down the row for each product type (batter/ dough) show significance ($P < 0.05$). 1 – 20 represent concentration (g) of bran in *magwinya* formulation. Control – 0 g bran in formulation.

The GI-lowering effect of bran types used in this study can be linked to their dietary fibre content which is composed mostly of non-starch polysaccharides such as β glucans (soluble fibre) in OB and arabinoxylan in WB (Onipe *et al.*, 2015; Kumar & Prabhasankar, 2018). Soluble fibre of OB acts through alteration of the food microstructure via restriction of starch gelatinisation because of its high-water absorption capacity which in turn limits water available to starch granules in the food matrix (Sozer *et al.*, 2014; Torres *et al.*, 2019). Wheat bran is composed of up to 58% insoluble dietary fibre which increases fecal bulk, reduces transit time in the small intestine and is resistant to enzymatic hydrolysis due to its heterogeneous structure (Onipe *et al.*, 2015); thus, lowering eGI with its increasing concentration in our fried products. The eGI of WB samples were lower than OB samples for both fried batter and dough; thus, deducing that WB is a better choice at lowering the GI of *magwinya* made from either dough and/or batter. Despite the similarity between both type of products, their eGI showed high disparity which was affected by the difference in ingredient (bran type used) and

initial moisture content of the products which resulted in different degrees of starch gelatinisation and rates of carbohydrate digestion and their respective eGI values.

6.3.5 Relationship between estimated glycemic index, and other variables in the study

The relationship between eGI, glucose, starch and total oil content of *magwinya* were estimated using Pearson's correlation to observe and report linear trends amongst these properties.

6.3.5.1 Wheat bran *magwinya*

Correlation among available glucose (RAG, SAG, UG), digestible starch (RDS, SDS, RS), HI and eGI of wheat bran *magwinya* is presented in Table 23. Total starch correlated positively with RDS of WB *magwinya* (Table 23). The effect of WB concentration on the properties were not as strong as initial moisture content. For complete gelatinisation during heat treatment, water is essential to hydrate starch molecules and cause a disruption of the crystalline structure of amylopectin. When there is not enough water, gelatinisation becomes incomplete which affects starch digestion (Dona *et al.*, 2010). Fat content of WB *magwinya* was correlated to their glucose and starch contents. Fat content correlated positively to digestible starch fractions (Table 23) of WB *magwinya* and negatively to RS ($r = -0.618$, $P < 0.01$). Individually in fried dough, a negative correlation was established between FC and RS ($r = -0.749$, $P < 0.01$), while no significant correlation existed in WB fried batter. Increase in fat content also may have led to an increase in eGI ($r = 0.495$, $P < 0.01$). This is accounted for by fried batter samples which had higher eGI values. A possible explanation could be the enzyme used which catalysed. There was a perfect positive correlation between HI and eGI ($r = 1$, $P < 0.05$).

6.3.5.2 Oat bran *magwinya*

Correlation among available glucose (RAG, SAG, UG), digestible starch (RDS, SDS, RS), HI and eGI of oat bran *magwinya* is presented in Table 24. Results showed RAG and RDS contributed strongly to eGI of OB *magwinya* as indicated by significant positive correlations ($r = 0.962$, 0.976 , $P < 0.01$). Initial moisture content had a great influence on glucose, starch and eGI of OB *magwinya* as shown (Table 24) by significant correlation of initial moisture content to most of the starch properties except UG and RS where an inverse relationship is noted. In a low-moisture system like fried dough in this study, an increase in RS may be as a result of incomplete starch gelatinisation. Abundant water and heat are two important factors that aid starch gelatinisation and in turn the extent of starch digestion during hydrolysis. Exposure of starch molecules to heat in excess water leads to disruption of the crystalline structure thereby linking the hydroxyl group of starch and water molecules via hydrogen bonding (Magallanes-Cruz *et al.*, 2017). This, in turn, solubilises the starch granule, leading to increased swelling

and subsequent leaching of amylose which is easily accessible to amylolytic enzymes during digestion (Singh *et al.*, 2010). Correlation of fat content to glucose and starch contents revealed similar results as WB fried products.

Table 23. Correlation coefficients of independent variables, glucose, digestible starch and glycemic index of wheat bran *magwinya*

	Initial moisture content	wheat bran concentration	RAG	SAG	UG	RDS	SDS	RS	TS	K	HI	eGI
RAG	.931**	-.310*										
SAG	.596**	.488**	.313*									
UG	-.985**	0.118	-.957**	-.574**								
RDS	.765**	-.389*	.896**	0.119	-.808**							
SDS	.575**	.527**	0.281	.992**	-.545**	0.102						
RS	-.971**	0.166	-.955**	-.565**	.995**	-.795**	-.534**					
TS	0.142	-0.181	0.264	-0.192	-0.169	.656**	-0.167	-0.128				
K	.851**	-.374*	.962**	0.182	-.885**	.937**	0.140	-.871**	.415**			
HI	.865**	-0.211	.916**	.338*	-.892**	.943**	.308*	-.866**	.536**	.955**		
eGI	.865**	-0.211	.916**	.338*	-.892**	.943**	.308*	-.866**	.536**	.955**	1.000**	
Fat	.561**	-0.087	.542**	.403**	-.590**	.487**	.393*	-.618**	0.107	.444**	.495**	.495**

RAG, SAG & UG represent rapidly available, slowly available & unavailable glucose (N = 18)

RDS, SDS, RS & TS represent rapidly digestible, slowly digestible, resistant and total starch

K, HI & eGI represent equilibrium, hydrolysis index and estimated glycemic index

*Correlation significance at the 0.05 level, **Correlation significance at the 0.01 level.

RDS correlated positively to eGI ($r = 0.705$, $P < 0.01$), indicating RDS had significantly influence on eGI of OB *magwinya*. RS correlated negatively to fat ($r = -0.701$, $P < 0.01$) and positively to eGI ($r = 0.655$, $P < 0.01$). There was perfect correlation between HI and eGI ($r = 1$, $P < 0.01$).

Opposing results on the effect of deep-frying on RS content of potato products have been reported. On the one hand, deep-frying significantly increased RS of potato products (Mangala *et al.*, 1999; Ghidurus *et al.*, 2010), mainly because deep-frying increases fat available for amylose-lipid complex formation in the food. Resistance of digestion enzymes to this complex reduces digestible starch which in turn increases RS. On the other hand, Yadav (2011) observed that deep-frying had a prominent effect on RS reduction. In this study, however, percentage fat content was compared to the starch contents and an inverse relationship existed between RS and fat content for OB and WB fried products. The negative correlation may be linked to starch gelatinisation which was more pronounced in batter samples (having higher fat content) because of higher initial water content. Inclusion of bran in *magwinya* formulation also contributed to fat reduction and consequential increase in RS.

Table 24. Correlation coefficients of independent variables, glucose, digestible starch and glycemic index of oat bran *magwinya*

	Initial moisture content	Oat bran concentration	RAG	SAG	UG	RDS	SDS	RS	TS	K	HI	eGI
RAG	.942**	-0.195										
SAG	.557**	0.095	.397**									
UG	-.963**	0.155	-.981**	-.565**								
RDS	.951**	-0.145	.986**	.400**	-.969**							
SDS	.608**	0.116	.437**	.959**	-.593**	.463**						
RS	-.916**	0.239	-.972**	-.544**	.987**	-.948**	-.571**					
TS	.885**	0.125	.790**	.440**	-.802**	.872**	.554**	-.724**				
K	.874**	-0.258	.964**	.319*	-.932**	.931**	.319*	-.927**	.687**			
HI	.985**	-0.052	.962**	.518**	-.973**	.976**	.570**	-.932**	.897**	.915**		
eGI	.985**	-0.052	.962**	.518**	-.973**	.976**	.570**	-.932**	.897**	.915**	1.000**	
FC	.636**	-.361*	.702**	0.148	-.661**	.705**	0.249	-.671**	.576**	.649**	.655**	.655**

RAG, SAG & UG represent rapidly available, slowly available & unavailable glucose (N = 21)

RDS, SDS, RS & TS represent rapidly digestible, slowly digestible, resistant and total starch

K, HI & eGI represent equilibrium, hydrolysis index and estimated glycemic index

*Correlation significance at the 0.05 level, **Correlation significance at the 0.01 level.

6.4. Conclusion

The study hypothesised that WB and OB will have a reduction effect on the eGI of *magwinya*. because of their HI and GI-reduction effect attributable to high dietary fibre content. Reduction in RAG values and concomitant increase in UG values of WB and OB fried products points to the delayed effect of the fibres in the release of glucose of the fibres during hydrolysis reaction. This means oat and WB are suitable proponents for blood glucose management and delayed carbohydrate absorption in the bloodstream. Oat and WB had a similar effect on reduction of RAG and RDS values of fried dough. However, WB had improved effect in fried batter products. The fat content of fried products was inversely related to RS and directly proportional to eGI – indicative of amylose-lipid complex formation in fried dough and starch gelatinisation in fried batter products. *Magwinya* is widely known as an unhealthy food and proposed to be a high GI. However, The reduction of *magwinya* GI from high (80.03) to medium (< 70) because of bran addition and moisture variation proves that improvements to the food is possible. The outcomes of this study can serve as baseline information or an indicator to *magwinya* (or fried food) processors, nutritionists and consumers. *In vitro* studies only cater for estimation of starch digestion and glycemic index. However, to get a true sense of *magwinya* GI, *in vivo* study using human or animal subjects is highly recommended. Extraction and purification of fibres of oat and WB and incorporation in *magwinya* formulation is also suggested for future studies.

CHAPTER 7: MAPPING OIL, CARBOHYDRATES AND OTHER CHEMICAL CONSTITUENTS OF *MAGWINYA* USING NEAR INFRA-RED HYPERSPECTRAL IMAGING TECHNOLOGY

Abstract

Distribution of chemical components of *magwinya* was visualised using NIR hyperspectral imaging (HSI). Fried dough and batter enriched with 1 – 20% oat and wheat bran were captured with a short wave infra-red camera and raw images were mean-centred. Principal component analysis (PCA) was applied to mean-centred data for pixel-wise classification using spectral scattering (standard normal variate) and 1st and 2nd derivatives of Savitsky-Golay method. There was little separation observed in the PCA score plots in the results due to a large similarity between classes. Prominent bands related to oil were featured at 1400, 2305 and 1716 nm, while those related to starch were featured at 1449, 1776, and 2261 nm. 1941 (related to moisture), and 2117 nm (related to protein). Bands related to protein were featured at 1509, 1994, 2223, 2229 nm and water at 1450, 1934 and 1940nm. Aromatics, phenols (1422 nm) and benzene were also identified, albeit minimum. The processing methods and some principal components assisted in mapping the chemical components of *magwinya*. Visualisation of *magwinya* chemical constituents using HSI shows good promise for further research in modelling and predictions.

Keywords: NIR, spectra images, wavelength, hyperspectral imaging, *magwinya*.

7.1. Introduction

Deep-fried foods are considered unhealthy owing to the content of saturated fat, cholesterol and myriad of chemical compounds from thermal oxidation, hydrolysis, and other complex chemical reactions (Choe and Min, 2007). Oil content, coupled with the crispiness of fried foods contributes to its palatability, flavour and taste. Chemical compounds like alkanals, lactones, dienals and other cyclic hydrocarbons which are oxidation products of linoleic acid contribute immensely to the flavour of fried foods (Ghidurus *et al.*, 2010).

Vibrational spectroscopy is a valuable non-invasive imaging technology for quality observation of food products through the acquisition of data based on the interaction between incident light and molecules in the food sample (Sun, 2010; Cheng & Sun, 2014; Mahajan & Kamalapur, 2019). Hyperspectral imaging combines spectral information of spectroscopy and spatial data of digital imaging to form a three-dimensional image data known as a hypercube containing 2D spatial and 1D spectral information. The use of HSI holds the advantage in respect of building

chemical images, offering multi constituent information, and flexible extraction of spectral information when compared to other forms of spectral imaging (Feng & Sun, 2012). There are exhaustive reviews and papers on HSI application to the field of Food Science. These include but are not limited to quality evaluation of fruits and vegetables (Lu *et al.*, 2017), detection of contamination and quality examination of fish products (Menesatti *et al.*, 2010; Cheng & Sun, 2014; Cheng *et al.*, 2014b) detection of defects and contamination to ensure food safety (Feng & Sun, 2012), texture-based classification of maize kernels (Manley *et al.*, 2009; McGoverin & Manley, 2012; Williams & Kucheryavskiy, 2016), physicochemical analysis of fish products (Cheng *et al.*, 2014b), safety evaluation of cereals (Sendin *et al.*, 2018a), monitoring bread dough properties during mixing (Kaddour and Cuq, 2011), measurement of moisture content of bread (Liu & Møller, 2011), and maize kernels (Zhang & Gao, 2020), to name a few.

Several chemical reactions including oxidation, polymerisation, hydrolysis, and Maillard process occur in *magwinya* and these reactions influence nutritional value, flavour development and colour changes thereof. Conventional proximate and mineral composition of *magwinya* has been quantified in previous studies (Onipe *et al.*, 2018; 2019). However, the application of NIR HSI for quantitative and qualitative analysis of *magwinya* is a new research niche that needs further exploration, as there are currently no published studies on HSI in *magwinya* analysis. Therefore, the objective of this chapter was to investigate the potential of using near-infrared hyperspectral imaging for visualisation of chemical components (oil, starch, protein) of *magwinya* enriched with varying amounts of bran and moisture.

7.2. Materials and methods

7.2.1 *Magwinya* production

Magwinya was produced based on the method in section 4.2.3. Wheat and oat bran were substituted in 100 g wheat flour at 1 – 20 % and fried batter and dough were prepared according to the method of Kwindu *et al.* (2018). Cooled *magwinya* samples were sliced in halves and duplicate crumb image was taken for each sample type.

7.2.2 Scanning of *magwinya* using hyperspectral imaging system

The crumb of a cross-sectional slice of freshly fried *magwinya* cooled to ambient temperature (21°C) was scanned using a benchtop hyperspectral imaging system in reflectance mode following the method of Wu *et al.* (2012). Hyperspectral images of *magwinya* were acquired with a push broom HySpex SWIR-384 (short wave infrared) imaging system (Norsk Elektro Optikk, Norway) using Breeze® (Prediktera) software version 2019.1.0. The camera was mounted on a laboratory rack with a translation stage and fitted with a lens of 30 cm focal length. The field of view (FOV) for the SWIR camera is 95 mm. The imaging system comprised

of an imaging spectrograph which is coupled to an MCT (mercury cadmium telluride) sensor for the SWIR-384. The spectral range for the SWIR camera is from 850 nm to 2500 nm with spectral intervals of 5.45 nm resulting in a total of 288 spectral points. The spatial resolution for the SWIR-384 using a 30 cm lens is pixel size 0.247 mm and each image consists out of 384 pixels. Samples were illuminated by two 150 W halogen lamps (Ushio lighting Inc., Japan) with the capacity to emit light in the 400 – 2500 nm wavelength range. The integration time was manually set to 3000 μ s (SWIR). Radiometric calibration was performed in the Breeze software package. A 50% grey reflectance standard Zenith Allucore diffuse (SphereOptics GmbH, Germany) was used as a white reference. Dark references were also recorded and both references were used to correct for the uneven light intensity of different wavelength bands. The images were automatically calibrated using the white and dark references and converted to pseudo-absorbance before analysis (Colling, 2018).

7.2.3 Image analysis

Calibrated images were exported to Evince® processing software version 2.7.10 (Prediktera AB, Umea, Sweden). A PCA model was created in which the background of the images was removed to keep only pixels related to the region of interest (*magwinya*). Pixels about air gas cells were also removed. The spectral data were extracted by selecting only the region of interests to investigate the similarities and differences in oil and carbohydrates distribution in *magwinya* samples. Score images and wavelengths of interest were captured after the application of (1) Mean centring (MC) only, (2) MC and standard normal variate (SNV); and MC and Savitzky-Golay derivative pre-processing. The control samples were compared with bran enriched samples for molecular similarities and differences.

7.2.4 Pre-processing of hyperspectral images of *magwinya*

The main objective of pre-processing is to remove physical phenomena to improve exploratory analysis (Rinnan *et al.*, 2009). Principal component analysis (PCA) was applied to the mean-centred absorbance mosaic images. Score plots and score images were used interactively to identify unwanted pixels, e.g. outliers, sample background, dead pixels, and edge effects, which were subsequently removed from the dataset (Sendin *et al.*, 2018). The cleaned image was used in subsequent analysis. PCA was recalculated with additional components after obtaining a cleaned image.

Principal component analysis

After various pre-processing treatments were evaluated, Savitsky-Golay transformation (2nd order polynomial, 1st and 2nd derivatives, 7 points) and standard normal variate (SNV) transformation was applied to the data (Rinnan *et al.*, 2009). Data were analysed with pixel-

wise PCA (Williams & Kucheryavskiy, 2016), and the PC score images, score plots and loading line plots were examined. Spectral data pre-processing reduces unwanted background information and increases the signal of chemical information. NIR absorbance is dominated by functional groups containing hydrogen bonds, for example, OH, CH and NH related to water, protein, fat and carbohydrates. Hence the focus of this study was on oil, water, starch, protein and other components formed during frying. Molecules can transition to different vibrational levels depending on the amount of energy absorbed by the sample. Specific wavelengths for molecules were assigned using the NIR spectral Table (Osborne *et al.*, 1993; Fei *et al.*, 2017).

7.3 Results and discussion

The results of image analysis using NIR hyperspectral imaging is presented in the subsequent section. The control *magwinya* (no bran) were first compared and then comparison was made between each control sample and its bran-enriched counterparts for differences in oil, water and starch components.

7.3.1 Comparison of control fried batter and fried dough

Magwinya images were cleaned and the background and unnecessary pixels removed (Figure 29) and were subjected to principal component analysis (PCA) with paired combinations of 6 principal components (PC) from PC1 to PC6 through score images and plots. Control fried dough and batter are similar in composition except for the amount of water in their formulation. As little as the compositional difference is, there may exist differences in the chemical composition due to the reactions occurring during frying which results in the formation of new compounds in the food. Hence, a need to observe any differences and select the important wavelengths from the score images and loadings plots.

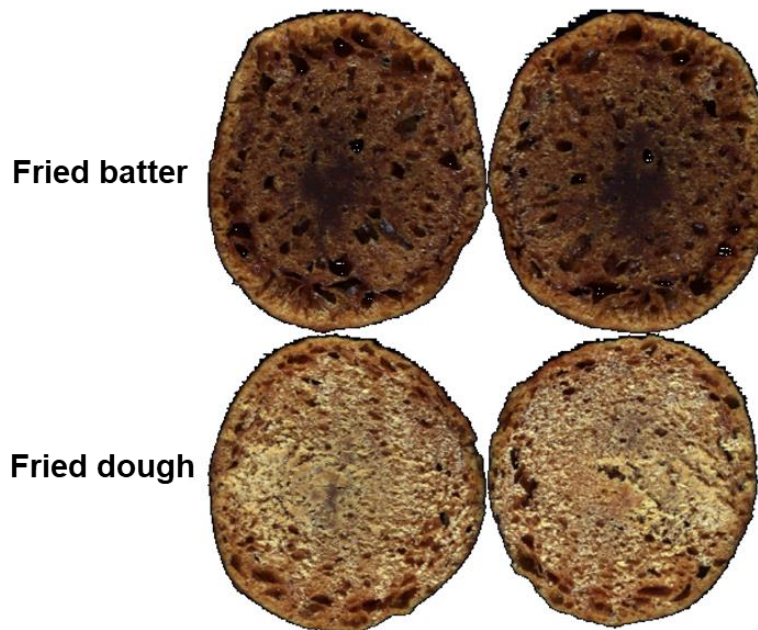


Figure 29. RGB images of control *magwinya* with no background

7.3.1.1 Mean centring pre-processing of control *magwinya*

The crumb of *magwinya* samples in MC pre-treatment loadings spectra was characterised by wavelengths ranging from 1394 nm to 2305 nm (Table 25) across seven principal components (PCs 1 – 7) in the following variations: PC1- 94.8%, PC2 – 3.68%, PC3 – 0.662%, PC4- 0.258, PC5- 0.202%, PC6 – 0.06, PC7 - 0.032%. The wavelengths associated with water ranging from 1928 to 1939 nm were present in PC1- PC7 in both negative and positive spectra of the PCs. The combination band of water molecules around 1920 nm were linked to C=O stretching in the second overtone of the CONH group and O-H stretching and deformation group at 1940 nm. Water is a critical component of *magwinya* for hydration and activation of starch-gluten matrix essential for the sponginess of the product. Also identified in the NIR regions were identified as starch was found in specific bands as 1547 nm (PC7) 1858 nm (PC4), 1885 nm (PC3), 1888 nm (PC1). Assignment of wavelengths to the peaks were done using the NIR spectral Table (Osborne *et al.*, 1993). As shown in Table 25, the absorption bands from 1449 nm to 1760 nm was a characteristic of 1st overtone initiated by the presence of O-H and C-H functional groups (Fei *et al.*, 2017; Kaddour and Cuq, 2011).

Oil was identified at wavelengths 1411, 1700, 1710, linked to C-H and O-H bonds in the first overtone and 2305 nm characterised by combination bond vibration. The loading line plots of PC6 for control fried dough showed 1939 nm as the prominent peak (in the negative axis of PC6), which signifies moisture distribution towards the crust, moisture concentration was noted at the centre of fried batter sample. The distribution of cellulose in the positive axis of PC1 and

PC6 shows the waveband 2339 nm (formed as a result of stretching and deformation of cellulose C-H bonds).

Table 25. Specific wavelengths in mean centring pre-processing for control *magwinya* crumb

Mean centring	Wavelengths (nm)	Structure	Group	Vibration bonds
PC 1	1888	Starch	O-H, C-H	Combination
PC2	1389	CH ₂	C-H	Combination
	1661	Cis-RCH=CHR1	C-H	1 st overtone
	1847	Cellulose	C-H	1 st overtone
	1934	H ₂ O		Combination
PC3	1710	Oil	C-H	1 st overtone
	1885	Starch	O-H, C-H	Combination
	1934	H ₂ O	O-H	Combination
PC4	1394	CH ₂	C-H, C-H	Combination
	1858	Starch	C-O, O-H	Combination
	1934	H ₂ O	O-H	Combination
PC5	1078	Benzene	C-H, C-C	Combination
	1389	CH ₂	C-H	Combination
	1656	Cis-RCH=CHR1	C-H	1 st overtone
	1721	CH ₂	C-H	1 st overtone
	1928	CONH	C=O	2 nd overtone
	2305	Oil	C-H	Combination
PC6	1144	Aromatic	C-H	2 nd overtone
	1324	CH ₃	C-H	Combination
	1411	ROH	O-H	1 st overtone
	1700	Oil	C-H	1 st overtone
	1885	Starch	O-H, C-H	Combination
	1939	H ₂ O	O-H	Combination
	2021	CONH ₂	C=O	2 nd overtone
	2125	Amino acid	N-H, C=O	Combination
	2239	Amino acid	N-H, NH ₃ ⁺	Combination
	2392	ROH	O-H	2 nd overtone
PC7	1345	CH ₃	C-H	Combination
	1547	Starch	O-H	1 st overtone
	1934	H ₂ O	O-H	Combination

PC – principal component. Assignments of wavelengths were done using NIR spectral table (Fei et al., 2017; Kaddour and Cuq, 2011).

The prominent waveband 1885 nm represents starch which was majorly distributed in the crumbs of fried dough and fried batter. The major component of wheat flour is starch, hence the obvious peak of starch (Nawrocka & Lamorska, 2013). Other chemical compounds identified in the spectra loadings are aromatics (1416 nm), amino acids (2125 – 2239 nm) and alcohols (2392 nm).

7.3.1.2 Mean centring and Standard normal variate processing of control *magwinya*

The application of MC and SNV processing to the crumb of *magwinya* featured wavelengths related to oil at 1209 nm, 1716nm, 1721nm, 2119 nm and 2305 nm linked to C-H stretching at first overtone and combination bands, respectively (Figure 30). Starch was also identified in the spectra loadings plots across PCS 1 to 7 at wavelengths 1383 nm, 1547 nm, 1896 nm, 1885nm, 2010 nm and 2016nm. Water was represented by wavelength in the negative spectrum at 1934 nm. The wavelengths in the spectra loading of PC4 ranged between 1209 nm and 2305 nm with the lowest absorption band representing oil and the highest band associated with starch (1885). The absorbance at 1416 nm is a combination band consisting of C-H stretching and deformation bonds identified as an aromatic. One of the sensorial features of *magwinya* that appeals to consumers is flavour which is formed from heterocyclic compounds produced from Maillard reaction occurring between amino acids, oxidation products and reducing sugars (Gillatt, 2001).

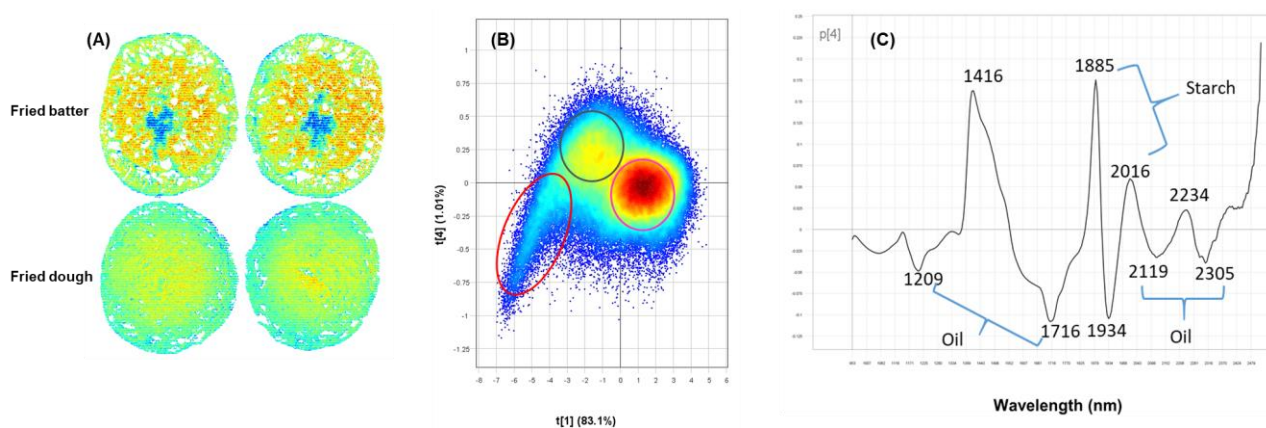


Figure 30. Score image (PC4) for control *magwinya* - fried batter (top) and fried dough (bottom) using mean centring and standard normal variate pre-processing (A). Score plot of PC4 vs. PC1 score plot showing clusters of pixels. Cyan blue colour characterised the crust section; yellow coloured pixels correspond to the crumb region while the red colour was assigned to the core of the fried products. (B) The loadings line spectra plot of PC4

7.3.1.3 Mean centring and Savitsky-Golay second derivative processing of control *magwinya*

The crumb of *magwinya* samples in MC/SG second derivative pre-treatment was characterised by wavelengths ranging from 1122 nm to 2283 nm the first principal component as shown in Figure 31. Assigned band at 1449 nm in the positive spectra region describes starch and water interaction associated with O-H stretching in the first overtone; while the band; while the band at 1923 nm can be linked to C=O stretching the second overtone of CONH structure. The band

1405 nm in the negative spectra space of PC1 represents oil which associated with N-H and O-H stretching in the first overtone. Bands representing starch (1874 nm) was the result O-H with C-O stretching vibration while 2234 nm, is linked to N-H stretching and NH₃⁺ deformation associated with protein matrix in the *magwinya*.

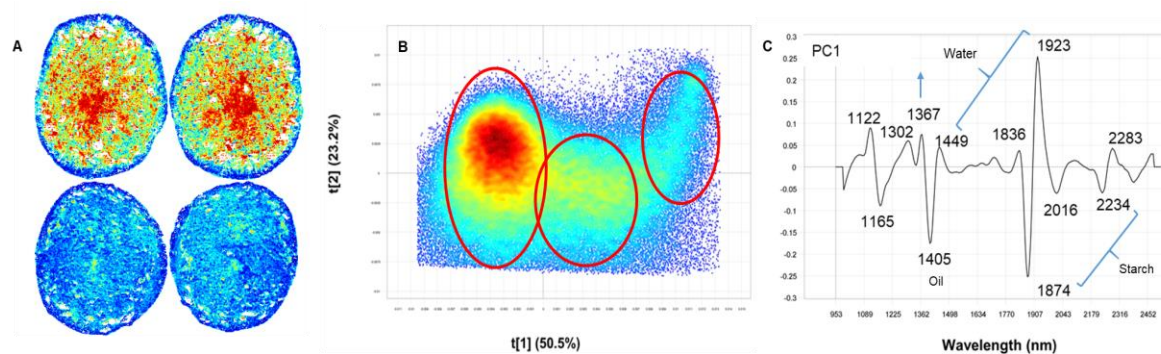


Figure 31. Score image (PC1) for control *magwinya*-fried batter (top) and fried dough (bottom) using Mean centring/ Savitzky-Golay 2nd derivative pre-processing (A). Score plot of PC1 vs. PC2 score plot showing clusters of pixels. Red colour – the crumb of fried dough and crust region of the fried batter; yellow colour – Crumb of fried batter; cyan blue – the core of fried batter (B); and the loadings line spectra plot of PC1 (C).

Application of MC, MC/SNV and MC/S-G pre-processing to *magwinya* crumb showed a lot of similarities. Hence, Major peaks identified in bran-enriched *magwinya* are discussed in the subsequent section under two major headings with regards to two pre-processing methods – MC/SNV and MC/S-G second derivative. The RGB images *magwinya* enriched with OB and WB are presented in Figure 32. The products were mostly similar in terms of water content; while minor differences were noticed in starch, oil and protein contents especially in the lower PCs.

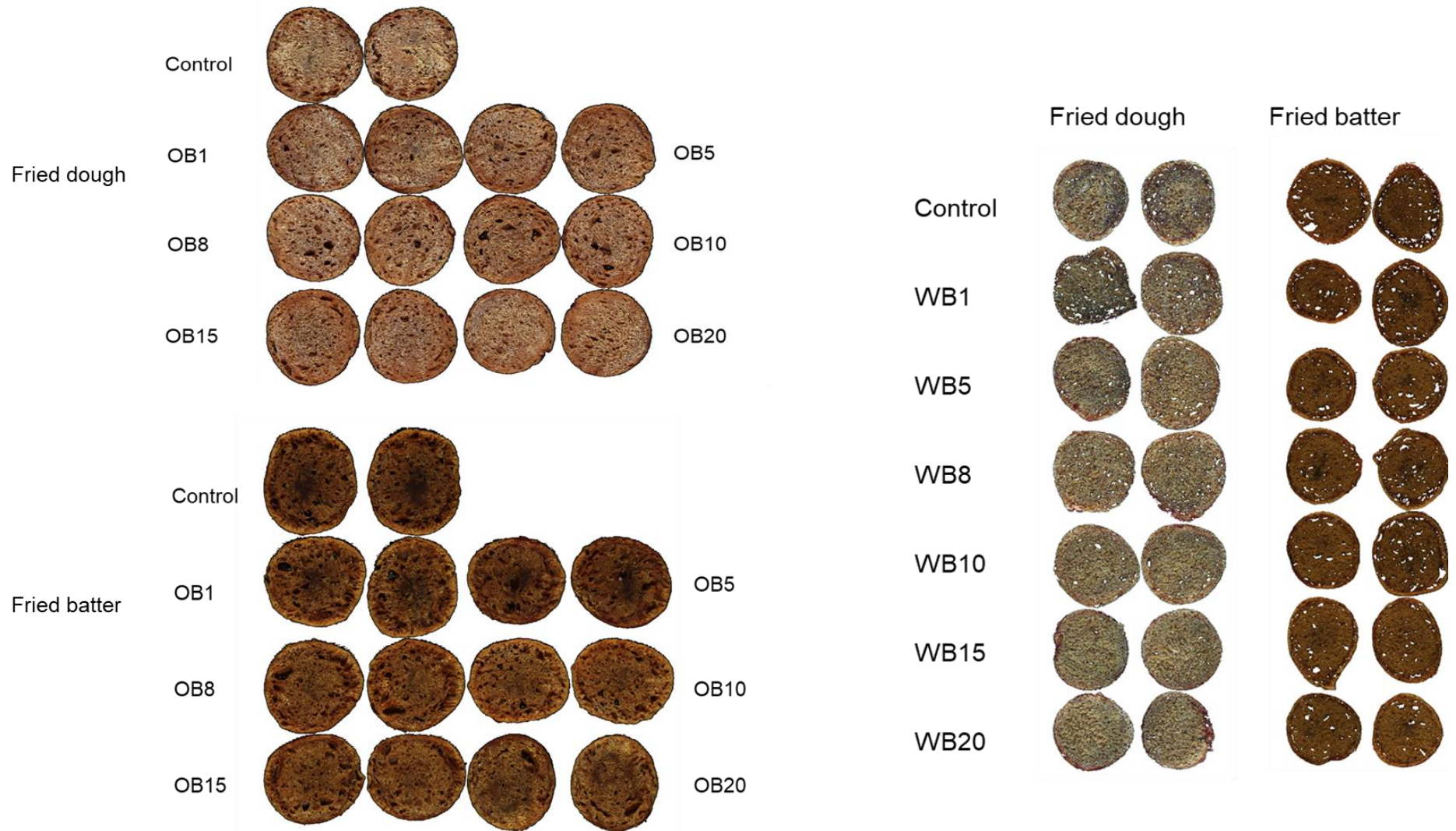


Figure 32. Clean RGB images of oat bran *magwinya*(left) and wheat bran *magwinya* (right). OB1 – OB20 and WB1- WB20 represents oat and wheat bran concentration (g) in fried dough formulation

7.3.2 Mean centring and standard normal variate processing of bran enriched *magwinya*

Mean-centred data was not presented as there was minimal separation amongst the control and bran-enriched samples. Therefore, SNV and Savitsky-Golay derivatives were discussed as they presented a clearer separation than only mean-centring. The PCA plots were observed for the processing method that presented the best pixel-wise separation and the loadings line was checked for corresponding prominent peaks. The conforming wavelength for the specific peaks based on the clusters noticed in the PCA space were assigned functional groups using the NIR spectral table (Fei *et al.*, 2017).

7.3.2.1 Control versus fried dough samples

The application of SNV pre-processing in the crumb of *magwinya* featured one prominent peak (1934 nm) in the positive spectra (OB fried dough) and negative spectra of WB fried dough (PC4) related to water distribution in the samples as represented by the cyan blue colour which reduced with increasing bran concentration (Figure 33).

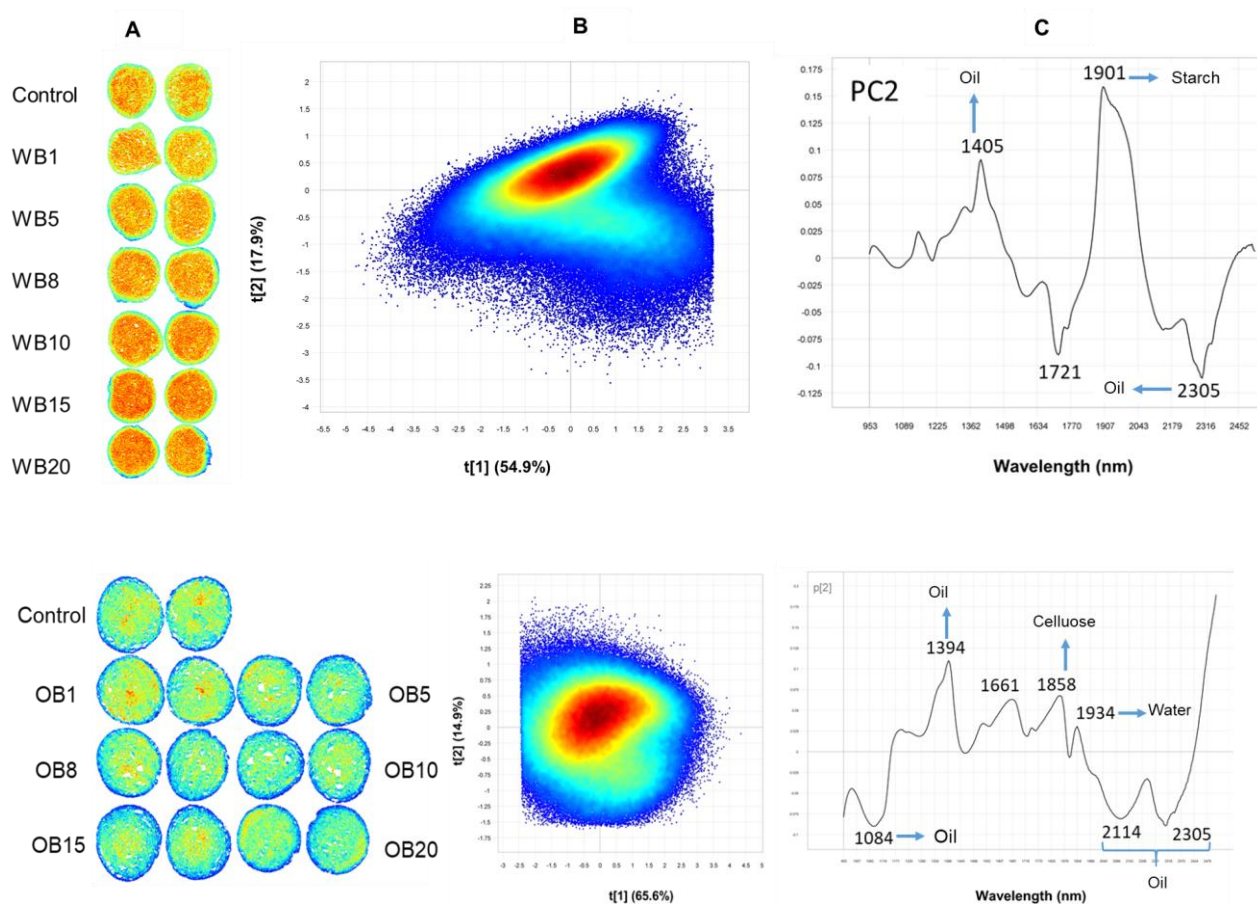


Figure 33. Score image (PC2) using mean centring/ standard normal variate processing for fried dough enriched with wheat bran (top) and oat bran (bottom) showing

similarities in *magwinya* crumb (A). Score plot of PC2 vs. PC1 score plot showing clusters of pixels. Cyan blue characterised the crust section; green coloured pixels correspond to the crumb region (B); and the loadings line spectra plot of PC2 (C). OB1 – OB20 and WB1 – WB20 represents concentration (g) of oat and wheat bran in *magwinya* formulation

Absorption peaks related to oil in both OB and WB fried dough were 1084 nm, 1089, 1209 nm, 1394 nm, 1716 nm, 2114 and 2305 nm (C-H stretching 2nd overtone and C-H stretching with C-H deformation). The peaks 1084, 2114 nm and 2305 nm represented oil distribution in the negative spectra (Figure 33) depicted in the outer region of the samples where oil uptake is highest; like penetrated oil content quantified in Chapter 4, and supported by previous studies (Pedreschi *et al.*, 2008; Moreno and Bouchon, 2013). Additionally, the presence of cellulose was characterised by absorption bands at 1498 nm (first overtone O-H intramolecular hydrogen bond), 1858 nm (stretching of O-H and C-O bonds); mostly linked to the presence of bran in the samples (Onipe *et al.*, 2015). The prominent peak (1934 nm) in the negative spectra of PC3 is represented by water molecules in the core of OB and WB fried dough associated with bond vibrations in O-H stretching and deformation. This reduced with increasing bran concentration (Onipe *et al.*, 2019). The peaks for oil 1721 nm and 2299 are characterised by C-H stretching in the first overtone and combination bands of N-H and C=O stretching. In PC5, major bands showed the distribution of water (1934 nm) and starch (1542 nm, 1879 nm, 1890 nm, and 2016 nm) comprising of O-H and C-O stretching and combination bands in the core of the samples. 1084 nm could also be classified as benzene with stretching bands of C-H and C-C vibration bonds located in the outer crumb region of the samples. Benzene is a carcinogenic aromatic compound formed during the frying process and is regarded as unsafe (Choe and Min, 2007).

7.3.2.2 Control versus fried batter samples

The Score image and spectra loadings of *magwinya* crumb using MC/SNV processing is shown in Figure 34. Major peaks in the negative space of PC4 spectra (Figure 34) relates to oil (1209, 1716 and 2305 nm) and structurally corresponds to the crust region depicting the penetrated surface oil (PSO) of *magwinya* (See chapter 4). Penetrated surface oil was highest in fried batter samples and this was corresponded by absorption peaks which depicts lipid spectrum in the NIR region. Concentration of oil at the penetrated surface is majorly linked to suction forces linked to oil uptake in the cooling phase (Ziaifar *et al.*, 2008).

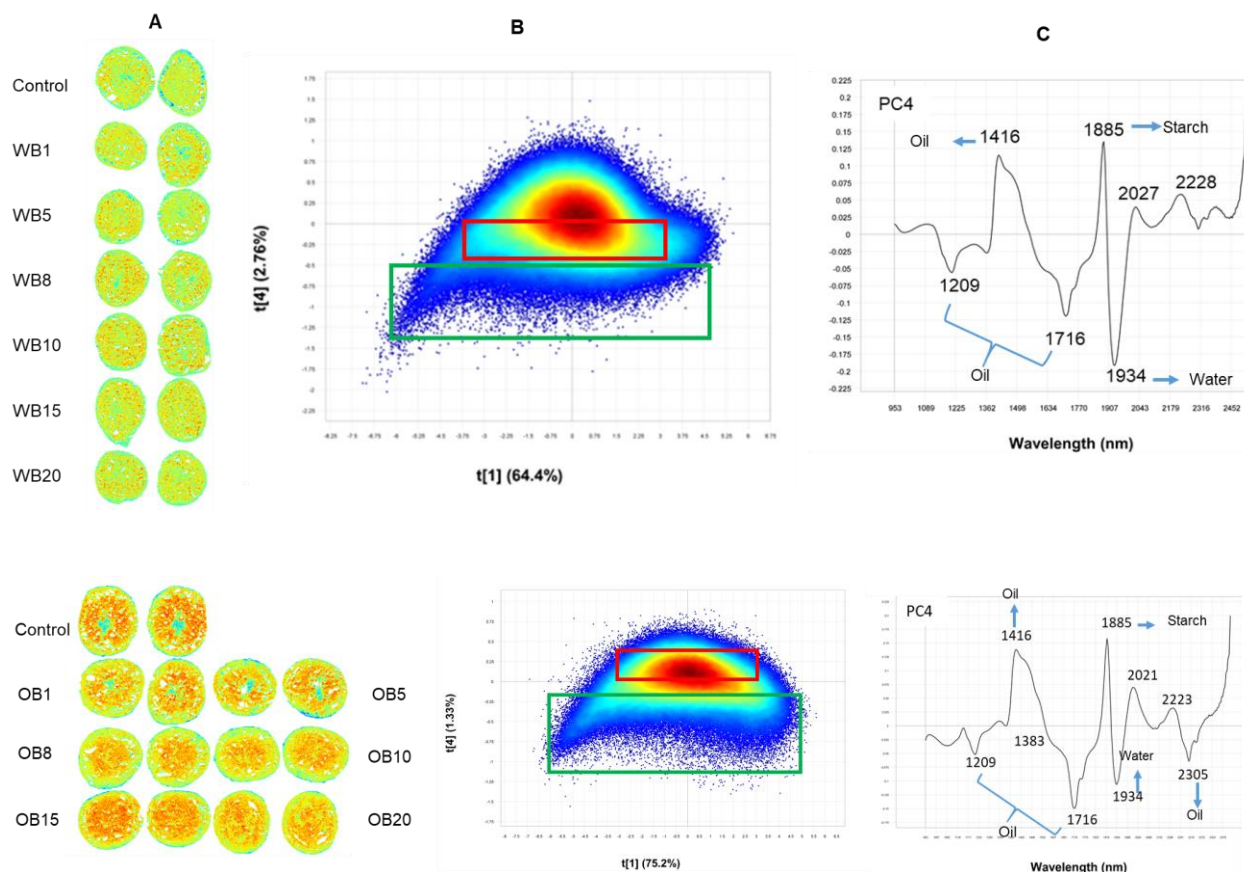


Figure 34. Score image (PC4) using mean centring/ standard normal variate processing for fried batter enriched with wheat bran (top) and oat bran (bottom) showing similarities in *magwinya* crumb (A). Score plot of PC4 vs. PC1 score plot showing clusters of pixels. Red colour (in red box) corresponds to crumb while cyan blue (in the green box) corresponds to crust region (B); and the loadings line spectra plot of PC4 (C). OB1 – OB20 and WB1 – WB20 represents concentration (g) of oat and wheat bran in *magwinya* formulation

Loadings plot both OB and WB fried batter looks similar to a minor difference in terms of wavebands related to oil in the negative spectra. Water was related to a prominent peak at 1934 nm in all PCs. Wavebands related to oil ranged from 1383 to 2305 nm, especially at PC4. First overtone stretching of O-H by intramolecular hydrogen bonds at 1547 nm and O-H and C-O stretching at 1863, 1885 and 1896 nm were related to starch and cellulose (1841 nm) majorly located in the crumb of the samples. Moreover, N-H stretching and NH_3^+ deformation was related to protein in the NIR region of 2223 and 2229 nm. Cellulose was also related to the first overtone of C-H stretching at 1776 nm. The absorption peak at 1176 nm is

characteristic of the second overtone of C–H stretching vibration of HC=CH chemical group; while 1460 featured the first overtone of N-H stretching vibration of CONH₂ functional group.

7.3.3. Mean centring and Savitsky-Golay (SG) second derivative processing of bran-enriched *magwinya*

7.3.3.1 Control versus fried dough samples

The application of MC/SG second derivative to *magwinya* crumb across the principal components revealed wavelengths ranging from 1116 to 2435 nm, with higher absorption peaks related to starch, cellulose water and oil. MC and S-G 2nd derivative featured wavelengths related to oil at 1411, 1405, 1427, 1710 1716, and 2392 nm (Figure 35). These oil absorption bands were mainly based on C-H stretching in the first overtone, second overtone and combination vibration bonds. The spectrum range for lipids in the NIR region by Fei *et al.* (2017) corresponds to the range in this study. Lipids with spectrum range of 1400 – 1720 are the result of C-H stretching with peaks of polyunsaturated fatty acids at 1720 range. However, wavelengths predicting oil were observed in the region between 1400 nm and 1430 nm based on the hydroxyl group which may be associated with alcohols- which are by-products of oxidation reaction during the frying process (Diop *et al.*, 2014). Spectra of fish oil, animal fats and pure triglycerides have been reported within 1714 – 1760 nm range (Sato, 1994; Garrido-varo *et al.*, 2008).

Wavelengths that featured starch 1438 1547, 1879, 1897, 1901, 2016 nm (as a result of O -H and C-O stretching and deformation), 2228, 2234 (O-H stretching and deformation) 2435 nm (O-H, C-O stretching), and 2228 nm (C-H, C=O bond of –CHO structure). The wavelengths 1858, 2386 and 2397 nm were related to cellulose content of *magwinya*, majorly influenced by bran incorporation. Highest absorption band of the three was 2397 nm was observed in the positive spectra of PC3 and its distribution in the spectra image showed distribution in the entire crumb of *magwinya*.

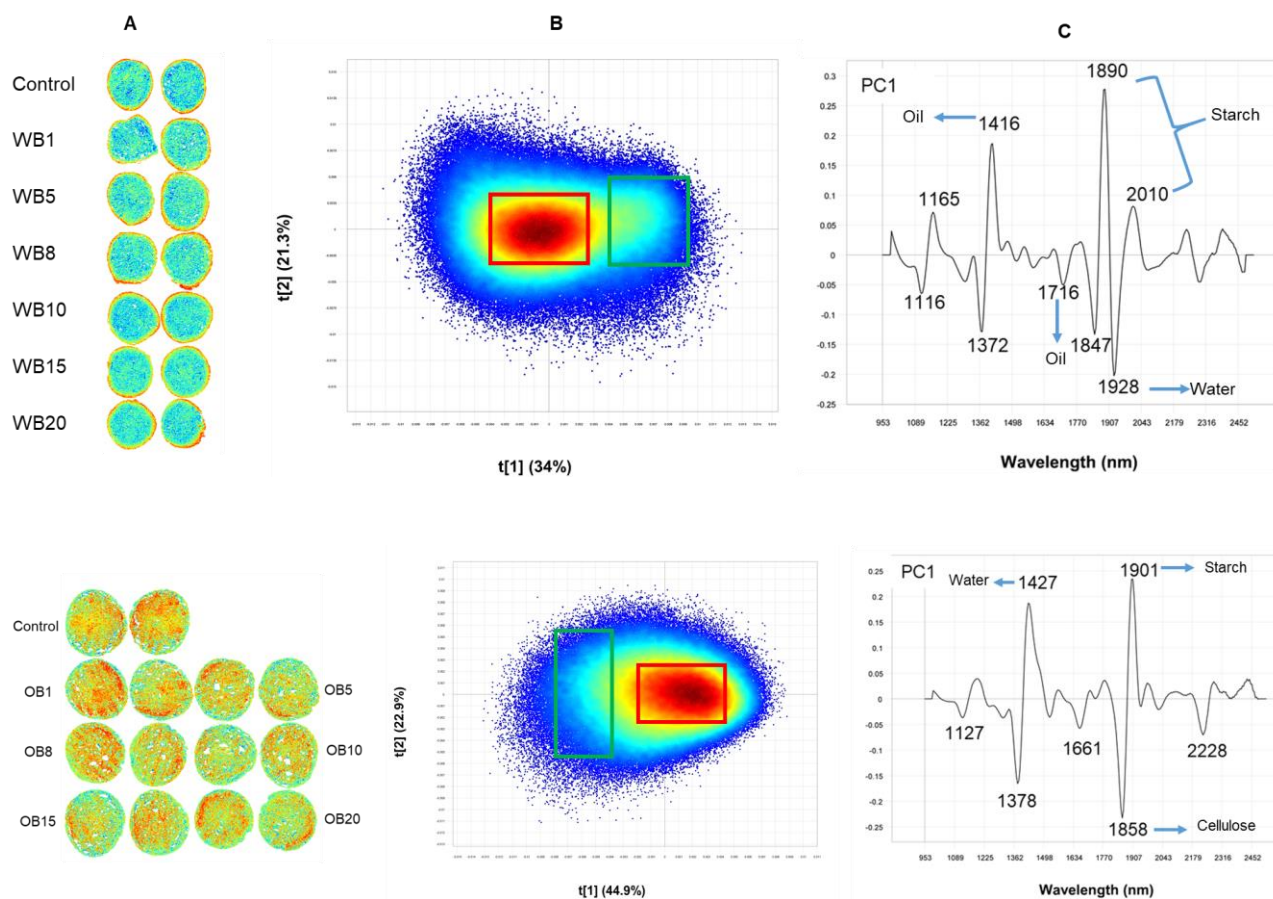


Figure 35. Score image (PC1) using mean centring/ Savtzky-Golay 2nd derivative processing for fried dough enriched with wheat bran (WB) -top and oat bran (OB) – bottom; showing similarities in *magwinya* crumb (A). Score plot of PC1 vs. PC2 score plot showing clusters of pixels. Red colour (in red box) corresponds to crumb in OB samples and crust in WB samples (B); and the loadings line spectra plot of PC2 (C). OB1 – OB20 and WB1 – WB20 represents concentration (g) of oat and wheat bran in *magwinya* formulation

The score image of WB fried dough showed red pixels in the crust section which corresponded to oil in the loadings line; while in OB samples, the red pixels correspond to starch content (Figure 35). The peaks associated with protein in *magwinya* crumb were 1509 (first overtone N-H stretching) and 1994 nm (N-H asymmetric stretching + amide II). The crumb of *magwinya* is linked to gas cell formation and retention during processing from the viscoelastic structure of gluten proteins. The presence of aromatics in *magwinya* crumb was due to C-H stretching in the second overtone at 1116, 1122, 1127, and 1133 nm. Thermal oxidation that occurs during deep-frying leads to the formation of aromatic compounds (Choe and Min, 2007). Absorption peaks related to water were related to C=O stretching in the second overtone of

chemical group CONH (1928 nm) and O-H stretching and deformation vibration bonds (1934 and 1945 nm). Other prominent wavebands 1165, 1171, 1302, and 1367 nm are characteristic of the second overtone of C-H stretching vibration of various chemical groups ($-\text{CH}_2$, $-\text{CH}_3$, $-\text{CH}=\text{CH}-$). The wavelength 1660 nm is an absorption band of fatty acids with *cis* double bonds of the $\text{RCH}=\text{CHR}_1$ chemical group (Fei *et al.*, 2017).

7.3.3.2 Control versus fried batter samples

Score images and spectra loadings line using application of MC and S-G second derivative is presented in Figure 36. Absorption peaks observed in oat bran fried dough are quite similar in composition to oat bran fried batter.

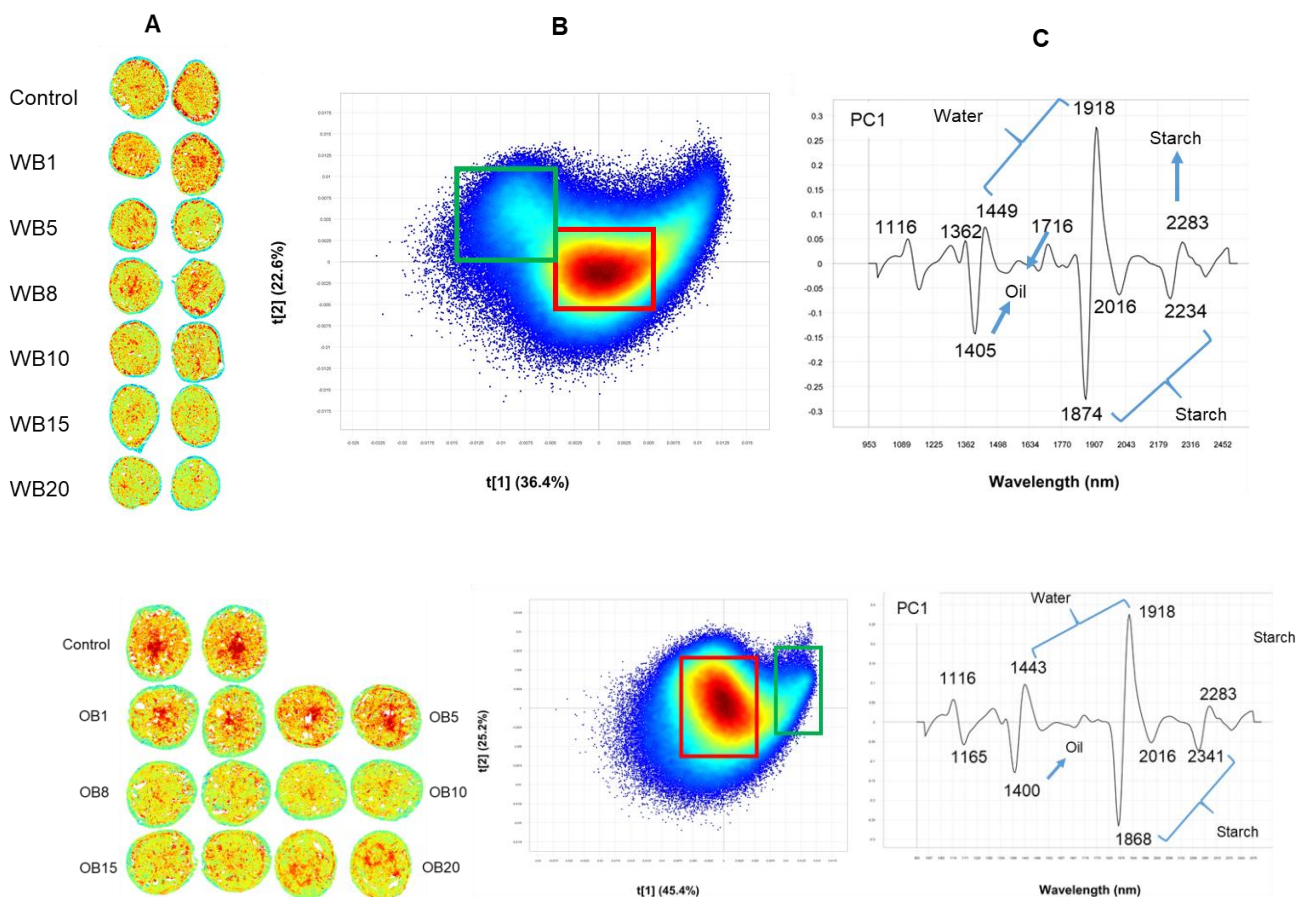


Figure 36. Score image (PC1) using mean centring/ Savtzy-Golay 2nd derivative processing for fried batter enriched with wheat bran (WB) – top and oat bran (OB) – bottom; showing similarities in *magwinya* crumb (A). Score plot of PC1 vs. PC2 score plot showing clusters of pixels. Red colour (in red box) corresponds to crumb and cyan blue corresponds to the crust region (B), and the loadings line spectra plot of PC2 (C) OB1 – OB20 and WB1 – WB20 represents concentration (g) of oat and wheat bran in *magwinya* formulation

The red colour in the score image of *magwinya* showed similarity in control, OB1 and OB5 fried batter samples (Figure 36) as well as control, WB1-WB8 fried batter. This similarity is mostly due to the water content of the samples which diminished with an increase in bran concentration. Maximum absorption of PC1 featured starch (1868 - 2283 nm), oil (1400, and 1716 nm). Absorption peaks in other PCs featured protein (1988 nm), cellulose (2337 nm), which was almost evenly distributed in the crumb (Figure 36), while maximum peaks in positive spectra featured oil (1204, 1710 nm), starch (1863 nm), alcohols (2370), aromatics (1422 nm), amino acids (2299). The loading line plots of fried batter differentiating the PCs were dominated by the OH stretching and deformation combination mode for water (1920–1940 nm) which is an important feature of fried batter samples.

7.4. Conclusion

Hyperspectral imaging was applied for classification of *magwinya* based on bran concentration. The pixel-wise PCA results were influenced by similarity in the samples, which overshadowed the less prominent differences in chemical composition between the closely related classes. The main sources of variance identified in the initial PCs were due to differences within *magwinya* type (fried dough and batter) and not due to differences between the classes (bran concentration). There were obvious structural differences between both sets of products. Fried batter samples were characterised by more gas cells in the crumb than fried dough which had more compact crumb structure. Meaningful trends were identified in spectral data analysed with SNV and S-G mostly identifying bands related to oil at the crust perimeter, while water molecules were distributed within the crumb of *magwinya*. Other compounds including aromatics, alcohols, were also featured in the wavebands. The use of PCA in mapping the distribution of these compounds in *magwinya* is a preliminary and effectual step towards future quantitative analysis and model prediction of oil, moisture and chemical components of *magwinya*.

CHAPTER 8: GENERAL CONCLUSION

8.1. Conclusion

Magwinya is a streetwise popular African snack that has been in existence for a long time and is likely to continue for generations to come. *Magwinya* production and sales generate income for producers; while consumers get ready-to-eat food at convenience. Thus, the survey carried out in this study generated knowledge on *magwinya* processing, sales and consumption patterns which provided viable information repository for future research. It was found out that technological challenges still exist in the production process due to lack of an automation system for mixing, proofing, frying and draining. Considering the fourth industrial revolution, and feedback from *magwinya* producers in Thohoyandou area, development of *magwinya*-production machine encompassing all unit operations is recommended.

Wheat flour was partially substituted with oat and wheat bran at 1 – 20% for quantification of the oil fractions SO, PSO and STO. Reduction of total oil content in OBFB was noted at 10% OB and from 5% in OBFD. The PSO fraction contributed mostly to the total oil content of all the samples at 46% for Wbfd, 53% and 57% for Wbfb and OBFb respectively. Oil uptake in *magwinya* is a surface-related phenomenon, but the distribution of the oil fractions varied from one snack-type to the other. Furthermore, confocal microscopy revealed notable differences in terms of oil distribution, depth of oil penetration, structure and pore properties of the fried products, thus emphasising the impact of ingredient formulation (water and bran variation) on oil uptake reduction in *magwinya*. Porosity is a contributing structural factor to oil influx in *magwinya*. In comparison to the control sample, a reduction in porosity was observed for all samples. Cubic polynomial relationship was established between PO_{ia}, porosity and PO_{sox} for *magwinya* crumb, as well as between FD and crust hardness and surface oil for the crust. Penetration of oil into the crumb reduced and varied among the samples with a reduction of initial moisture content and increase in bran concentration, albeit a minimal effect was observed for bran type.

Furthermore, *in vitro* assay of *magwinya* revealed significant reduction in RAG, RDS and eGI of *magwinya* with bran addition. This means OB and WB are suitable proponents for blood glucose management and delayed carbohydrate absorption in the bloodstream. Oat and WB had a similar effect on reduction of RAG and RDS values of fried dough. However, WB had an improved effect in fried batter products. *Magwinya* is widely known as an unhealthy food and proposed to be a high GI. The reduction of *magwinya* GI from high (80.03) to medium (< 70) due to bran addition and moisture variation proves that improvements to the food is possible.

Through the application of different pre-processing methods in the PCA model space, wavebands depicting mostly oil, protein, water and starch content of *magwinya* were identified. Other compounds including aromatics, alcohols, were also featured in the wavebands. The use of PCA in mapping the distribution of these compounds in *magwinya* is a preliminary and effectual step towards future quantitative analysis of chemical components of *magwinya*.

8.2. Contribution to knowledge and recommendations

The outcomes of this study, for the most part, is the first of such on *magwinya* research, and therefore, make the following scientific contributions and recommendations to the study of *magwinya* or other fried dough/ batter foods.

1. Data repository for production and consumption statistics of *magwinya* in Thohoyandou area.
2. Oil uptake in *magwinya* is surface-based and can be reduced through ingredient modification. This is especially important to *magwinya* (or fried food) processors for future improvements.
3. Estimation of *magwinya* GI which can serve as baseline information to nutritionists and consumers. *In vitro* studies only cater for estimation of starch digestion and glycemic index. Nevertheless, *in vivo* study using human or animal subjects is highly recommended to obtain *magwinya* GI.
4. Non-invasive technologies such as CLSM and HSI for visualisation and quantification of chemical properties of *magwinya*. These methods were effective for evaluation of crumb features, oil penetration and mapping chemical constituents of *magwinya*. The use of other software such as MATLAB for HSI data analysis and modelling and C-Cell for crumb grain features is recommended.

Considering the wealth of information provided in this thesis, there exist rich prospects for future studies and improvements to the work done.

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APPENDICES

Appendix I. Sample size calculation

Determine Sample Size

Confidence Level:	95% ▾	i
Population Size:	58000000	i
Proportion:	0.5	i
<input type="radio"/> Confidence Interval:	0.03847	i
Upper	0.53847	
Lower	0.46153	
<input type="radio"/> Standard Error	0.01963	i
<input type="radio"/> Relative Standard Error	3.93	i
<input checked="" type="radio"/> Sample Size:	650	i

(<https://www.abs.gov.au/websitedbs/D3310114.nsf/home/Sample+Size+Calculator>)

Appendix II. *Magwinya* Production Survey

Instruction: Please answer all questions by ticking or writing the appropriate answer in the answer space provided.

Section 1: Demographic information

Name of respondent/ Restaurant (name will be kept confidential)		
Position of respondent		
Location		
Province		Limpopo
Age (Years)		20 - 30 31 - 40 41 - 50 51 - 60 61 - above
Gender		Male Female
Qualification Level	Certificate	
	Diploma	
	Degree	
<i>Magwinya</i> production experience (years)	Less than 1 year	
	1 – 5	
	6 - 10	
	11 - 15	
	15- above	
Date		

Section 2: *Magwinya* production process

- Where did you learn the art of making *magwinya*?
a. Catering school__ b. Home__ c. Online__ d. Others (specify)_____
- What type of flour do you use in *magwinya* production?
a. Bread flour__ b. Cake flour__ c. Whole flour__ d. Others (specify)_____
- Do you measure the ingredients used in *magwinya* production?
a. Yes____ b. No____
- If yes to **question 3**, which of the following measurements do you use?
a. Cups__ b. Kitchen scale__ c. Instinct__ d. Other (specify)_____
- What is the proportion of ingredients used
a. Flour____ b. sugar ____ c. yeast____ d. salt____ water____
- Any other ingredient used
a. Spices _____
b. Flavours _____
- Mode of mixing
a. Manual____ b. Kitchen mixer____ c. Industrial mixer____
- How long do you allow the dough to rise?
a. 45 min__ b. 1 hr__ c. 2 hr__ d. Others (specify)_____
- Where do you put the dough to rise?
a. Counter-top__ b. Proofing cabinet__ c. Other (specify)_____

10. How long is the frying time?
 a. 3 min____ b. 5 min____ c. 10 min____ d. Other (specify)____
11. What is the shape of your *magwinya*?
 a. Round____ b Flat____ c. Other (specify)_____
12. Do you use any special equipment in *magwinya* production?
 a. Yes____ b. No____
13. If yes to above question, please list the equipment

Section 3: Sales

14. How many *magwinya* do you make per bag of flour?
 a. 2.5 kg____ b. 5 kg____ c. 10 kg____ d. 25 kg____
15. How many bag(s) of flour do you use up per day?
 a. 2.5 kg____ b. 5 kg____ c. 10 kg____ d. 25 kg____
16. What is the size of your *magwinya*?
 a. Small____ b. Medium____ c. Large____
17. How many *magwinya* do you sell per day?
 a. Small____ b. Medium____ c. Large____
18. What is the price of one (1) *magwinya*?
 a. Small____ b. Medium____ c. Large____
19. How many days per week do you sell *magwinya*? _____
20. What time of the day is your peak sales?
 a. Morning____ b. Afternoon____ c. Evening____
21. Do you meet your daily sales target?
 a. Yes____ b. No____
22. After daily sales is there demand for more?
 a. Yes____ b. No____
23. If yes to **question 22**, what limits your production
 a. Labour _____
 b. Finance _____
 c. Others (specify) _____
24. Do you have left overs
 a. Yes____ b. No____
25. If yes to question 23, what happens to the left overs
 a. Thrown away _____
 b. Re-sold _____
 c. Re-fried _____

Comment/Remarks

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Thank you for your time!

Appendix III. *Magwinya* Consumption Survey

Instruction: Please answer all questions by ticking or writing the appropriate answer in the answer space provided.

Section 1: Demographic information

Name of respondent (name will be kept confidential)		
Title		
Occupation		
Location		
Province		Limpopo
Age (Years)		16 - 20 20-30 31-40 41-50 51-60 61- above
Gender		Male Female
Highest qualification	Grade 12	
	Diploma	
	Degree	
	Post graduate	
Date		

Section 2: *Magwinya* consumption

- How much do you like *magwinya*?
 - Like extremely
 - Like very much
 - Like moderately
 - Neither like nor dislike
- How often do you eat/consume *magwinya*?
 - Everyday
 - Twice a week
 - Thrice a week
 - Once a month
- How do you eat *magwinya*?
 - Main meal
 - Snack
 - Side dish
 - All of the above
- If the answer to **question 3** is **main meal**, which one is it?
 - Breakfast
 - Lunch
 - Dinner

All of the above

5. What size do you consume?

- Small
- Medium
- Large
- All of the above

6. Do you eat your *magwinya* plain?

- Yes
- No
- Sometimes
- Most of the time

7. If no to question 6, what do you garnish it with?

- Curry
- Fish
- Meat
- Polony
- Cheese
- Russian
- Atchaar
- Other (specify) _____

8. Which drink do you consume *magwinya* with?

- Soft drink
- Coffee
- Tea
- Energy drink
- Water
- Other (specify) _____

Section3: *Magwinya* purchase

9. Where do you like to buy *magwinya* around Thohoyandou?

- Univen cafeteria
- Univen tuck shop
- Roadside vendors
- Other (specify) _____

10. Based on your answer to **question 9**; why do you purchase at that location?

- Proximity/ closeness to the seller
- Price
- Cleanliness
- Size of *magwinya*
- Others (specify) _____

11. Compared to other snacks, why do you buy *magwinya*?

- Price
- Personal likeness
- Satisfaction

Other (specify)

Section 4: *Magwinya* characteristics

12. What is your favourite characteristic of *magwinya*?

- Taste
- Shape
- Smell
- Oiliness
- Other (specify) _____

13. Do you like the oiliness of *magwinya*?

- Yes
- No
- To an extent

14. If **no to question 13**, will you prefer *magwinya* with reduced oil?

- Yes
- No

15. Are you aware of the health implications of regular consumption of fried foods like *magwinya*?

- Yes
- No
- To an extent

16. If the oiliness of *magwinya* is reduced, will you purchase it?

- Yes
- No

Comment/Remarks

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Thank you for your time!

Appendix IV. Consumer responses across age groups

Information		Age						Sig*
		≤20	21-30	31-40	41-50	51-60	61+	
<i>Magwinya</i> likeness	Like extremely	41	47	6	2	0	1	<0.001*
	Like very much	111	52	5	0	0	0	
	Like moderately	87	183	14	6	1	1	
	Neither like nor dislike	39	30	6	2	0	0	
Consumption frequency	Everyday	94	44	6	0	0	1	<0.001*
	Twice a week	93	93	13	3	0	0	
	Thrice a week	40	74	1	1	0	0	
	Once a month	51	101	11	6	1	1	
How <i>magwinya</i> is eaten	Main meal	102	88	7	2	0	1	0.05*
	Snack	96	131	19	6	0	1	
	Side dish	62	57	3	2	1	0	
	All the above	18	36	2	0	0	0	
Main meal type	Breakfast (B)	90	80	8	2		1	0.65
	Lunch (L)	53	33	1	0		0	
	Dinner (D)	3	1	0	0		0	
	B + L + D	8	17	2	0		0	
	B + L	4	4	0	0		0	
Size consumed	Small	62	18	1	2	0	0	<0.001*
	Medium	121	29	11	3	1	2	
	Large	83	261	19	5	0	0	
	All of the above	12	4	0	0	0	0	
Eat <i>magwinya</i> plain	Yes	51	141	20	6	0	1	<0.001*
	No	106	57	4	3	0	0	
	Sometimes	108	99	6	1	0	1	
	Most of the time	13	15	1	0	1	0	
<i>Magwinya</i> garnish	Curry	3	1	0	1	0	0	0.06
	Fish	4	8	0	1	0	0	
	Meat	4	3	0	0	0	0	
	Polony	44	15	3	0	0	0	

	Cheese	5	7	0	0	0	0	
	Russian	25	6	1	0	1	1	
	Atchar	63	44	3	2	0	0	
	Potato chips	54	17	1	0	0	0	
	Avocado	1	0	0	0	0	0	
Accompanying drink	Soft drink/Juice	149	158	10	3	1	0	0.15
	Coffee	24	19	2	1	0	0	
	Tea	40	70	11	4	0	2	
	Energy drink	10	6	1	0	0	0	
	Water	52	58	6	2	0	0	
Where <i>magwinya</i> is purchased	School cafeteria	34	148	9	3	0	0	<0.001*
	School tuck shop	56	128	12	5	0	1	
	Roadside vendors	49	22	5	1	1	1	
	School market	135	3	2	0	0	0	
	Supermarket	4	11	3	0	0	0	
Reason for purchase	Proximity	72	115	11	3	1	0	0.13
	Price	75	49	4	0	0	1	
	Cleanliness	68	78	9	3	0	1	
	Size	56	47	6	2	0	0	
	Better taste	6	13	1	1	0	0	
	Less fat	1	7	0	0	0	0	
<i>Magwinya</i> purchase compared to other snacks	Price	86	74	7	1	0	0	<0.001*
	Personal likeness	124	116	9	4	0	0	
	Satisfaction	68	120	15	3	1	1	
	Cravings	0	1	0	1	0	1	
Favourite <i>magwinya</i> characteristics	Taste	227	259	20	5	0	1	0.03*
	Shape	17	15	7	1	0	1	
	Smell	27	30	2	3	1	0	
	Oiliness	6	12	1	0	0	0	
	All of the above	0	3	0	0	0	0	
Like <i>magwinya</i> oiliness	Yes	76	31	3	2	1	1	<0.001*
	No	189	256	21	6	0	0	
	To an extent	13	25	7	2	0	1	
Prefer <i>magwinya</i> with less oil	Yes	183	262	24	6	-	-	<0.001*

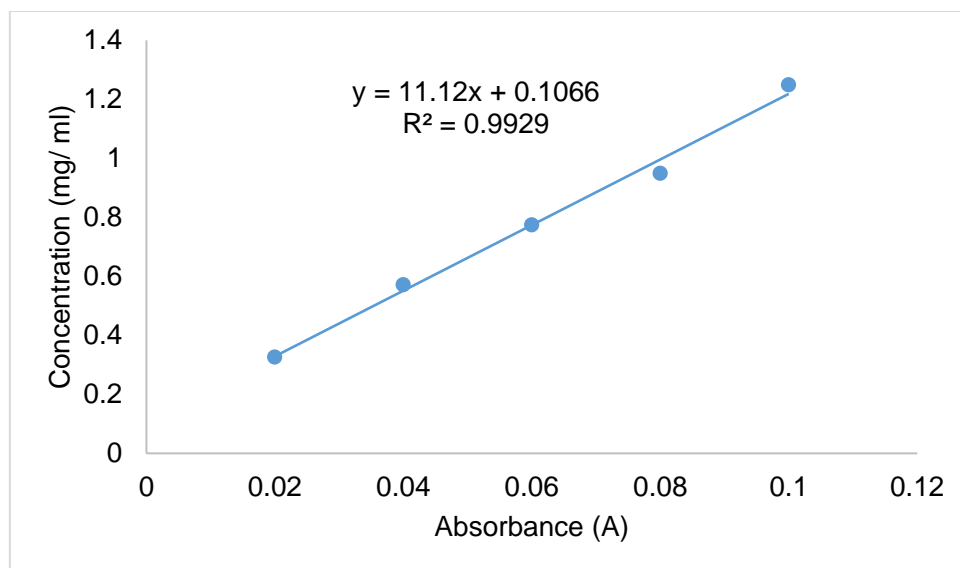
	No	36	10	1	0	-	-	
Awareness of health implications of frequent fried food consumption	Yes	183	236	21	6	0	1	0.06
	No	77	56	5	3	1	1	
	To an extent	18	20	5	1	0	0	
Will purchase low fat <i>magwinya</i>	Yes	218	294	28	9	0	0	<0.001*
	No	59	17	3	1	1	2	

*Pearson's Chi square test of significance at $P < 0.05$

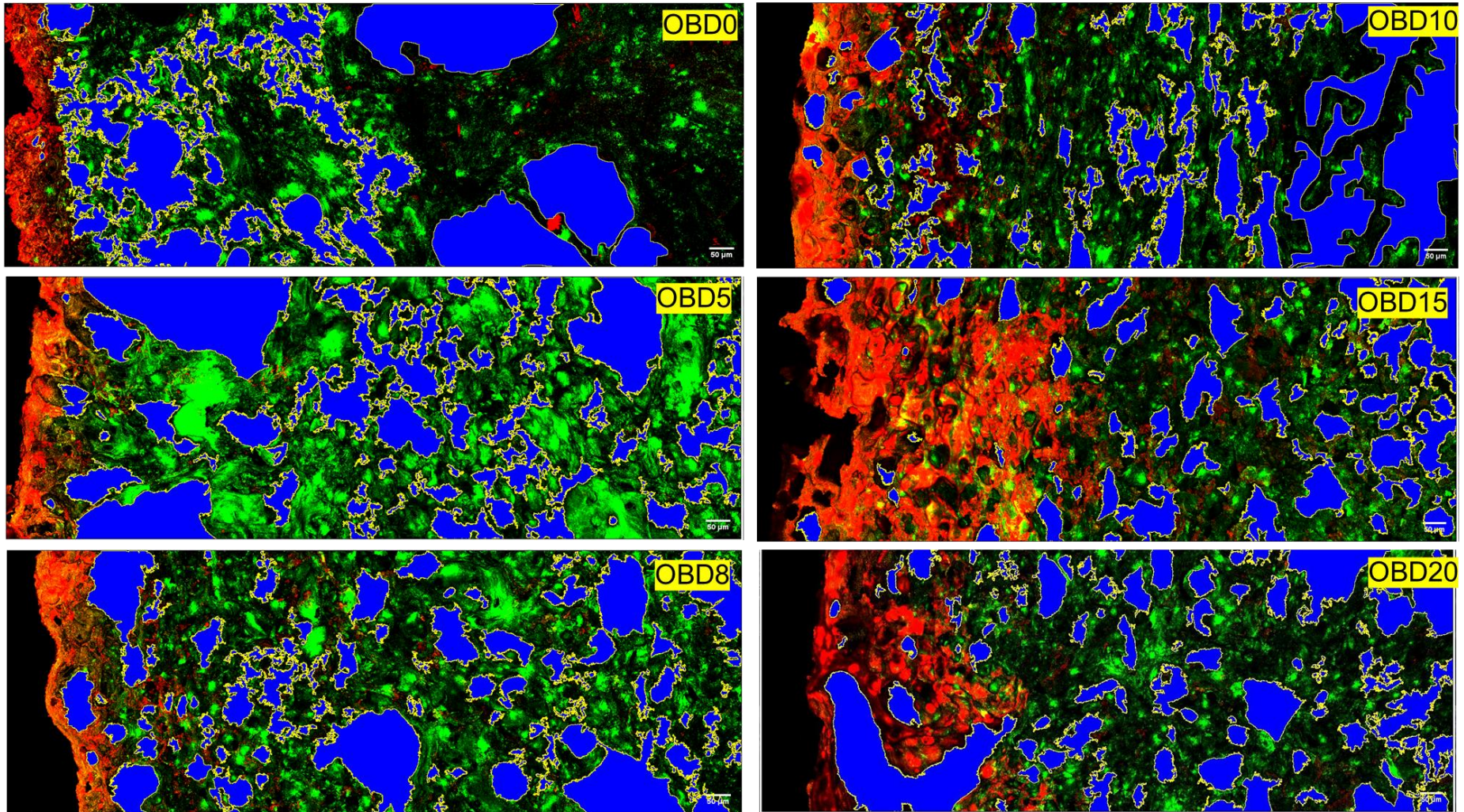
Appendix V. Preliminary findings for proofing temperature of *magwinya*

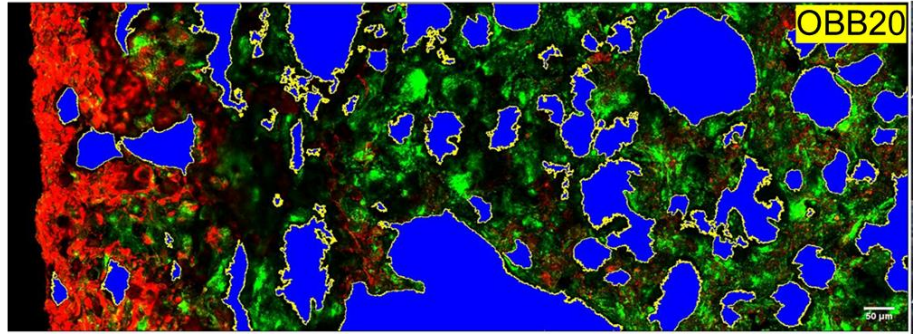
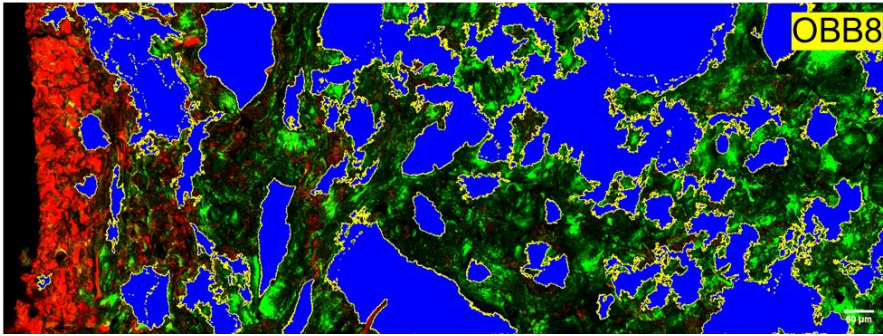
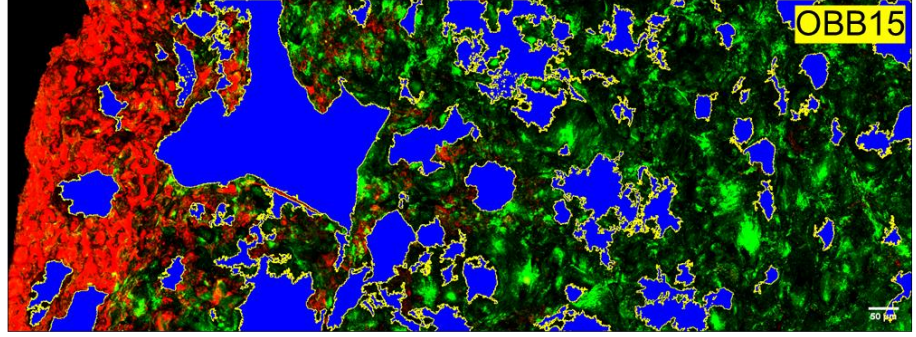
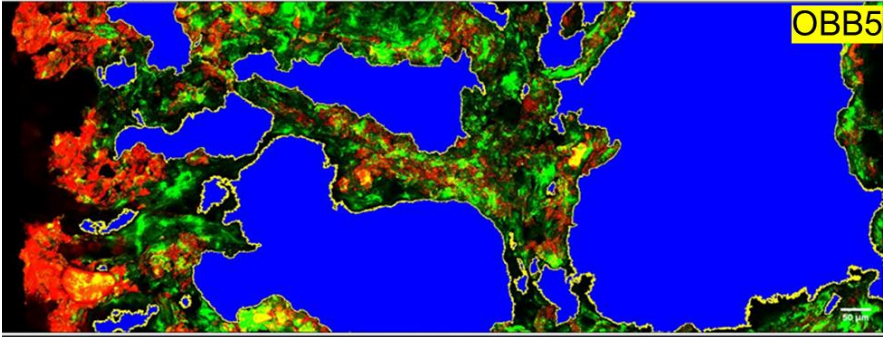
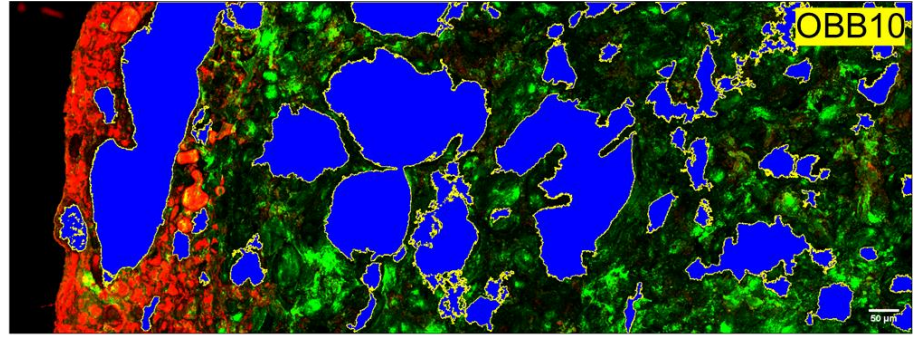
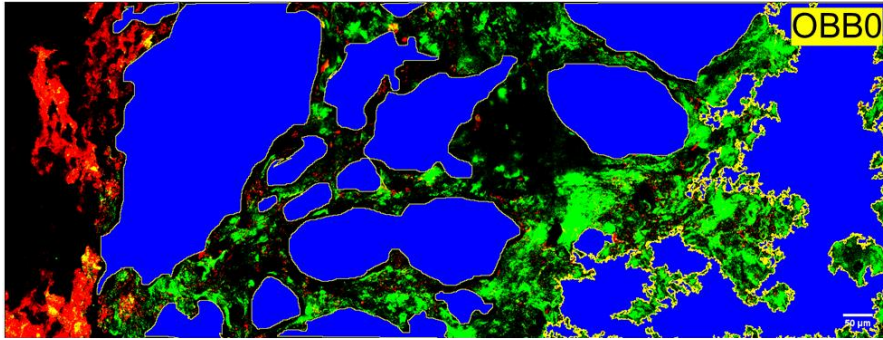
The aim of this preliminary study was to determine the effect of wheat bran addition, fermentation (FET) and frying temperature (FRT) on oil absorption of *magwinya*. Substitution of wheat bran (WB) in wheat flour was done at 1, 8, and 15%, and dough was fermented at 30°C, 33°C and 35°C for 1 h and fried at 160°C, 170°C and 180°C for 5 min. Analysis were done in triplicate and for oil content in duplicate. Statistical analysis was done using SPSS version 24 and the data obtained was measured by multivariate analysis of variance. Resultant *magwinya* was analysed for ash, moisture, oil content, dry matter, colour and texture analysis (hardness). Control sample was *magwinya* without WB, fermented at 35°C and fried at 180°C. Results indicated that moisture content ranged from 31.78 to 47.98%, dry matter from 52.02 to 67.03%, ash content from 2.76 to 4.49%, oil content from 1.15 to 7.87%, crust hardness from 43.94 to 64.00g and crumb hardness from 68.01 to 85.10g at FRT of 160 to 180°C, FET of 30, 33 and 35°C and WB addition of 1 to 15 g. The L* value of the crust ranged from 27.98 to 48.89 and the crumb from 50.40 to 63.46, the a* value of the crust ranged from 10.55 to 18.48 and for the crumb ranges from 1.83 to 7.99, the crust b* value ranged from 10.16 to 30.83 and for the crumb from 18.80 to 21.70 at FRT of 160 to 180°C, FET of 30, 33, 35°C and WB addition of 1 to 15 g. Moisture content and dry matter were significantly higher ($P < 0.05$) with the interaction effect of FRT and WB addition and no significant difference ($P > 0.05$) in ash content, crust hardness and colour attributes (L*, a*, H° and ΔE) of the crumb including H° and ΔE of the crust with the interaction effect of FET, FRT and WB. The crumb hardness, colour attributes (L*, a*, b*, and C) of the crust and oil content were significantly lower ($P < 0.05$) with the interaction effect of FET, FRT and WB addition. Higher FET and FRT resulted in high oil absorption of *magwinya* and was optimal at the **FET of 30°C** and FRT of 170°C. Incorporation of WB initially reduced the oil content of *magwinya* reaching optimal at 8 g but increased at 15 g. Optimum WB, FET and FRT conditions generated in this study can be considered for future processing of *magwinya* for oil reduction.

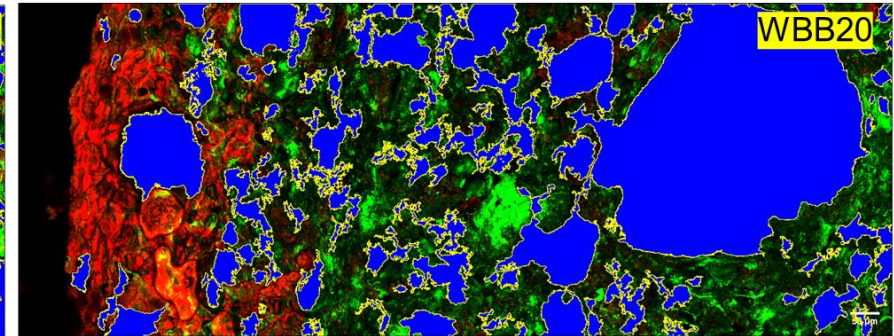
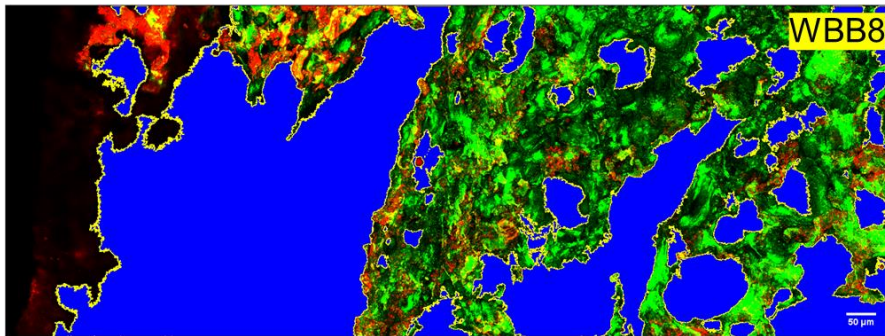
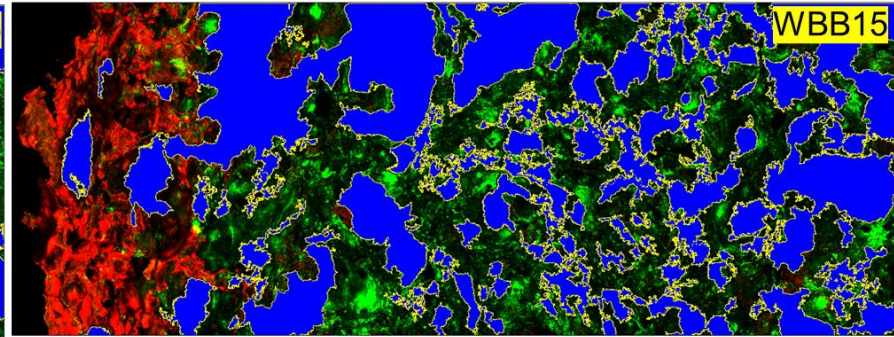
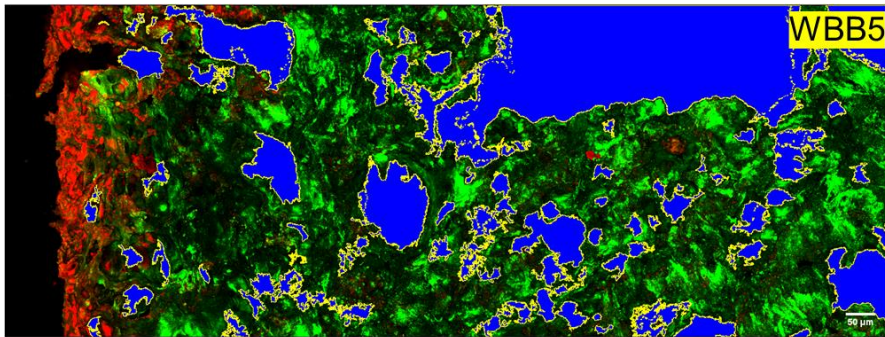
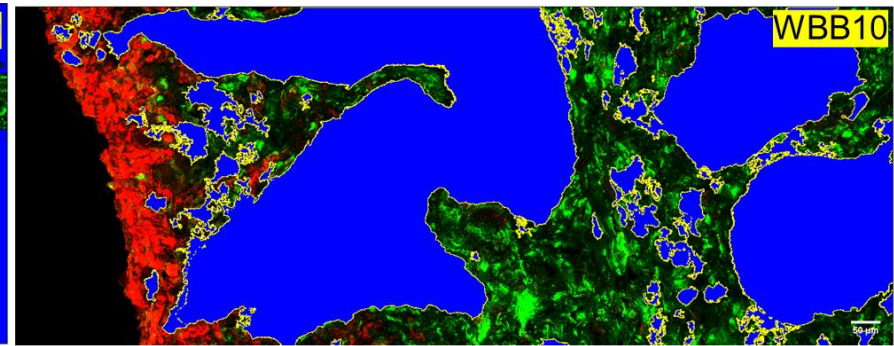
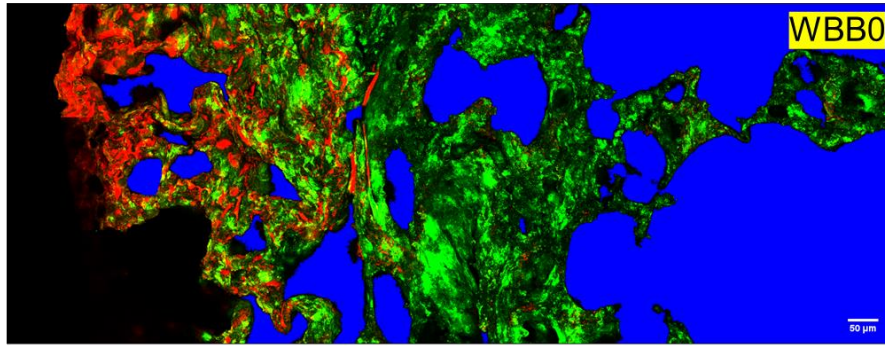
Appendix VI. Standard curve of Sudan III dye concentrations against absorbance

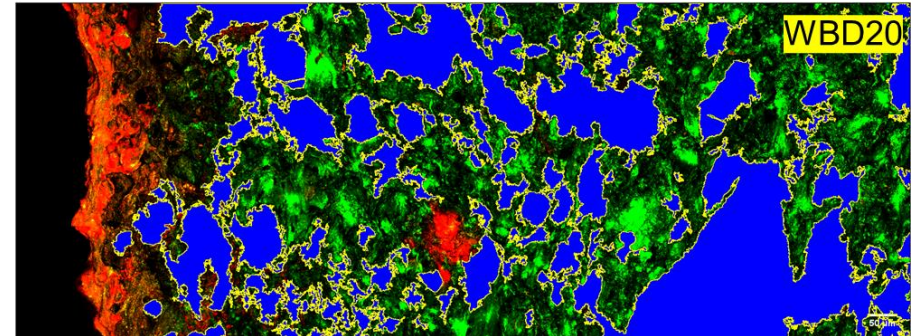
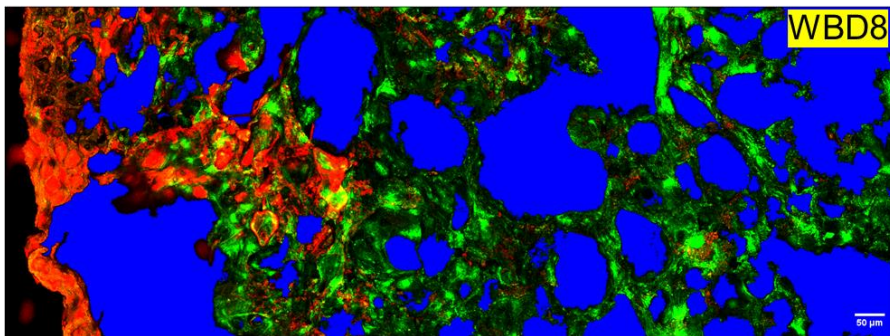
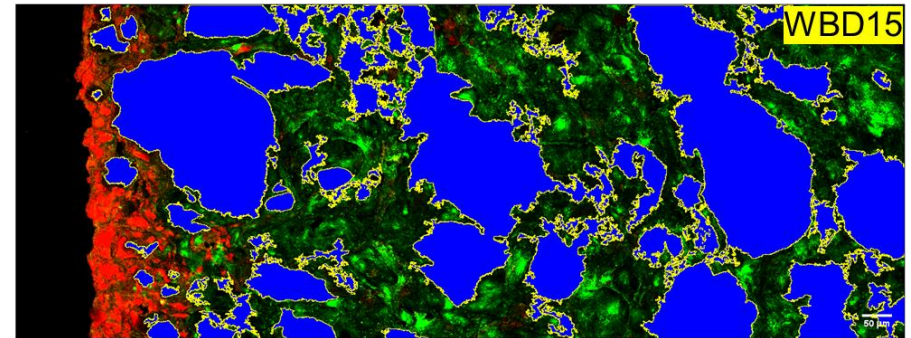
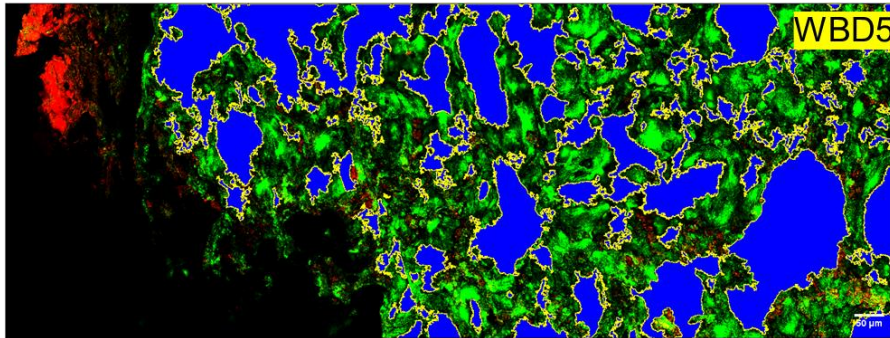
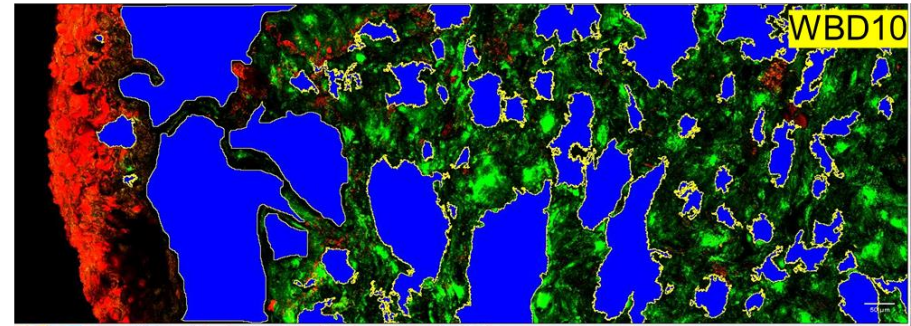
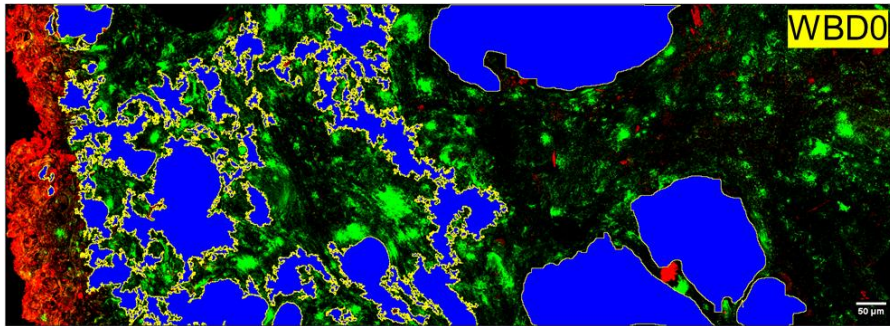


Appendix VII. Segmented pores of *magwinya* cross-section micrographs









Appendix VIII: Multivariate analysis showing main and interaction effects of independent variables on fractal dimension

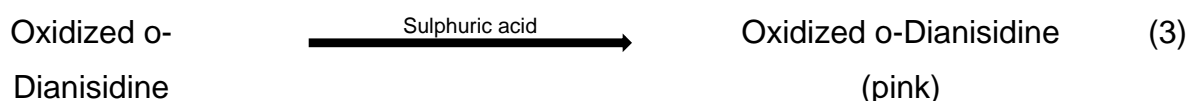
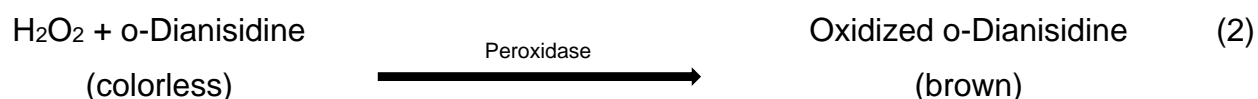
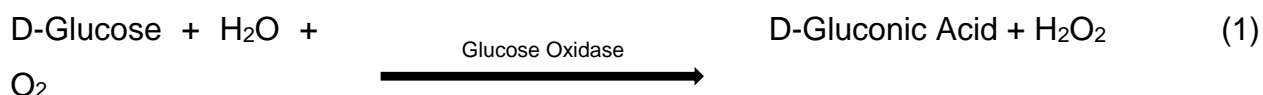
Source	Type III Sum of Squares	df	Mean Square	F	Sig. ^b
Corrected Model	.342 ^a	23	0.015	4.213	< 0.001
Intercept	258.125	1	258.125	73229.375	< 0.001
X	0.01	1	0.01	2.705	0.104
Y	0.064	1	0.064	18.127	< 0.001
Z	0.053	5	0.011	3.013	0.016
X * Y	0.067	1	0.067	19.016	< 0.001
X * Z	0.046	5	0.009	2.617	0.031
Y * Z	0.078	5	0.016	4.402	0.001
X * Y * Z	0.024	5	0.005	1.378	0.243
Error	0.254	72	0.004		
Total	258.72	96			
Corrected Total	0.595	95			

X = bran type, Y = initial moisture content, Z = Bran concentration (g)

(a) $R^2 = 0.60$ (b) Computed using alpha = .05

Appendix IX. Measurement of glucose using glucose-oxidase reagent kit

Oxidation of glucose to gluconic acid and hydrogen peroxide through the action of glucose oxidase (Equation 1). The hydrogen peroxide in turn reacts with colourless o-dianisidine in the presence of peroxidase to form a brown-coloured product (Equation 2). The final reaction takes place between oxidized o-dianisidine reacts and sulfuric acid to form a more stable pink coloured product (Equation 3) – whose intensity was measured at 530 nm and is proportional to the original glucose concentration in the sample.



The GAGO kit was prepared according to the manufacturer's instructions thus: Content of one (1) Glucose Oxidase/Peroxidase Reagent (G 3660) capsule (containing 500 units of glucose oxidase (*Aspergillus niger*), 100 purpurogallin units of peroxidase (horseradish) and buffer salts) was dissolved in an amber bottle with 39.2 ml of deionized water. The solution was prepared prior to use and the excess was stored at 2 – 8°C and was discarded when turbidity developed. Next, the o-Dianisidine Reagent (D 2679) vial containing 5 mg of o-dianisidine dihydrochloride powder was reconstituted with 1 ml of deionised water and inverted several times to dissolve properly. The vial was wrapped in foil paper to avoid exposure to light. The o-danisidine reagent (0.8 ml) was transferred to the 39.2 ml glucose oxidase reagent in the amber bottle and contents were mixed by inverting several times.