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Nutritional Qualities, Health Benefits and the Potential for Complementary Food Formulation of Three Types of *Amaranthus Caudatus* Grain Cultivated in Ethiopia as Affected by Processing

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Submitted to Center for Food Science and Nutrition for the Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Food Science and Nutrition

June, 2015

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Acknowledgement

I would like to express my deepest gratitude to my advisor professor Gulelat Desse. He is not only a good mentor but also a best friend and a father throughout my stay in the Center. I did not forget his welcoming approach starting from the day I joined the Center. He really strives for my success and encourages me a lot to run over the success truck. I owe you Dr. Gulelat!

I am indebted to Dr. Claire Mouquet-Rivier, for her unreserved support and follow up of my research. Dr. Claire, I am happy to have you as an advisor. I am lucky to be hosted in your lab and conduct the major part of the research. I really appreciate your patience, dedication to follow my experiments in the lab, motivation to design my research and reviewing my manuscripts despite your busy schedule. The smile in your face and happiness when I succeed in the lab is really unforgettable. Dr. Claire, you had a significant contribution in my academic excellence, so thank you very much.

I am also grateful to Dr. Abdualziz Adish for his supervision and designing of my project. His positive feedback and encouragement help me a lot to complete my project. I really appreciate your viewpoint when you comment my work.

Dr. Kaleab Baye, I couldn't forget your support throughout my project especially when we were in France. You inspire me when I am down with frustration and made my stay in France enjoyable. This work is completed with your motivation and advice in how to treat challenges.

I would like to acknowledge Mr. Tilahun Bekelle, Chair of the Center for Food Science and Nutrition for his support to complete my dissertation. Nothing was impossible whenever I share it with you. Thank you again!

My gratification should also goes to staffs of IRD; Dr. Jean-Pierre Guyot, Dr. Jacques Berger, Dr. Christele Vernierre, Ms. Issabelle Rochette, and Mr. Christian Picq for their support during my stay at IRD, Montpellier, France.

I would like to thank Mr. Kalkidan, Mr. Abdu and Mr. Hailegebriel, former staffs of Tepi agricultural research center, who helped me during sample collection in Bench Majji. They travelled to the sample collection site with me and simplify my life. It would have been tough to collect the samples if they were not there.

I would also like to thank Mr. Debebe, Mr. Muluneh, Ms. Woinishet for their support when I conduct experiments at the Center for Food Science and Nutrition. Mrs. Yismualem and Ms. Tinsae should also deserve appreciation for facilitation of office requirements.

My wife, Mrs. Mina Endris took the lion share of all the effort to complete this dissertation. Your patience and love energizes me every time and step up my effort to break the impediment. I pass all the challenges with your motivation and encouragement. Thus this work is not only mine but also yours. My sweet daughter, Eliana and lovely son, Keab give me the pleasure and strength in times of stress and hardship. I love you so much! Mina, I can't tell you how much I felt happiness to have those kids with you.

My friends; Dr. Yihunie Ayele, Dr. Hirut Assaye, Mrs. Yenenesh Ayalew, Mr. Alemu Gonfa, Mr. Ephrem Mengistu, Mr. Abera Belay, Mr. Abebe Ayelign, Mr. Engida Dessalegn, Ms. Zewetir Abebe, Mr. Tadesse Alemu, Mr. Tesfaye Deme, Dr. Ashagire Zewdu, Dr. Aweke Kebede, Dr. Abebe Haile, all deserves special appreciation for sharing me your life and research experience. We had enjoyable and real memorable times. Don't forget that such a friendship should never die!!!

I would also like to thank my friend Mr. Desta Woldetsadik for your motivational thoughts, discussions and encouragement you had with me on my project. I wish you success in your PhD.

I am also grateful to my best friends Mr. Tizazu Wolde and Mr. Sintayehu Yitina for their support and encouragement throughout my life. Tize and Sinte, a PhD work is a long journey and requires patience, your enthusiasm to see me completing my PhD was appreciated. I am also indebted to my friend Mr. Yidnekachew Zewdie whom also was keen to see my work completed. Yidne congratulates I am done now!

I am also thankful to the Office of Graduate Programs of Addis Ababa University for the financial support for the study and providing grant for my stay in France. I am also grateful to Wolaita Sodo University for funding my research.

Last but not least, I want to express my gratitude to my family W/ro Abaynesh Maru (mother), Ato Amare Kifle (father), Tigist Amare, Banchayew Amare, Bezawit Amare, Eyerusalem Amare, Natinael Amare and Hilina Amare for their unconditional love, support, encouragement and understanding.

Table of Content

<i>Acknowledgement</i>	<i>i</i>
LIST OF FIGURES	<i>x</i>
LIST OF TABLES	<i>xii</i>
<i>Abbreviations/Acronyms</i>	<i>xv</i>
<i>Summary</i>	<i>xx</i>
1. Chapter One: Introduction	1
1.1. Background	1
1.2. Rationale of the study	4
1.3. Objective	7
1.3.1. General Objective.....	7
1.3.2. Specific Objectives.....	7
2. Chapter Two: Literature Review	9
2.1. Food and Nutrition Security	9
2.2. Global Malnutrition Situation	11
2.3. Malnutrition Situation in Ethiopia.....	13
2.4. Strategies to Tackle Malnutrition	14
2.4.1. Supplementation.....	14
2.4.2. Food Fortification	15
2.4.3. Biofortification.....	16
2.4.4. Dietary diversity.....	16
2.5. Amaranth: the solution to world hunger	17
2.5.1. Overview	17
2.5.2. Botanical Description.....	18
2.5.3. Botanical Distribution in Ethiopia	20
2.5.4. Worldwide Distribution	21
2.5.5. Edible part(s) of amaranth and mode of consumption.....	21
2.5.6. Vegetable amaranth.....	22
2.5.7. Grain Amaranth.....	22
2.5.8. Seed/Grain.....	23
2.5.9. Significance of amaranth in the past and in the present.....	24
2.5.10. Nutritional benefits of Amaranth	25
2.5.10.1. Protein.....	25
2.5.10.2. Amino acids	25
2.5.10.3. Lipids	30
2.5.10.4. Fatty acids.....	31
2.5.10.5. Squalene.....	32
2.5.10.6. Dietary Carbohydrates	32
2.5.10.7. Dietary Fiber	39
2.5.11. Mineral Absorption Inhibitors	45

2.5.11.1.	Phytic acid/Phytates.....	45
2.5.11.2.	Polyphenols.....	62
2.5.12.	Determination of antioxidant activity.....	62
2.5.12.1.	Mechanism of action of antioxidant in ABTS Assay.....	64
2.5.12.2.	Mechanism of action of antioxidant in DPPH Assay.....	65
2.5.13.	Complementary feeding strategies.....	65
2.5.13.1.	Complementary food in developing countries.....	65
2.5.13.2.	Characteristics of Complementary Food.....	66
2.5.13.3.	Amount and Frequency of Complementary Feeding.....	67
2.5.13.4.	Complementary Feeding Age.....	69
3.	Chapter Three-Results and Discussion.....	71
3.1.	Nutritional qualities of three types of <i>Amaranthus caudatus</i> grains cultivated in Ethiopia and the effect of popping and fermentation.....	71
3.1.1.	Introduction.....	71
3.1.2.	Materials.....	72
3.1.2.1.	Description of sampling areas.....	72
3.1.2.2.	Sample collection.....	73
3.1.3.	Methods.....	74
3.1.3.1.	Grain Characterization.....	74
3.1.3.2.	Sample preparation.....	74
3.1.3.3.	Proximate Composition Analysis.....	75
3.1.3.4.	Mineral Analysis.....	79
3.1.3.5.	Determination of Antinutrients.....	80
3.1.3.6.	Estimation of mineral bioavailability.....	81
3.1.3.7.	Quality assurance and quality control.....	81
3.1.4.	Statistical Analysis.....	82
3.1.5.	Results and discussion.....	82
3.1.5.1.	Analytical data quality assurance.....	82
3.1.5.2.	Grain Characterization.....	83
3.1.5.3.	Proximate composition of raw amaranth grains.....	83
3.1.5.4.	Effect of popping and fermentation on proximate composition.....	84
3.1.5.5.	Mineral contents of raw amaranth grains.....	85
3.1.5.6.	Effect of popping and fermentation on mineral contents.....	86
3.1.5.7.	Mineral absorption inhibitors in raw amaranth samples and the effect of processing.....	86
3.1.5.8.	Estimation of mineral bioavailability using molar ratio.....	88
3.1.6.	Conclusion.....	90
3.2.	Nutrient and Energy Adequacy of Amaranth based Complementary Foods ...	91
3.2.1.	Introduction.....	91
3.2.2.	Materials and Methods.....	91

3.2.2.1. Sample collection	91
3.2.2.2. Calculation of energy value.....	91
3.2.2.3. Calculation of iron, zinc, protein, fat and energy requirement from complementary foods	92
3.2.3. Results and Discussion.....	92
3.2.3.1. Description of Commercially available CFs	92
3.2.3.2. Proximate composition of commercially available CFs.....	93
3.2.3.3. Contribution of commercial CFs and amaranth-based CFs to the estimated nutrient requirement from complementary food for 6-23 month old children.....	95
3.2.4. Conclusion	98
3.3. Combined treatment of germination and soaking may improve both energy density and phytate degradation in amaranth porridge.....	99
3.3.1. Introduction	99
3.3.2. Materials and methods	100
3.3.2.1. Sample collection	100
3.3.2.2. Sample preparation.....	101
3.3.2.3. Extraction of phytases	101
3.3.2.4. Determination of free phosphorus.....	102
3.3.2.5. Measurement of phytase activity.....	102
3.3.2.6. Experimental Design	102
3.3.2.7. Determination of phytic acid.....	104
3.3.2.8. Kinetics of phytic acid degradation using optimum conditions	105
3.3.2.9. Preparation of porridge and viscosity measurement	105
3.3.3. Data Analysis	106
3.3.4. Results and discussion	106
3.3.4.1. Estimation of Phytase activity.....	106
3.3.4.2. Determination of optimal condition for phytic acid degradation in amaranth 108	
3.3.4.3. Phytic acid degradation under optimum conditions of temperature and pH	108
3.3.4.4. Effect of flour type, malt addition and soaking on phytic acid degradation	109
3.3.4.5. Effect of malt addition on apparent viscosity and energy density.....	111
3.3.5. Conclusion	112
3.4. Effect of processing on free and total amino acid profile, protein digestibility, protein extraction efficiency and fractionation of <i>Amaranthus caudatus</i> grain cultivated in Ethiopia	114
3.4.1. Introduction	114
3.4.2. Materials and methods	116
3.4.2.1. Materials.....	116
3.4.2.2. Solvents, chemicals and reagents	116
3.4.2.3. Sample preparation.....	117

3.4.2.4. Preparation of porridge.....	117
3.4.2.5. Protein and dry matter measurement.....	117
3.4.2.6. Free and total amino acid analysis.....	118
3.4.2.7. Protein extraction	119
3.4.2.8. Determination of in vitro protein digestibility	120
3.4.2.9. Amino acid score and protein digestibility corrected amino acid score.....	121
3.4.2.10. Calculation of the percentage contribution of amaranth to estimated amino acid requirements	121
3.4.3. Data Analysis	122
3.4.4. Results and Discussion.....	122
3.4.4.1. Protein, total and free amino acid content in amaranth grains	122
3.4.4.2. Effect of popping and fermentation on total amino acid content	124
3.4.4.3. Effect of popping and fermentation on free amino acid content	127
3.4.4.4. Effect of popping and fermentation on protein extraction efficiency	129
3.4.4.5. Effect of popping and fermentation on amaranth protein fractions	130
3.4.4.6. Effect of popping and fermentation on in vitro protein digestibility (IVPD) and protein digestibility corrected amino acid score (PDCAAS)	133
3.4.4.7. Percentage contribution of amaranth as an ingredient in complementary foods to the daily amino acid requirement of 6-23 month old children.....	135
3.4.5. Conclusion	137
3.5. In vitro starch digestibility and functional property of <i>Amaranthus caudatus</i> grain as affected by popping and fermentation.....	138
3.5.1. Introduction.....	138
3.5.2. Materials and Methods.....	140
3.5.2.1. Sample collection	140
3.5.2.2. Solvents, Chemicals and reagents	140
3.5.2.3. Sample preparation.....	140
3.5.2.4. Preparation of porridge by cooking and with instant procedure	141
3.5.2.5. Viscosity Measurement	141
3.5.2.6. Calculation of energy density.....	142
3.5.2.7. Total starch	142
3.5.2.8. Free sugars.....	142
3.5.2.9. Preparation of enzyme mixture	143
3.5.2.10. In vitro Starch digestibility	143
3.5.3. Statistical Analysis.....	144
3.5.4. Results and Discussion.....	145
3.5.4.1. Free sugars, total starch and starch fractions of raw <i>Amaranthus Caudatus</i> grain porridge	145
3.5.4.2. Effect of processing on free sugars, total starch and starch fractions in amaranth grain porridge	146

3.5.4.3. In vitro starch digestibility in raw amaranth	147
3.5.4.4. Effect of processing on BAS, RAG, SD and SDRI in amaranth.....	149
3.5.4.5. Correlation.....	150
3.5.4.6. Effect of processing on the consistency of amaranth grain porridge	151
3.5.4.7. Evaluation of the instant character of popped amaranth flour	153
3.5.4.8. Energy Density of amaranth porridge and the effect of processing	155
3.5.5. Conclusion	157
3.6. Bioactive components and in vitro antioxidant activity of <i>Amaranthus caudatus</i>	
grain as affected by processing.....	158
3.6.1. Introduction.....	158
3.6.2. Materials and Methods.....	159
3.6.2.1. Sample collection	159
3.6.2.2. Solvents, Chemicals and reagents	160
3.6.2.3. Sample preparation.....	160
3.6.2.4. Sample extraction	161
3.6.2.5. Determination total phenolic content	161
3.6.2.6. Determination of Total flavonoid.....	162
3.6.2.7. Determination of γ -aminobutyric acid (GABA).....	162
3.6.2.8. Determination of Antioxidant capacity (AC).....	163
3.6.3. Data Analysis	163
3.6.4. Results and Discussion.....	164
3.6.4.1. Total phenolics and total flavonoid contents of raw amaranth grains.....	164
3.6.4.2. Effect of processing on total polyphenol and total flavonoid of <i>Amaranthus</i> caudatus grains.....	165
3.6.4.3. Antioxidant activity of three types of raw <i>Amaranthus caudatus</i> grains.....	166
3.6.4.4. Effect of processing on antioxidant capacity of <i>Amaranthus caudatus</i> grain 167	167
3.6.4.5. Correlation between total polyphenol and antioxidant activity in amaranth	168
3.6.4.6. Effect of popping, germination and fermentation on the content of GABA in three types of <i>Amaranthus caudatus</i> grain	170
3.6.5. Conclusion	171
3.7. Physicochemical property, nutritional quality and sensory acceptability of teff-	
amaranth blended injera	173
3.7.1. Introduction.....	173
3.7.2. Materials and Methods.....	174
3.7.2.1. Sample collection	174
3.7.2.2. Preparation of amaranth and teff flour	174
3.7.2.3. Composite flours	174
3.7.2.4. Preparation of Injera.....	174
3.7.2.5. Physicochemical properties.....	176

3.7.2.6. Determination of proximate composition.....	177
3.7.2.7. Mineral analysis	177
3.7.2.8. Phytic Acid Determination in Injera	178
3.7.2.9. Sensory Evaluation of Injera Samples.....	179
3.7.3. Statistical analysis	180
3.7.4. Results and discussion	180
3.7.4.1. Physicochemical properties of teff, amaranth and a blend of teff and amaranth flour	180
3.7.4.2. Proximate composition of teff and teff-amaranth blended Injera	183
3.7.4.3. Mineral content of teff and teff-amaranth blended Injera	183
3.7.4.4. Phytic acid content of teff and teff-amaranth blended Injera	184
3.7.4.5. Estimated mineral bioavailability of teff and teff-amaranth blended Injera	185
3.7.4.6. Sensory acceptability of teff and amaranth-teff blended Injera	185
3.7.5. Conclusion	188
4. Chapter Four: General Conclusion and Recommendation.....	189
4.1. Conclusion	189
4.2. Recommendation	192
References.....	193
Annexes	220

LIST OF FIGURES

Chapter 2

Figure 2.1 The changing distribution of hunger in the world: Total numbers and shares of undernourished people by region, 1990-92 and 2012-14.....	9
Figure 2.2 Evolution of Food Security Dimensions.	11
Figure 2.3 Framework of the relations between poverty, food insecurity, and other underlying and immediate causes to malnutrition and its short-term and long-term consequences.....	13
Figure 2.4 <i>Amaranthus caudatus</i>	19
Figure 2.5 Potential growing areas of <i>Amaranthus caudatus</i> in Ethiopia.....	20
Figure 2.6 Distribution of <i>Amaranthus</i> in America.....	21
Figure 2.7 <i>A. cruentus</i> seed in (left) cross- and (right) longitudinal sections as viewed in a light microscope.....	23
Figure 2.8 Major structure of amino acid.....	25
Figure 2.9 Health benefits of dietary fiber.....	44
Figure 2.10 Structure of myo-inositol 1,2,3,4,5,6 hexakis[dihydrogen phosphahate.....	46
Figure 2.11 Hydrolysis of phytic acid by plant and fungal phytases to inositol and phosphoric acid by phytase.....	59
Figure 2.12 Classification of antioxidants.	63
Figure 2.13 Mode of action of DPPH radical with antiradical compound (RH).....	65

Chapter 3

Figure 3.1.1 Map of SNNPR (A) and Map of Ethiopia (B).....	73
Figure 3.1.2 The three different <i>Amaranthus caudatus</i> grains.....	73
Figure 3.1.3 Effect of processing on molar ratios of IP6:Fe (A), IP6:Zn (B), IP6:Ca (C) and IP6*Ca:Zn (D).....	89
Figure 3.2.1 Percentage contribution of commercial CFs and amaranth-based CFs to the recommended nutrient requirement from CF.....	97
Figure 3.3.1 Flow chart for kinetics of phytic acid degradation under optimum conditions.	105

Figure 3.3.2 Effect of malt addition on apparent viscosity of amaranth porridge	112
Figure 3.4.1 Schematic diagram for sequential extraction of amaranth protein fractions	120
Figure 3.4.2 Effect of processing on protein fractions expressed as percentages of total extracted protein.....	132
Figure 3.4.3 Effect of processing on in vitro protein digestibility (%) in amaranth..	133
Figure 3.5.1 Viscosity of porridge prepared from three types of <i>Amaranthus caudatus</i> grain.	152
Figure 3.5.2 Viscosity of three types of popped amaranth measured with cooking and instant procedure.....	154
Figure 3.6.1 Effect of processing on antioxidant capacity of <i>Amaranthus caudatus</i> grain.. .	167
Figure 3.6.2 Correlation between total polyphenol content and DPPH assay (A), total polyphenol content and ABTS assay (B) and DPPH and ABTS assay (C).....	169
Figure 3.7.1 Flow diagram for injera preparation	175
Figure 3.7.2 pH kinetics for teff and teff-amaranth flour blend doughs during fermentation.	182

LIST OF TABLES

Chapter 2

Table 2.1 Classification of the principal dietary carbohydrates.....	34
Table 2.2 Glycemic Index of common foods.....	36
Table 2.3 Some Typical Amylose-Amylopectin Mass Ratios for Starches.....	36
Table 2.4 Dietary reference intake for total fiber by life stage	45
Table 2.5 Distribution of phytate in morphological components of cereals and legumes.....	46
Table 2.6 Phytate Contents of Cereals, legumes and their products.....	48
Table 2.7 Phytase activity of common cereals and legumes.....	61
Table 2.8 Minimum dietary energy density required to attain the level of energy needed from complementary foods.....	68
Table 2.9 Breastfeeding rates (%) in the developing world by region.....	70

Chapter 3

Table 3.1. 1 Quality assurance results for nutrient analysis of amaranth grain	82
Table 3.1.2 Proximate composition of three types of raw <i>Amaranthus caudatus</i> grains.....	83
Table 3.1.3 Effect of processing on mean proximate composition of the three types of <i>Amaranthus caudatus</i> grains.....	85
Table 3.1.4 Mineral content of three types of raw <i>Amaranthus caudatus</i> grains	86
Table 3.1.5 Effect of processing on mean mineral content of three types of <i>Amaranthus caudatus</i> grains	86
Table 3.1.6 Mineral absorption inhibitors in raw <i>Amaranthus caudatus</i> grains.....	87
Table 3.1.7 Effect of processing on mean mineral absorption inhibitors of three types of <i>Amaranthus caudatus</i> grain.....	88
Table 3.2.1 Product Description of commercially available complementary foods in Ethiopia	93
Table 3.2.2 Proximate composition and energy contents of commercial complementary foods	93
Table 3.2.3 Mineral, mineral absorption inhibitors and estimated mineral bioavailability of commercially available complementary foods.....	94

Table 3.3.1 Specification matrix of the second order design.....	104
Table 3.3.2 Endogenous phytase activity of raw and processed white amaranth grain flour	107
Table 3.3.3 Optimum conditions and predicted value of responses at optimum conditions.	109
Table 3.3.4 Effect of flour type, malt addition (10%), and incubation time on phytic acid degradation in amaranth grain.....	110
Table 3.4.1 Total and free amino acid profile (mg.g-1 protein) of three types of raw <i>Amaranthus caudatus</i> grain.....	123
Table 3.4.2 Effect of popping and fermentation on total amino acid content of <i>Amaranthus caudatus</i> grain (mg.g-1 protein).....	126
Table 3.4.3 Effect of popping and fermentation on free amino acid content of <i>Amaranthus caudatus</i> grain	128
Table 3.4.4 Effect of processing on protein extraction efficiency of amaranth grain.....	129
Table 3.4.5 Percent protein digestibility corrected amino acid score (PDCAAS) for three types of raw and processed amaranth grains	134
Table 3.4.6 Percentage contribution of raw amaranth to the daily indispensable amino acid requirements.....	136
Table 3.5.1 Total starch and its fractions in raw and processed <i>Amaranthus caudatus</i> grain	147
Table 3.5.2 Bioavailable and rapidly available glucose, starch digestibility and starch digestibility rate index of raw and processed <i>Amaranthus caudatus</i> grain porridge	149
Table 3.5.3 Selected relationships among nutritional components of <i>Amaranthus caudatus</i> grain.....	151
Table 3.5.4 Energy density and minimum daily number of meals required to attain the level of energy needed from amaranth based complementary foods of different viscosity for children with average breast milk energy intake according to age group.....	156
Table 3.6.1 Total polyphenol and total flavonoid contents in three types of raw <i>Amaranthus caudatus</i> grain	164

Table 3.6.2 Effect of processing on the content of total polyphenols and total flavonoids in <i>Amaranthus caudatus</i> grain.....	166
Table 3.6.3 Antioxidant activity of three types of raw <i>Amaranthus caudatus</i> grain	166
Table 3.6.4 Effect of processing on γ -Aminobutyric acid content of <i>Amaranthus caudatus</i> grain.....	170
Table 3.6.5 Amount of amaranth based food required to meet recommendation for physiological function.....	171
Table 3.7.1 Functional properties of teff, amaranth and a blend of teff-amaranth flour	181
Table 3.7.2 Kinetic parameters of pH change during injera sourdough fermentation.....	182
Table 3.7.3 Proximate composition of teff and teff-amaranth blended injera	183
Table 3.7.4 Iron and Zinc and phytic acid content of teff and teff-amaranth blended injera	184
Table 3.7.5 Sensory acceptability test for teff and teff-amaranth blended injera	187

Abbreviations/Acronyms

AAS	Amino acid score
ABTS	2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)
ADF	Acid detergent fiber
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BAS	Bioavailable starch
BME	Breat milk energy
CD	Celiac disease
CE	Capillary electrophoresis
CF	Complementary Food
CRC	Colorectal cancer
DAGs	Diacylglyceroles
DASH	Dietary Approach to Stop Hypertension
DM	Dry matter
DP	Degree of Polymerisation
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDHS	Ethiopian Demographic Health Survey
FAO	Food and Agriculture Organization
FFAs	Free Fatty acids
GABA	γ -aminobutyric acid
GAE	Gallic acid equivalent
GI	Glycemic index
HBA1C	Glycosylated hemoglobin
HPLC	High performance liquid chromatography
IGN	Intestinal gluconeogenesis
IP6	<i>myo</i> -inositol hexakisphosphate
IVPD	In vitro protein digestibility
MAGs	Monoacylglycerols
MDG	Millenium development goal

MUFAs	Monounsaturated fatty acids
NDF	Neutral detergent fiber
NIDDM	Non insulin dependent diabetes mellitus
NSP	Non starch polysaccharides
OAC	Oil absorption capacity
PDCAAS	Protein digestibility corrected amino acid score
PU	Phytase unit
PUFAs	Polyunsaturated fatty acids
RAG	Rapidly available glucose
RDA	Recommended dietary allowance
RDS	Rapidly digestible starch
RS	Resistant starch
SD	Starch digestibility
SDRI	Starch digestible rate index
SDS	Slowly digestible starch
SNNPR	Southern Nations, Nationalities and Peoples Region
TAGs	Triacylglycerols
TKW	Thousand kernel weight
TS	Total starch
WAC	Water absorption capacity
WHO	World Health Organization

ORGANIZATION OF THE THESIS

Chapter 1: describes the background information, the rationale, main and specific objectives of the study.

Chapter 2: presents the literature review that covers a brief description of food and nutrition security, global, regional and country specific issues of malnutrition and tackling strategies for that, an overview about amaranth: its origin and distribution, nutritional qualities and health benefits and ways to improve that and complementary feeding strategies.

Chapter 3: presents the findings of the study in sub chapters as follows.

Chapter 3.1: Nutritional qualities of three types of *Amaranthus caudatus* grains cultivated in Ethiopia and the effect of popping and fermentation. This chapter evaluates the overall nutritional quality (proximate composition, mineral content and mineral absorption inhibitors), in-vitro mineral bioavailability based on molar ratio of phytate to minerals for three types of amaranth: white, red and brown in their seed color.

Chapter 3.2: Nutrient and Energy Adequacy of Amaranth based Complementary Foods. This chapter attempts to show adequacy of nutrients in a sole amaranth-based complementary food prepared from popped amaranth as utilized by children in the study area and comparison was made with few selected locally processed commercial complementary foods.

Chapter 3.3: Combined treatment of germination and soaking may improve both energy density and phytate degradation in amaranth porridge. Based on the results of chapter 3.1 on the level of phytic acid in amaranth, this chapter attempts to show strategies to degrade phytic acid content of amaranth and the consequences on energy density of the subsequent porridge prepared after the treatment.

Chapter 3.4: Effect of processing on free and total amino acid profile, protein digestibility, protein extraction and fractionation of *Amaranthus caudatus* grain cultivated in Ethiopia. This chapter attempts to show the protein quality of the three types of *Amaranthus caudatus* grain cultivated in Ethiopia referring to the free and total amino acid profile, and protein digestibility. The impact of popping and fermentation on the aforementioned parameters was also assessed. Moreover, the protein fractions that play a role in the functional property during food formulation was also determined in raw and processed amaranth based on Osborne classification.

Chapter 3.5: In vitro starch digestibility and functional property of amaranth grain as affected by popping and fermentation. The rate of starch digestion in foods allows the selection of that particular food for a specific target group. For instance, diabetic patients require foods of slow rate of starch digestion to manage the rise in blood glucose levels. While infants and children, due to their limited gastric capacity, requires foods that could be digested faster so that it will promote the rapid return of hunger and encourages them to eat frequently to meet their energy requirement. Therefore, this chapter evaluates the different starch fractions and starch digestibility as influenced by popping and fermentation. Besides, the functional property and energy density of porridge prepared from raw and processed amaranth was evaluated.

Chapter 3.6: Bioactive components and in vitro antioxidant activity of *Amaranthus caudatus* grain as affected by processing. Food bioactive compounds have several health promoting effects by acting as antioxidant, anticancer, antidiabetic, anticholesterolemic, antihypertensive etc. This chapter, therefore, determines some of the bioactive compounds such as total polyphenols, total flavonoids and γ -aminobutyric acid (GABA) in three types of raw and processed amaranth. The associated health promoting effect, i.e. antioxidant activity, mainly related to the first two bioactive compounds was evaluated in raw and processed amaranth.

Chapter 3.7: Physicochemical property, nutritional quality and sensory acceptability of teff-amaranth blended injera. Injera is the staple food of almost all Ethiopians. It is also

served for children as a complementary food. The preparation of injera uses single cereal flour or a blend of one or two cereal flours and fermentation as a major process parameter that has beneficial effect in improving the nutritional and sanitary qualities. Injera preparation from a blend of amaranth and teff is not common in Ethiopia. Therefore, this chapter evaluates the nutritional quality and sensory acceptability of teff-amaranth blended injera compared to the control injera prepared from 100% teff.

Chapter 4: Presents summary of conclusions and recommendations.

Summary

Amaranth is a valuable cereal crop with high biodiversity, distribution and productivity. Unlike other commonly utilized cereals, amaranth has high tolerance to arid conditions and poor soils, resistance to drought, pests and ability to adapt to environments that are not conducive to conventional cereals. The crop is underutilized in many parts of the world despite it has all the good attributes mentioned. Although it is known long time ago as a food and medicine in some parts of Ethiopia, it is still an underutilized crop and known as an “invasive weed” by agricultural experts. Therefore, this study evaluates the nutritional qualities and health benefits of three different types of *Amaranthus caudatus* grain cultivated in SNNPR, Ethiopia for a possible use as an ingredient in complementary food formulation. The macro and micronutrient contents, mineral absorption inhibitors (IP6 and Iron binding polyphenols: galloyls and catechols), free and total amino acid profiles, protein fractions and digestibility, starch fractions and digestibility, bioactive compounds (total polyphenol, total flavonoid and γ -aminobutyric acid (GABA)) and antioxidant activity using DPPH and ABTS assays were determined. The effect of commonly utilized processing methods for cereal food preparation, popping and fermentation, on the overall nutritional qualities and health benefits was evaluated. Moreover, combining treatments such as soaking (at optimum pH and temperature for endogenous phytase enzyme) and malt addition was applied in order to see the quality changes consequent to phytate degradation and starch hydrolysis.

The results of the study showed that amaranth has higher protein (14.0-15.5 g/100g DM) compared to maize, rice and barley but comparable with the content present in wheat and teff. The fat content is in the range of 7.5-7.7 g/100g DM and this value is two-fold higher than that present in commonly utilized cereals such as maize, barley, rice, sorghum, wheat and teff. The carbohydrate, acid detergent fiber, neutral detergent fiber and ash contents were in the range of 60-68, 5.5-14.2, 7.1-14.8 and 2.6-3.1 g/100g DM, respectively. Iron, zinc, calcium and magnesium contents were obtained in the range of 12-21, 2.7-3.4, 102-215, 292-340 mg/100g DM, respectively. A surprisingly too high IP6 content ranging from 1.85 to 2.20 g/100g DM was found in raw amaranth limiting its application for the formulation of infant foods. The content of galloyls and catechols were in the range of 93-143 mg TE/100g DM and

24-54 mg CE/100g DM, respectively. The high content of IP6 was decreased by 39 and 77% after popping and fermentation, respectively with subsequent improvement in estimated mineral bioavailability. However, the residual level is still high enough to demonstrate inhibition of mineral absorption. Fermentation also demonstrated a much larger, by 96-100%, degradation of iron binding polyphenols than popping.

Experimental design aimed to achieve maximum phytic acid degradation by optimizing the best condition for enhanced activity of endogenous phytase using combined treatment of malt addition (10%) and soaking resulted in complete degradation of the phytic acid in 8.36 h at optimum temperature of 46 °C and pH of 5.2. The complete phytic acid degradation achieved in such relatively short period is due to the higher enzymatic activity of added malt, 3x higher than raw amaranth. Furthermore, the malt also favors the hydrolysis of starch and found to lower the apparent viscosity. This consequently improves the energy and nutrient density of amaranth porridge.

The content of total essential amino acids, which include lysine, threonine, valine, leucine, isoleucine, histidine, sulphur and aromatic amino acid were in the range of 65.3-73.9, 35.0-40.3, 41.3-48.3, 59.5-67.1, 34.9-39.4, 33.9-37.2, 66.5-79.5 and 88.4-99.4 mgg⁻¹ protein, respectively. This result shows that all, except leucine in raw red and brown amaranth, are above WHO reference pattern for children aged 1-2 years. However, when the digestible fraction of the protein was considered the contribution to the daily essential amino acid requirement is decreased by 18-29%, the maximum decrease being for the brown amaranth due to its lower digestibility compared to the other two amaranth samples. Both popping and fermentation caused substantial decrease in cysteine, lysine and methionine contents. The content of free essential amino acid obtained was below 0.2% and the highest was for histidine. Popping decreased all the measured essential free amino acids due to volatilization but fermentation increased all the essential free amino acids due to hydrolysis of large polypeptide molecules. Separation of amaranth seed protein fraction based on Osborne classification demonstrates that albumin + globulin are the major and prolamin is the minor fraction which is in extreme contrast to teff and sorghum grains affecting the functional property during food formulation

The free glucose (FG), total starch (TS), rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) contents of the three types of raw amaranth were found in the range of 0.64-0.67, 48-60, 27.4-33.2, 10.9-13.9 and 8.5-9.4 g/100 DM, respectively. Popping exhibited significant improvement in RDS, SDS and TS contents and no significant change on FG and RS content. On the other hand, fermentation increased the FG, RDS and SDS and decreased the TS and RS contents. Consequent to the increase in RDS and SDS, the starch digestibility also increased from 83 to 86 and 96% during popping and fermentation, respectively. The starch digestibility of the raw sample was relatively high and the further improvement during popping and fermentation was associated with degradation of phytic acid during both processing methods and enhanced microbial enzyme activity during fermentation. Moreover, both popping and fermentation modifies the rheological property of amaranth. Therefore, the energy density of gruel prepared from raw, popped and fermented amaranth was found to follow the order; fermented > popped > raw.

Total polyphenol and total flavonoid contents were highest for the brown amaranth (257 mg GAE/100g DM and 680 µg CE/g DM) followed by red (158 mg GAE/100g DM and 600 µg CE/g DM) and white (146 mg GAE/100g DM and 520 µg CE/g DM). Antioxidant capacity (AC) that was determined using DPPH and ABTS assays also showed that brown amaranth had highest AC (43.29±3.22 and 93.08±0.72 mg TE/100g DM, respectively) followed by red (35.52±1.88 and 85.66±3.59 mg TE/100g DM, respectively) and white (16.65±1.54 and 79.61±1.43 mg TE/100g DM, respectively) amaranth. All processing methods, popping, germination and fermentation showed tremendous improvement on the antioxidant activity due to the production of maillard reaction products during popping and increased extractability of phenolic compounds during germination and fermentation. A remarkable increase in γ -aminobutyric acid (GABA), that has an antihypertensive and antidiabetic effect, was observed during germination and fermentation indicating that malted and fermented amaranth are preferred ingredient in functional food preparation especially for celiac patients due to the absence of gluten in amaranth.

Realizing the good nutritional qualities of amaranth compared to commonly utilized cereals, the attempt made to prepare injera from a blend of teff and amaranth was successful with

acceptable nutritional and sensory qualities until 20% amaranth blending thereby contributes to the actions to ensure food and nutrition security. Generally all the three types of cultivated *Amaranthus caudatus* grains were found to have good nutritional and health benefits, the brown amaranth being superior in many of the parameters analyzed. Moreover, the processing techniques applied were also contributed to the improvement in the nutritional quality and health benefits. However, due to the presence of high levels of phytic acid in the studied amaranth, utilizing them as an ingredient in complementary foods should be done with great caution. Selection and breeding of low phytic acid containing amaranth is also strongly recommended to exploit the potential of the crop.

1. Chapter One: Introduction

1.1. Background

Amaranthus spp is an ancient plant belonging to the family of Amaranthaceae which is believed to have originated from Central and South America (Gamel et al., 2006). It is an underutilized plant in which both the grain and leaves are edible. It produces a large amount of biomass in a short period of time (Kauffman and Weber, 1990) and therefore has the potential to contribute to a substantial increase in world food production. It is a pseudocereal with excellent nutritional and functional properties, high tolerance to arid conditions and poor soils, resistance to drought, pests and ability to adapt to environments that are not conducive to conventional cereals (Capriles et al., 2008) and grows from sea level to 3500 m (Taylor and Emmambux 2008; Townsend, 2000). The plant includes more than 60 species (Townsend, 2000; Stallknecht & Schulz-Schaeffer, 1993) and several of which are cultivated as leafy vegetables, grains or ornamental plants, while others are weeds (Kauffman and Weber, 1990; Townsend, 2000). The main species grown as vegetables are *Amaranthus tricolor*, *Amaranthus dubius*, *Amaranthus lividus*, *Amaranthus cruentus*, *Amaranthus palmeri* and *Amaranthus hybridus* while *Amaranthus hypochondriacus*, *Amaranthus cruentus* and *Amaranthus caudatus* are the main grain species (Teutonico and Knorr, 1985).

Amaranth requires only 3-4 months to harvest and can be cultivated twice annually with rainfall in Ethiopia (personal communication). Although there is no FAO statistics about amaranth grain production, the major producers of amaranth are China and Russia with 300 000 and 100 000 ha cultivated, respectively (Cai et al., 2004). In Uganda grain yield of upto 5.0 t.ha⁻¹ has been reported (Stallknecht and Schulz-Schaeffer, 1993). In Mexico, yield ranging between 3.0 and 5.9 t.ha⁻¹ was reported (Bressani, 2003). According to a report by Williams and Brenner (1995), upto 3.0 t.ha⁻¹ can be obtained with good agronomic practices and good seed sources, but 1.5 t.ha⁻¹ is more the norm. Unpublished sources revealed that the grain yield of amaranth in Ethiopia was estimated to reach 1.1 to 4.0 t.ha⁻¹.

Amaranth was declared as one of the future promising crop to feed the global population (National Academy of Sciences, 1975). Due to its high nutritional value as well as some

agricultural advantages such as relative high grain yield, resistance to drought, and short production time (Mendoza & Bressani, 1987), amaranth has got the attention of many researchers.

In Ethiopia, very few species such as *Amaranthus caudatus*, *Amaranthus hybridus*, *Amaranthus spinosus*, *Amaranthus dubius* and *Amaranthus viridus* are grown in many parts of the country (Townsend, 2000; Teketay, 2010). In the past years it has been used in many places of the country, particularly in the Southern Nations Nationalities and Peoples Region (SNNPR), mainly for its medicinal purpose especially for mothers at their early stage of lactation with the assumption that it strengthens their bones (personal communication). In some places of the country, grain amaranth is fermented to make alcoholic beverage, e.g. beer ('tella') in Benishangul-Gumuz region and another local beverage known as 'Chaqa' in konso. The cooked seeds could also be made into porridge, and ground seeds are mixed with 'teff' to prepare pancake-like bread ('injera') (Teketay, 2010).

Amaranth has been reported to have high protein, fat and mineral contents (Rastogi and Shukla 2013; Mustafa et al., 2011; Segura-Nieto et al., 1994; Pedersen et al., 1987). Martirosyan et al. (2007), Muyonga et al. (2008) and Kunyanga et al. (2012) also showed that amaranth has excellent amino acid and fatty acid profile with high amount of lysine and high percentage of unsaturated fatty acids. Amaranth oil also contains high amount of squalene, an intermediate in cholesterol biosynthesis which is found mainly in shark liver oil and olive oil, compared to any other cereals or legumes (He et al., 2002; Ryan et al., 2007).

Moreover, in the contemporary world the prevalence of celiac disease (CD), an inflammatory disease of the small intestine triggered by gluten proteins, is rising and 1:100 to 2:100 persons are victims of CD globally (Schuppan et al., 2009). Celiac patients, especially children, following a strict gluten-free diet, are often undernourished because of the reduced intake of energy which is largely taken from wheat-based foodstuffs in a western diet. Additionally, they suffer from micronutrient deficiency because of the previous malabsorption in active CD, as well as the actual low content of micronutrients in the alternative flours used in gluten-free formulations (Thompson et al., 2005). Therefore, it is very important to develop gluten-free

foodstuffs with high nutritional quality ingredients. Amaranth, being a gluten free cereal (Calderón de la Barca et al., 2010), could be used as a good candidate to be incorporated as an ingredient in gluten free functional food for celiac patients.

Although, the crop has high content of both micro and macro nutrients as compared to commonly available cereals, it also appears to have high content of antinutrients especially phytate (Pedersen et al., 1987; Egli et al., 2002), which is a potent inhibitor of iron, zinc, calcium, magnesium and other divalent metals (Hurrell et al., 2003; Egli *et al.*, 2004; Koréissi-Dembélé et al., 2013). This will possibly limit the application of the crop for a diversified uses. However, due to the high content of micro and macronutrients, grain amaranth of different species have been utilized to develop complementary food in different countries (Rathod and Udipi, 1991; Bhuvaneswari and Sharda, 2004; Macharia-Mutie et al., 2011; Mburu et al., 2011). In Ethiopia too, grain amaranth has been used to prepare complementary food despite information about the nutritional profile is lacking. Given the very high incidence of malnutrition, especially micronutrient deficiency, that could arise from inadequate intakes, impaired absorption or utilization, or excessive losses during complementary feeding period, provision of complementary food without any notion about the nutritional qualities of ingredients used for the formulation may jeopardize the health status of children and may thus contribute to the high prevalence of stunting in children.

Therefore, during the period of complementary feeding (6-23 months), micronutrient deficiency is a very common problem and in an attempt to tackle such a problem through diversifying the diet, care should also be taken to reduce the content of mineral absorption inhibitors. The use of different traditional processing methods such as soaking, germination, fermentation, and hydrothermal treatment has been found to be effective methods so far and thus applying these techniques along with the use of nutrient rich underutilized crops as a nutritional intervention mechanisms to improve energy and micronutrient density of complementary food could tackle micronutrient deficiency and associated problems.

1.2.Rationale of the study

Malnutrition and particularly undernutrition has been clearly identified as a major public health problem over large areas of the world, especially in developing countries and particularly amongst low socio-economic groups (Black et al., 2013). It is highly affecting infants, children, pregnant and lactating women throughout the world as these age groups are very sensitive to nutritional deficiencies (Whitney and Rolfes, 2008). Malnutrition during early life can lead to permanent stunting in growth (Onis and Blossner, 1999) expressed by low height-for-age (WHO, 2006). During the first two years of life, malnutrition has a profound effect on child growth and development particularly during the first phase of complementary feeding (6-12 months) at which time foods of low nutrient density begin to replace breast milk and rates of diarrhea due to food contamination are at their highest (WHO, 2001).

According to the United Nation Food and Agriculture Organization` (FAO) report, undernourishment is prevalent globally reaching to 11% and a predicted number of 805 million people are suffering from it in the year 2012/14 (FAO, 2014). Despite the progress observed over the past decades, one in every nine people in the world still has insufficient food for an active and healthy life. Developing countries account for 98% of the world's undernourished people and nearly 60% of the undernourished people live in only seven countries (Bangladesh, China, the United Republic of Tanzania, Ethiopia, India, Indonesia and Pakistan) (FAO, 2014).

In line with this, the global consequences of malnutrition resulted in the death of 6.6 million children in the year 2012, where about 3.2 million deaths were from Sub-Saharan African countries. The rate is translated in to nearly 18,000 child death per day in the world (UNICEF, 2014). Ethiopia, being one of the developing countries in the Sub-Saharan region, childhood malnutrition is prevalent in many parts of the country. According to the recent Ethiopian Demographic and Health Survey report, under five mortality rate is about 88 per 1000 live birth in 2011 being highest in the urban areas than in the rural areas (Central Statistical Agency and ICF International, 2012). The figure shows that the country is on good track to meet the millennium development goal 4 (i.e Reducing under-five mortality by two thirds,

between 1990 and 2015) prior to the deadline. However, the report also showed that among children who are in the age from 6 to 59 months, 44 percent were stunted (low height-for-age), 10 percent were wasted (low weight-for-height) and 29 percent were underweight (low weight-for-age) (Central Statistical Agency and ICF International, 2012). This shows that although mortality rate decreased as planned in the MDG, the indicators of malnutrition; stunting, underweight and wasting are still high.

Although there are many contributing factors for the prevalence of malnutrition in infants and young children, inappropriate complementary feeding is an important determinant factor (Daelmans et al., 2003). Complementary feeding period is the time when children are introduced to additional food, named complementary food (CF), to breast milk mostly at the age of 4-6 month (Yeung, 1998) or exactly right after 6 month according to WHO recommendation (WHO, 2001). In order to meet the recommended dietary allowance (RDA) for infants, the CF should be nutritionally dense, well-balanced in macronutrients and, with high content and bioavailability of minerals and vitamins, and microbiologically safe for the child (WHO, 1998). Moreover, the CF should provide sufficient amount of energy 356, 479, and 772 kcal/day for average breast milk energy intake of children aged 6–8, 9–11 and 12–23 month, respectively based on total energy requirement of US longitudinal data plus 25% (Dewey and Brown, 2003).

In Ethiopia, particularly in the urban areas, there are different types of commercially available complementary foods which are either imported or processed by local industries. These complementary foods are prepared with the aim to complement breast milk so as to meet the daily requirement of infants and young children. However, they are too expensive and are always far from the reach of most Ethiopian families to feed their baby. Hence, many depend on inadequately processed traditional foods consisting mainly of cereal porridges made from different starchy cereals and tubers (Igah, 2008). In some households, legumes are also supplemented with cereals but without a good knowledge of the cereal-legume blending proportion resulting in poor quality CFs. In CFs, containing high proportion of starch based cereals, the starch will often provides the principal source of energy, but when heated with water, starch granules gelatinize to produce a bulky, thick (viscous) porridge. As a result, the

porridge will be diluted with an attempt to get a consistency that could be easily consumed by children. However, dilution makes the CFs being low energy and nutrient density. The low energy and nutrient density means that large volumes of food have to be consumed to meet the infant's requirements but this is not usually possible, owing to the infant's limited gastric capacity and to the limited number of meals offered per day. On the other hand, the content of antinutritional factors is also relatively high that can inhibit absorption of certain micronutrients and macronutrients thereby causing nutritional deficiencies in children (Gibson and Hotz, 2000) who are at a physiologically vulnerable state. Deficiencies in this window period may have long term effect on the work capacity and intellectual performance during their adulthood stage (Martorell, 1997).

As part of an intervention strategy to tackle malnutrition in general and micronutrient deficiencies in particular strategies such as supplementation, fortification, biofortification and dietary diversification were implemented (Nestel et al., 2006). Though difficult to attain, among all the aforementioned strategies, ensuring adequate dietary diversity could be an affordable and sustainable strategy by many people especially in developing countries. One way of ensuring dietary diversity is to search for underutilized food sources of high nutritional value as they grow year to year with minimal agricultural inputs at a very high yield and potentially contribute to sustainably feed the growing population. Those underutilized plants have an under-exploited potential to contribute to food and nutrition security, health, income generation and environmental services (Jaenicke and Höschle-Zeledon, 2006). It is believed that one of the many underutilized plants that offers important promise for feeding the world's hungry is amaranth. This crop has been suggested as one among the 36 of the world's most promising crops to feed the world (National Academy of Sciences, 1975). Amaranth is high yielding, drought resistant and provides edible seeds and leaves but known as an "invasive weed", in Ethiopia. Nevertheless, in the Southern Nations, Nationalities and Peoples Region (SNNPR) a typical ethnic group named Me'enit use amaranth as food crop by cultivating it along with maize and sorghum mainly for the preparation of complementary food for feeding children in the form of porridge. Such practice is in line with the suggestions to mitigate infant and young child malnutrition. However there are few or no research reports on the

nutritional quality of *Amaranthus spp* grown under the agro climatic conditions of Ethiopia in order to promote to a wider community to utilize the crop as an ingredient in infant food.

Therefore, this study attempted to evaluate the overall nutritional quality, i.e. the amount and availability of nutrients, and health benefits of three different types of amaranth grain for a possible use of the crop as an ingredient in complementary food formulation. Moreover, the effect of different traditional processing methods on the overall nutritional quality and health benefits was assessed.

1.3.Objective

1.3.1. General Objective

To evaluate the nutritional quality, health benefits and potential use for complementary food formulation of three types of *Amaranthus caudatus* grain cultivated in Ethiopia.

1.3.2. Specific Objectives

Chapter 3.1

- i. Evaluate the nutritional quality of raw and processed (i.e. popped and fermented) amaranth grain.
- ii. Asses the effect of processing (popping and fermentation) on mineral absorption inhibitors (phytate and iron binding polyphenols) in amaranth grain.

Chapter 3.2

- i. Determine the nutrient adequacy of amaranth based complementary food to the daily nutrient requirement from CFs for 6-23 month old children and comparison to selected commercial complementary foods processed in Ethiopia.

Chapter 3.3

- i. Investigate the effect of hydrothermal treatment on phytic acid degradation, energy density and viscosity of amaranth porridge.

Chapter 3.4

- i. Evaluate the effect of processing on free and total amino acid profile, protein fractions and in vitro pepsin and pancreatin digestibility of amaranth.

Chapter 3.5

- i. Assess the effect of processing on starch fractions and starch digestibility in amaranth porridge.
- ii. Evaluate the effect of processing on rheological property and energy density of amaranth porridge.

Chapter 3.6

- i. Determine major bioactive compounds and in vitro antioxidant capacity of three types of raw amaranth grain.
- ii. Assess the effect of popping, germination and fermentation on the content of bioactive compounds and antioxidant activity of amaranth grain.

Chapter 3.7

- i. Formulate teff-amaranth blended injera and study the nutritional quality and sensorial property.

2. Chapter Two: Literature Review

2.1. Food and Nutrition Security

According to the current FAO estimates about 805 million people were estimated to be chronically undernourished in 2012–2014 (FAO, IFAD and WFP, 2014). It decreased over the last decade by about 100 million. However, about one in every nine people in the world still has insufficient food for an active and healthy life. The vast majority of these undernourished people live in developing countries, where an estimated 791 million were chronically hungry and/or one in eight people in these countries are chronically food insecure (FAO, IFAD and WFP, 2014). Figure 2.1 shows what changes have been observed on the number of hungry people in the entire region in the world since 1990. The number of hungry people increased by 22% in Sub-Saharan Africa, 138% in Western Asia and 117% in Northern Africa in 2012-14 than it was in 1990-92. The increase might be associated with the rapid rate of population growth, and political instability. Southern and Eastern Asia, and Sub-Saharan Africa accounts for more than 80% of the hungry people in 2012-14.

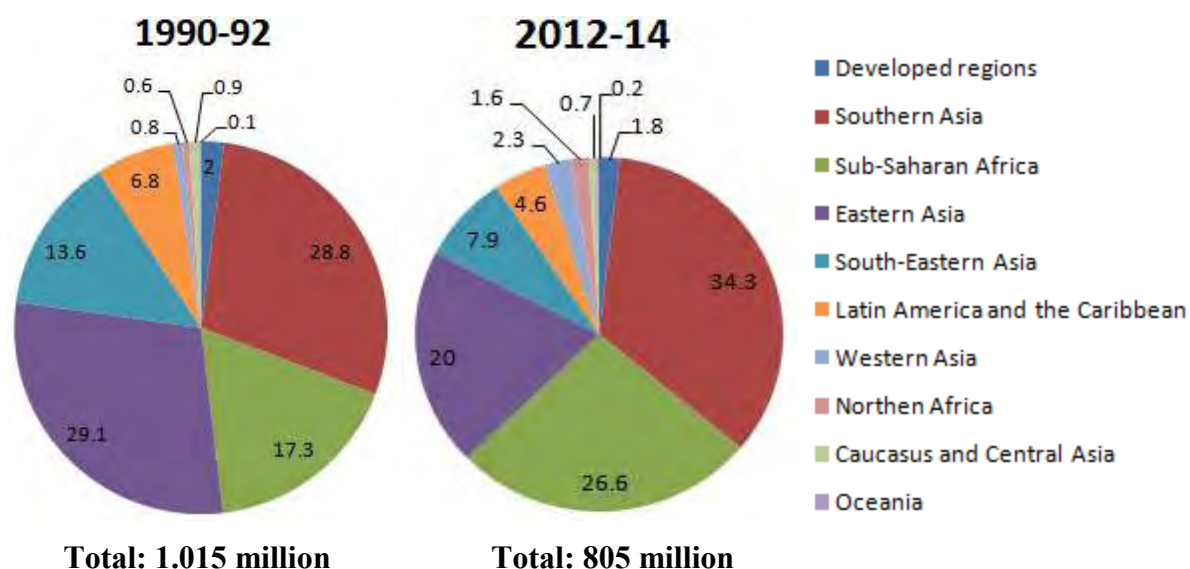


Figure 2.1 The changing distribution of hunger in the world: Total numbers and shares of undernourished people by region, 1990-92 and 2012-14 (Source: FAO, IFAD and WFP, 2014).

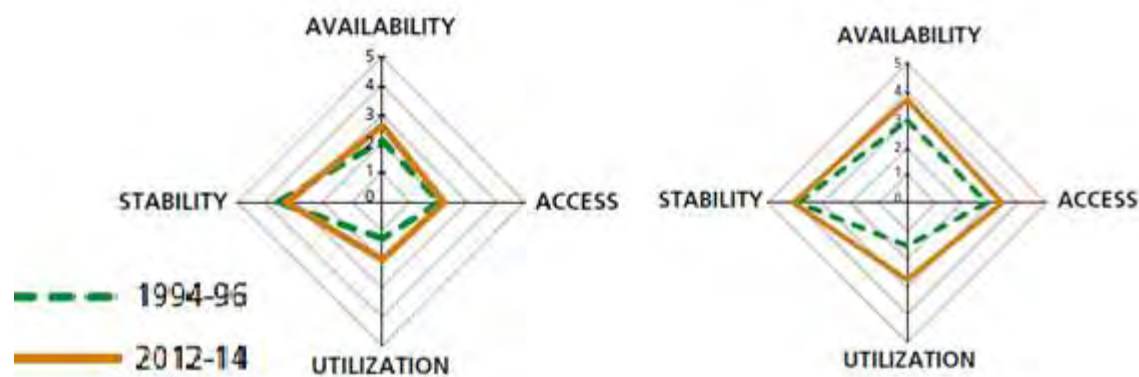
With the global population expected to reach over 9 billion by 2050, there will be a continuous need to increase food production and buffer stocks to meet the growing demand and efficiently cope with volatilities in food production and prices. It has been projected that global food production will need to increase by 70% in order to meet the average daily caloric requirement of the world's population in 2050 (FAO, 2006). However, the current global food system seemed to be challenged by several factors to feed the world. Some of the major factors are climate change, loss of species and genetic diversity, soil degradation, increasing urbanization, social conflict and extreme poverty. The stakeholders are, therefore, required to collaboratively work to address food and nutrition security at the global level (Hunter and Fanzo, 2013).

According to FAO definition, food security exists when all people at all times have physical, social and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life (FAO, 1996). The definition of food security encompasses four dimensions: availability, access, utilization by the body and stability of supply (FAO, 2008).

Depending on the duration, food insecurity could be categorized into two: Chronic and transitory food insecurity. The chronic food insecurity can occur when people are unable to meet their minimum food requirements over a sustained period of time. It could be resulted from extended periods of poverty, lack of assets and inadequate access to productive or financial resources. Transitory food insecurity is a short term and temporary situation that occurs when there is a sudden drop in the ability to produce or access enough food to maintain a good nutritional status. Short-term shocks and fluctuations in food availability and food access, including year-to-year variations in domestic food production, food prices and household incomes could cause transitory food insecurity (FAO, 2008).

Food security status of countries will be measured taking in to consideration of the food security indicators for all the four dimensions and measured on a scale from 1 to 5. Accordingly, as seen in Figure 2.2, Sub-Saharan Africa showed progress since 1994/6 in the availability and utilization of food. The status of food security in all developing countries also

shows progress in availability, access and utilization dimensions but the price volatility and political instability doesn't guarantee these countries to sustainably ensure food security (FAO, IFAD and WFP, 2014).



Sub Saharan Africa

All developing countries

Figure 2.2 Evolution of Food Security Dimensions (Source: FAO, IFAD and WFP, 2014).

In a quantitative short and long term food security status assessment report by Meade and Rosen (2013), in 76 low and middle income countries showed that the total number of food insecure people is estimated at 707 million in 2013 and the figure is projected to increase nearly by 23% to 868 million in 2023. The share of the population that is food insecure is projected to increase from 20.4% in 2013 to 21.5% in 2023. The distribution gap, the amount of additional food needed to bring people in all income deciles up to the nutritional target roughly 2,100 calories/day, is projected to increase by 28 percent in 2023, meaning that food insecurity in these countries is expected to intensify over the next 10 years. Despite improvements over the years, Sub-Saharan Africa is projected, by 47%, to remain the most food-insecure region in the world followed by Asia, 11%.

2.2.Global Malnutrition Situation

The World Health Organization defines malnutrition as the cellular imbalance between supply of nutrients and energy and the body's demand for them to ensure growth, maintenance, and specific functions (WHO, 2001). The imbalance between supply and demand for nutrients and energy would result in measurable adverse effects on body composition, function and clinical

outcome (Saunders et al., 2010). Malnutrition is one of the main causes for child death, where by about 2.6 million children are dying every year. Many more children survive, but grow up malnourished, without even having enough nutritious food to eat and without getting the nutrients they need to be healthy (Save the Children, 2012). According to Lancet series report, globally, 165 million children are stunted, 52 million are wasted and more than 100 million are underweight in the year 2011 (Black et al., 2013). The global prevalence of stunting, wasting and underweight is 26, 8 and 16%, respectively in which all are good indicators of malnutrition (Black et al., 2013).

Malnutrition particularly that caused due to deficiency of micronutrients is highly prevalent and even increasing in parts of several developing countries. Factors of immediate and direct influence to these nutritional disorders are inadequate food consumption and diseases, which usually interact in a mutually reinforcing manner (Saunders et al., 2010; Save the Children, 2012). In countries where household food security is ensured, adequacy of nutrient intake will not be guaranteed. This is because bioavailability of many nutrients in plant foods is usually low, and this will significantly contribute to the nutritional inadequacy.

Overnutrition is also another arm of malnutrition that is related to overconsumption of nutrients. Some nutrient when consumed to a level much beyond the recommendation, toxicity could occur and totally affect the health status of the consumer. For instance, excess intakes of vitamin A overtime do cause fracturing of bones or osteoporosis in any individual and birth defect if it occurs during pregnancy. Overconsumption of nutrients is also the major cause for the manifestation of chronic diseases such as cardiovascular diseases, diabetes, hypertension, kidney disease and etc (Whitney and Rolfes, 2008).

The basic and underlying causes of undernutrition are indicated by a framework developed by UNICEF (Figure 2.3) (WHO, 1998). According to this framework, it has been shown that malnutrition occurs when dietary intake is inadequate and health is unsatisfactory, being the two immediate causes of malnutrition. The immediate determinants of child nutritional status are, in turn, influenced by three underlying determinants manifesting themselves at the household level. These are food security, adequate care for mothers and children, and a

proper healthy environment, including access to health services. Associated with each is a set of resources necessary for their achievement. Finally, the underlying determinants of child nutrition are, in turn, influenced by basic determinants. The basic determinants include the potential resources available to a country or community, which are limited by the natural environment, access to technology, and the quality of human resources. Political, economic, cultural, and social factors affect the utilization of these potential resources and how they are translated into resources for food security, care and health environments and services (Smith and Haddad, 2000). Proper treatment of the basic and underlying causes of malnutrition could bring large changes on global undernutrition status.

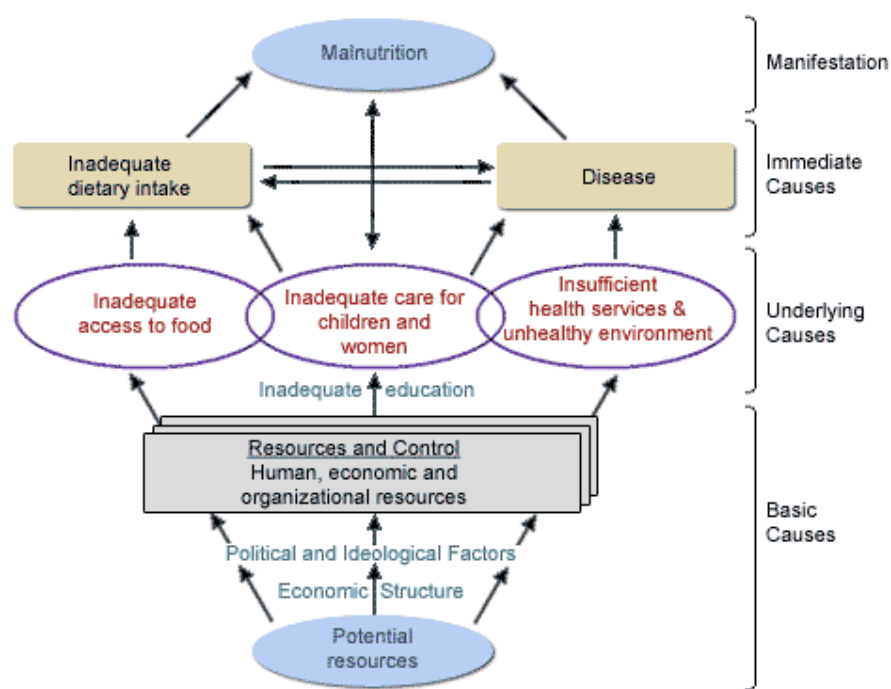


Figure 2.3 Framework of the relations between poverty, food insecurity, and other underlying and immediate causes to malnutrition and its short-term and long-term consequences (Adapted from WHO 1998).

2.3. Malnutrition Situation in Ethiopia

According to the recent Ethiopian Demographic Health Survey 2011 (EDHS 2011) report, among pre-school children, 44% are stunted, 10% are wasted, 29% are underweight and 2%

are overweight (Central Statistical Agency and ICF International, 2012). This implies that about 5 million pre-school children have shorter height for their age (stunted) (Central Statistical Agency and ICF International 2012; <http://scalingupnutrition.org>). The prevalence increases as the age of children increases. This is because children begin to feed complementary foods right after the age of six months and these complementary foods are not nutritionally adequate for the children. When this occurs frequently children become deprived of the essential nutrients for life this will in turn cause poor growth of children. Ethiopia loses around 16.5 percent of its GDP each year to the long-term effects of child malnutrition (<http://scalingupnutrition.org>).

2.4.Strategies to Tackle Malnutrition

2.4.1. Supplementation

This strategy involves the provision of specific nutrients in order to meet the immediate deficits of vulnerable groups (Shetty, 2011). For example, iron and folate supplements are provided to all pregnant mothers attending antenatal clinics in primary health care centers in several developing countries so as to reduce the risk of mortality and morbidity. Despite iron deficiency being a major public health problem, there has been very little progress in its control in the developing world. This is attributable to the low compliance with iron supplementation, insufficient targeted interventions for infants and young children, and lack of integration of nutritional interventions with other national programmes. The recent controversies regarding the safety of iron interventions related to the interaction between malaria and iron metabolism, and those pertaining to the role of iron in oxidative damage, have not helped (Shetty, 2011).

Mega dose vitamin A supplementation to preschool children and pregnant women could also lower the risk of vitamin A deficiency disorders such as xerophthalmia, infection, poor growth, anemia and mortality (West, 2003). Despite the progress observed on supplementation coverage recently, shortfall of the supplement and sustainability of vitamin A supplementation programmes are major challenges (Shetty, 2011). Zinc supplementation to young children in at risk populations is also expected to have a high impact and a course of

zinc supplements in conjunction with oral rehydration solutions is currently recommended by WHO/UNICEF for the treatment of acute diarrhea (WHO/UNICEF, 2004). A meta analysis by Brown et al. (2002) of thirty-three randomized controlled trial (27 in developing and 6 in developed countries) on children showed that Zn supplementation results in a positive response in linear growth and weight gain (but not weight-for-height). The study showed that the response is especially marked in those children who are stunted or underweight at baseline, emphasizing that a positive growth response to Zn supplementation is more likely to be apparent among children with pre-existing growth failure.

In general, although supplementation interventions have relatively rapid start-up times and can produce quick results in reducing morbidity and mortality, shortfall of the supplement, low accessibility of the supplement to the poor rural population in developing countries and sustainability of supplementation programmes are major challenges.

2.4.2. Food Fortification

Food fortification is usually regarded as the deliberate addition of one or more micronutrients to particular foods which are widely consumed, so as to increase the intake of these micronutrient(s) in order to correct or prevent a demonstrated deficiency and provide a health benefit (WHO and FAO, 2006). Wheat, rice and maize are the commonly available cereals used for fortification and some of the micronutrients falling short in the diets of many people and desired to be fortified on those cereal flours are iron, zinc, vitamin A, folic acid and vitamin B₁₂ (WHO, FAO, UNICEF, GAIN, MI, and FFI, 2009). Fortification is a mandatory activity in some countries while many others practiced it on voluntary basis.

The public health impact of food fortification depends on a number of parameters, but predominantly on the level of fortification, the bioavailability of the fortificants, and the amount of fortified food consumed. Many fortification activities are beneficial to the society but detail studies on the dietary intake of the consumer are needed before applying mandatory fortification as some of these nutrients may reach to toxic level after fortification.

2.4.3. Biofortification

Biofortification is the process of generating genetically improved staple food crops that are rich in bioavailable micronutrients, either through conventional breeding or genetic modification (Johns and Eyzaguirre, 2007). Agronomic biofortification can also provide temporary micronutrient increases through the application of fertilizers (Saltzman et al., 2013). The target crops for biofortification are mainly staple foods which predominate in the diets of the poor as a result the strategy could explicitly targets low income households. Some of the crops that were undergone biofortification are: orange fleshed sweet potato, maize, cassava, rice, wheat, pearl millet, lentil, cowpea, banana, sorghum and potato. These biofortified staple foods cannot deliver as high a level of minerals and vitamins per day as supplements or industrially fortified foods, but they can help by increasing the daily adequacy of micronutrient intakes among individuals throughout the lifecycle (Bouis et al., 2011). The initial investment of biofortification is very high, besides achieving a crop rich in micronutrients is a daunting task which could take several years. However, once the biofortified crops are in place and properly disseminated, they could be reproduced by farmers and automatically form part of the food chain (Nestel et al., 2006).

The strategy could also provides a feasible means of reaching malnourished rural populations who may have limited access to diverse diets, supplements, and commercially fortified foods. Generally, biofortification allows sustainable production of nutritionally improved varieties of staple crops and consumption year after year, even if government attention and international funding for micronutrient issues fade.

2.4.4. Dietary diversity

Dietary diversity is defined as the number of unique food categories consumed over a given period of time, and functions as an indicator of food security and a proxy for diet quality (Hoddinot and Yohannes, 2002; Rue, 2003). The diets of people in many developing countries rely on a very specific food groups predominately cereals and plant based foods. In order to enhance intakes and bioavailability of nutrients, promoting the consumption of animal source foods and increasing the diversity of plant based foods than restricting on very

few staple foods is strongly recommended. As a means of promoting food diversity, home gardening of different fruits and vegetables rich in micronutrients like orange fleshed sweet potato has been extensively promoted in rural areas of the developing world (Talukder et al., 2014).

2.5.Amaranth: the solution to world hunger

2.5.1. Overview

Amaranthus, which is a family of amaranthaceae, consists of approximately 60 species. The word is basically derived from the Greek word “Anthos” (Flower) which means never fading or unwilting (Rastogi and Shukula, 2013). Based on its use for human consumption, amaranth can be divided in to grain and vegetable amaranth (Venskutonis and Kraujalis, 2013). There are three species of the genus *Amaranthus* which are known for their seeds: *Amaranthus hypochondriacus*, *Amaranthus cruentus* and *Amaranthus caudatus* (Bressani, 2003). They produce relatively large inflorescences with often more than 50000 edible seeds per plant (Bressani, 2003). Grain amaranth is believed to originate from Central and South America (Cai et al., 2004). The main species grown as vegetable amaranth are *Amaranthus tricolor*, *Amaranthus dubius*, *Amaranthus lividus*, *Amaranthus cruentus*, *Amaranthus palmeri*, *Amaranthus edulis*, *Amaranthus retroflexus*, *Amaranthus viridus* and *Amaranthus hybridus* (Teutonico and Knorr, 1985; Bressani, 2003). They grow very well in the hot, humid regions of Africa, South-east Asia, Southern China, and India (Bressani, 2003).

During the pre-Columbian times, grain amaranth was one of the basic foods of Andean countries that was nearly as important as corn and beans (Rastogi and Shukla, 2013) and it was a staple food in Mexico during the Aztec civilization. The Aztecs believed that amaranth had magical properties and used it as a grain in religious practices. The seed was milled and mixed with honey or human blood and shaped it into forms of snakes, birds, mountains, deer, and gods that were eaten either during ceremonies at the great temples or in little family gatherings (National Academy of Science, 1984). After the arrival of the Spanish conquistadors in Latin America, the use of amaranth as a staple food ended and slowed the spread into world agriculture of a highly nutritious food. However, since the early 1980s, the

production of amaranth has rapidly expanded particularly in developing countries through introducing and cultivating different varieties of the crop (Cai et al., 2004).

2.5.2. Botanical Description

Amaranth is a dicotyledonous and fast growing plant, tolerant to drought, high temperature and pests. In this environment the plant produces higher yields of seeds than other conventional cereals (Barba de la Rosa et al., 1992).

Amaranthus caudatus

It is an erect annual, up to 1.5 m tall, all parts commonly red or purple; stem stout, often not or sparingly branched, glabrous or usually with long, multicellular hairs in the upper parts. Leaves: petiole up to 8 cm long, but not longer than the blade; blade: 2.5-15 x 1-8 cm, broadly to rhomboid-ovate or elliptic-ovate, glabrous or sparsely hairy on main veins below, apex: obtuse to sub acute. Flowers: in axillary and terminal spikes of crowded cymose clusters, the terminal inflorescence varying from a single, pendulous tail-like spike up to 30 cm or more long and 1.5 cm wide, to a panicle with a long terminal spike, male and female flowers intermixed. The seeds: are shining, varying from compressed and black to subspherical with a thick yellowish margin and translucent centre, almost smooth, 0.7-1.3 mm (Townsend 2000).

Local names: Aluma and Yeferenjtuf (Amharic), Lishalisho (Oromia), Zapina (Arsi), Gegebsa (Gambela), Haleba (Kambata), Passa (konso), Ennomariam (Tigray), Gagabsa (Wolaita) and Kattila (Maji) (Teketay, 2010).



Figure 2.4 *Amaranthus caudatus* (Townsend 2000).

Amaranthus hybridus

It is an erect, less commonly ascending, annual, up to 3 m tall in cultivated forms, but usually much shorter, usually with some red-tinted stems in part or throughout; stems stout, simple to much-branched, glabrous or with long multicellular hairs in upper parts. Leaves: petiole up to 15 cm long, broadly lanceolate to rhomboid or ovate, glabrous or thinly hairy on veins and margin of lower surface, apex obtuse to subacute. Flowers in axillary and terminal spikes of cymose clusters, that are densely crowded in upper parts, the terminal inflorescence varying from a single spike to a broad, much branched panicle, up to 45 cm long, uppermost spike sometimes nodding; male and female flowers intermixed. Seeds faintly reticulate around margins, lens-shaped (Townsend, 2000).

There are two subspecies under *A. hybridus*, namely *A. hypochondriacus* and *A. cruentus*. Both are known for their grains. *A. hypochondriacus* can grow at altitude of 1500-2400 m while *A. cruentus* grows at lower altitude (350-2350 m) (Townsend 2000).

2.5.3. Botanical Distribution in Ethiopia

Most *Amaranthus* species are widespread in tropical and subtropical regions of the world. They mostly grow as a weed of cultivation in degraded land and built-up areas, along rivers, roadsides and forest edges. The species grow in the low and midlands as well as on higher altitudes (900–2,600m) on a wide range of soils, but they are most common in middle and high altitudes (1,400 - 2,400m)

(http://www.africa.upenn.edu/faminefood/category3/cat3_Amaranthus_caudatus.htm).

Amaranthus caudatus grows at an altitude ranging from 500 to 2500m above sea level. Accordingly, as shown in the figure below this species can grow almost in every part of the country except some parts of the western, north eastern and south western Ethiopia (Townsend, 2000).

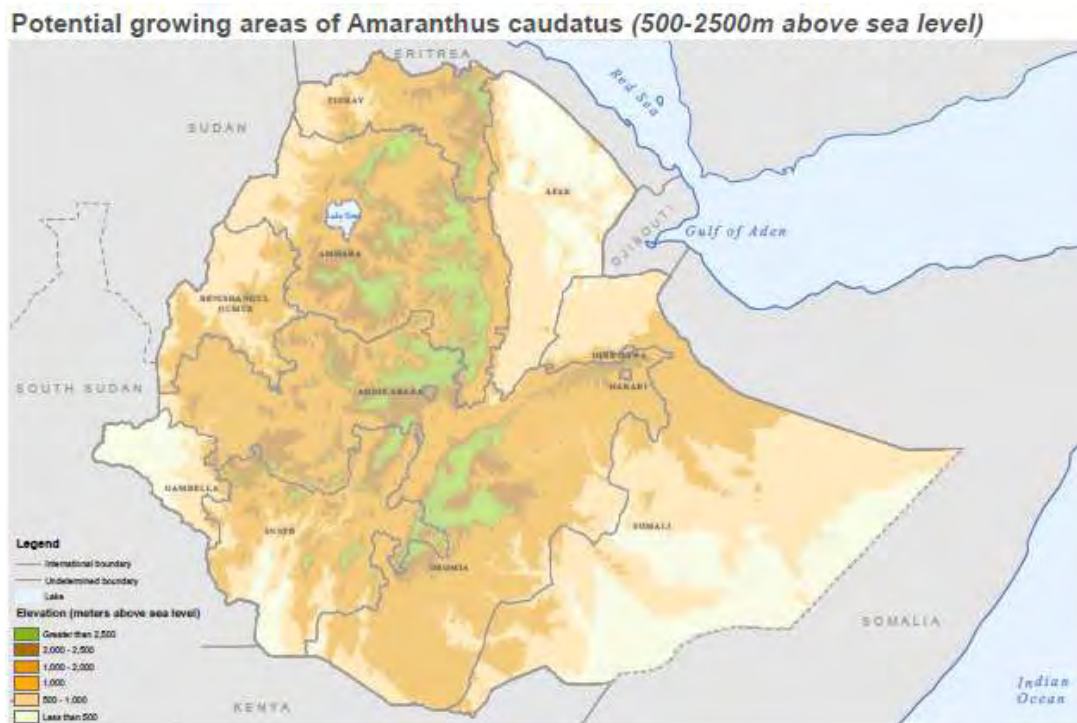


Figure 2.5 Potential growing areas of *Amaranthus caudatus* in Ethiopia

2.5.4. Worldwide Distribution

The native habitats of grain amaranth (mainly *Amaranthus cruentus* and *Amaranthus hypochondriacus*) are distributed throughout Mexico and extend into Guatemala and the southwestern United States. In South America (mainly *Amaranthus caudatus*) they are found in a band stretching from southern Ecuador through Peru and Bolivia into northern Argentina (Figure 2.6). Introduction of amaranth as a human food has been slow, but today it is produced and used as a grain or leafy vegetable in India, China, Southeast Asia, Mexico, the Andean highlands in South America and the United States. The Nebraska panhandle has become the most concentrated area of production of grain amaranth in the US.

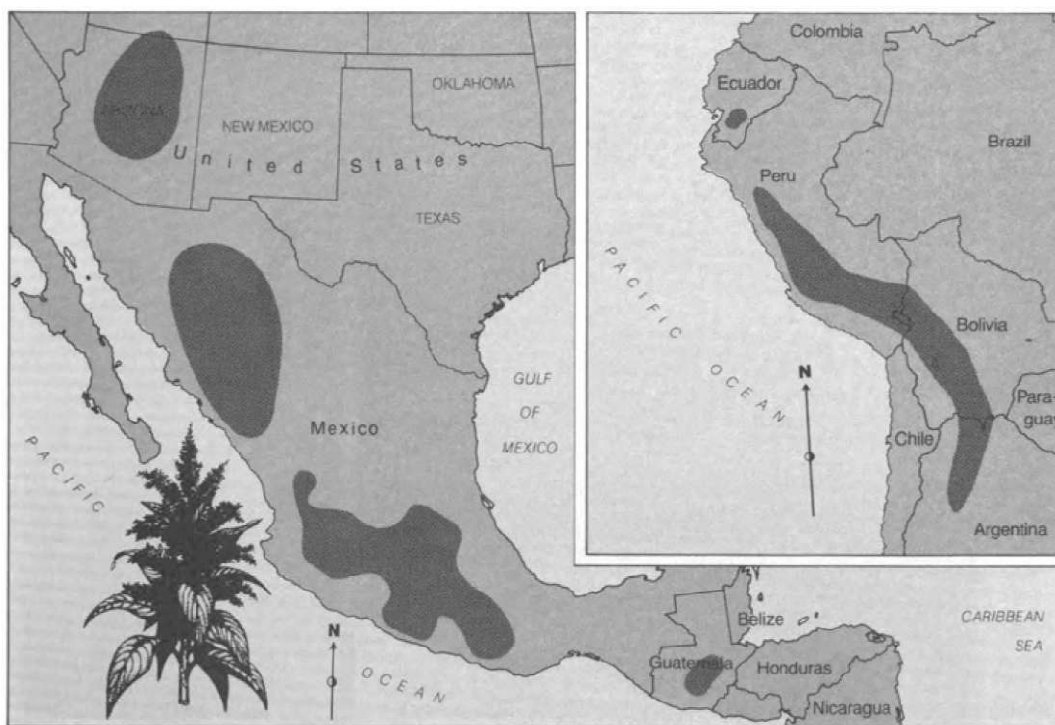


Figure 2.6 Distribution of *Amaranthus* in America (Source: National Academy of Science, 1984)

2.5.5. Edible part(s) of amaranth and mode of consumption

Amaranth is a multipurpose crop supplying high nutritional quality grains and leafy vegetables for food and animal feed; as possessing attractive inflorescence coloration, it also may be cultivated as an ornamental plant (Mlakar et al., 2009). The leaves and young shoots

are edible. They are used in salads and soups or eaten as spinach (Akubugwo et al., 2008). The seeds are made into breads, cakes, cookies, pancakes, crackers, pasta, confectionary and soups (Caselato-Sousa and Amaya-Farfán, 2012; Teutonico and Knorr, 1985). Kauffman and Weber (1990) also provided a description of the variety of products made from amaranth in different parts of the world. These include soups and stews from whole grain; *alegria*, a confection made from popped amaranth in Mexico; *atolea*, a fermented Mexican drink made from roasted amaranth flour; *chichi*, which is a form of beer made from amaranth in Peru; *sattoo*, a gruel consumed in Nepal, and *chapatti* made in different parts of Asia. In Ethiopia, the leaves are boiled and consumed as vegetable and the husk of the seed is also used as food in Konso while the young branches of the stalk are also eaten in South Omo by the Ari people. The plant seeds are also used for preparation of local beverage known as ‘Chaqa’ in Konso. The plant is very common and semi-domesticated on farm fields in Konso and South Omo. Some farmers have started to cultivate and intercrop the species on their farm fields near their homestead. In Konso the species is found intercropped with cotton, maize, sorghum and beans (http://www.africa.upenn.edu/faminefood/category3/cat3_Amaranthus_caudatus.htm).

Similarly, amaranth is better cultivated in Bench Majji Zone by a typical ethnic group called Me’enit society intercropped with maize and sorghum. The most frequent mode of consumption by these people is preparation of porridge from flour of popped amaranth grain.

2.5.6. Vegetable amaranth

Most *Amaranthus* species have edible leaves, and several species such as *A. lividus* L., *A. viridis* L.; *A. tricolor* L.; *A. gangeticus* L. and others are already widely used as potherbs (boiled greens). Their mild spinach-like flavour, high yields, ability to grow in hot weather, and high nutritive value have made them popular vegetable crops, perhaps the most widely eaten vegetables in the humid tropics of Africa and Asia (Mlakar et al., 2010).

2.5.7. Grain Amaranth

Grain amaranth belongs to a group of cereal-like grain crops or pseudocereals. The three principal species considered for grain production include *Amaranthus hypochondriacus* L.-

prince's feather; *A. cruentus* - bush greens, red amaranth and *A. caudatus* L. of two subspecies: subsp. *caudatus*; and subsp. *Mantegazzianus* (Mlakar et al., 2010).

2.5.8. Seed/Grain

The cross sectional and longitudinal sections of amaranth grain is shown in Figure 2.7. Amaranth grain is very small, lenticular in shape, 1.0-1.5 mm in diameter. The germ represents, together with the seed coat, around 25–26% of the weight of the seed, as obtained by milling techniques. This fraction is relatively rich in fat and protein. The perisperm represents 68–78% of the weight of the grain. In cereal grains, such as maize, the seed coat plus germ represent around 16–18% of the weight of the kernel, with the endosperm representing about 65%. The yield of amaranth could range between 1.1-1.5 t ha⁻¹ in some parts of the world. Better yield reaching to 3.0-5.9 t ha⁻¹ was obtained in Mexico (Bressani 2003). Ugandan amaranth also provides about 5 t ha⁻¹ (Muyonga et al., 2008). In Ethiopia, unpublished data showed that, the yield of the crop was estimated to range from 1.6 to 4 t.ha⁻¹. The global annual production of amaranth has been estimated at 470*10⁶ t per year world-wide (Bressani, 2003).

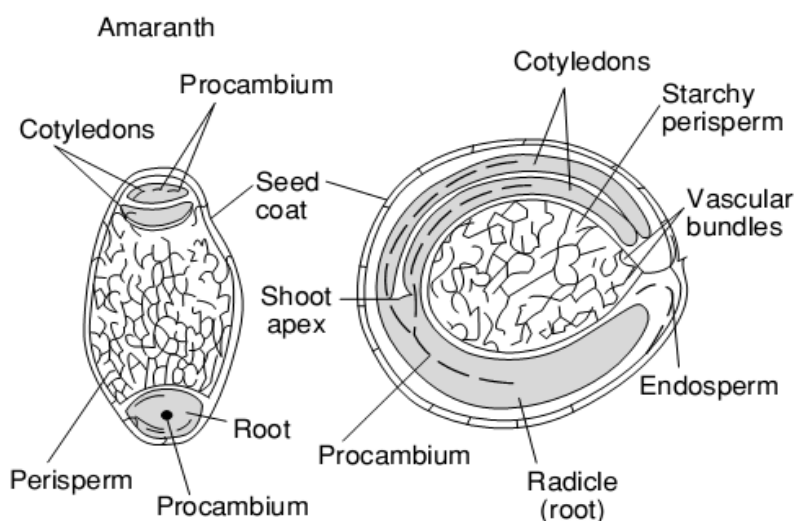


Figure 2.7 *A. cruentus* seed in (left) cross- and (right) longitudinal sections as viewed in a light microscope. Adapted from Bressani (2003).

2.5.9. Significance of amaranth in the past and in the present

In Pre-Columbian times, grain amaranth was one of the basic foods of the New World. It was nearly as important as corn and beans and was one of the principal items demanded as tribute. Amaranth was interwoven with legend and ritual. On various days of the religious calendar, Aztec women ground the seed, mixed it with honey or with human blood, and shaped it into forms of snakes, birds, mountains, deer, and gods that were eaten either during ceremonies at the great temples or in little family gatherings. After the arrival of the Spanish conquistadors in Latin America, the similarity between this communion ritual and Catholic Holy Communion, forced the Spaniards to prohibit the cultivation and use of amaranth by legislative fiat (National Academy of Science, 1984). After the migration of amaranth to Asia, Indians started to use amaranth by popping the grain and make it into confections, called laddoos, with honey or syrup, just the way the Aztec did. And among Hindus, popped amaranth grain soaked in milk was used on certain festival days when eating traditional cereals is forbidden. According to the report by Sauer (1967) amaranth was introduced into Spain in the 16th century, from where it had spread throughout the Europe. Around 1700s, it was known as a minor grain plant in central Europe and Russia and by the early 19th century it reached Africa and Asia. After that amaranth production in Europe declined and reduced to the state of an ornamental plant. The promotion of amaranth in the world was slowed down due to the aforementioned facts.

In the present day amaranth gets the attention of many scientists due to its good nutritional quality and health benefits. There exists a surprisingly large volume of literature available, particularly on the nutritional qualities of amaranth, health benefits, crop breeding, production and processing methods, development and commercialization of new amaranth products (Chaturvedi *et al.*, 1993; Berger *et al.*, 2003; Martinez *et al.*, 2014; Mburu *et al.*, 2011; Mustafa, *et al.*, 2011; Gamel *et al.*, 2005; Fritz *et al.*, 2011; Orsini Delgado *et al.*, 2011; Martirosyan, 2007). The strongest interest in amaranth (investigation and production) in Europe has been in Austria, Czech Republic, Slovak Republic, Germany, Hungary, Poland, Russia, Italy and Slovenia (Berghofer and Schoenlechner, 2002).

2.5.10. Nutritional benefits of Amaranth

2.5.10.1. Protein

Amaranth is often called the “small giant” because of its small grains with high nutritive value. Alike teff, the Ethiopian’s staple crop, amaranth grain is not susceptible for decortications and thus creates opportunity to utilize all the nutrients available in the grain. According to a report by Pedersen et al. (1987), amaranth has superior protein content than maize, sorghum and wheat. The diversity of amaranth is huge and thus there will exist a variation in the content of proteins but grain amaranth generally has a protein content ranging between 14.5-15.1g/100g (Rodas and Bressani, 2009). However, reports in different literatures used different values as a conversion factor that would result in differences in the values but Berghofer and Schoenlechner (2002) used a factor 5.85 with the assumption that there exist non proteneous nitrogen in amaranth.

2.5.10.2. Amino acids

Amino acids are the building blocks of proteins and can be defined as organic acid molecules that also carry at least one amino group. The amino group is attached to the alpha carbon relative to the carboxyl functional group. There is also another group that distinguishes one amino acid from another amino acid. The distinguishing groups are given the generic name of ‘side chains’ (‘R-groups’) (Amaya-Farfan and Acheco, 2003). Each amino acid is linked with a peptide bond to form a larger polypeptide called protein. The difference in sequence of same amino acids will produce a different polypeptide.

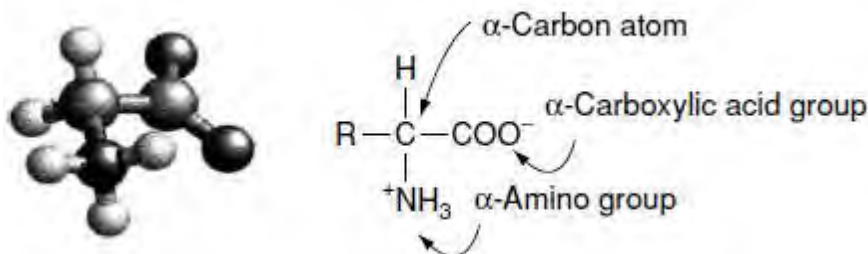


Figure 2.8 Major structure of amino acid

High content of protein in a typical food may not guarantee that it contains a balanced amino acid. For a food to be of good quality the amino acids should be present in a way to meet the daily requirement of an individual. Several studies conducted on different species of amaranth showed that amaranth had better amino acid profile than other cereals and legumes. It is rich in lysine and sulfur containing amino acids where these nutrients are lacking in cereals and legumes, respectively (Gamel et al., 2004). Due to its superior protein quality, supplementation of amaranth on wheat flour was found to improve the protein quality of the flour by about 45% (Abreu et al., 1994).

i. Method of Analysis for amino acids

The determination of amino acids usually followed after acid hydrolysis of the sample. This step resulted in the degradation of amino acids mainly tryptophan and thus a different step should be followed during the determination of tryptophan. Some of the major techniques applied during amino acid determination are high performance liquid chromatography, gas chromatography coupled with mass spectrometry, capillary electrophoresis coupled with mass spectrometry, liquid chromatography coupled with mass spectrometry.

HPLC

High performance liquid chromatography (HPLC) is the most popular method for analyzing amino acid components. Using UV detection for amino acids in most cases requires the absorption of the carboxyl group (-COOH) in the 200 to 210 nm range. Some amino acids with benzene rings can also be detected in the 250 to 280 nm range, but in general, they are difficult to analyze as-is with sufficient sensitivity and selectivity. Consequently, derivatization methods have long since been used. Since many amino acids contain amino groups (-NH₂ and -NHR) in their structures, a derivatizing reagent that selectively reacts with the amino group is used. The derivatization may takes place before separating the amino acid (pre-column) or after separating the amino acids (post-column) and the detection could be using UV/Vis or fluorescence detector. However, precolumn derivatization with orthophthaldialdehyde and/or 9-fluorenylmethyl chloroformate which is followed by reversed phase high-performance liquid chromatography (RP-HPLC) and fluorescence detection is the most widely used technique (Nimbalkar et al., 2012).

GC-MS

Analysis of amino acids using GC requires derivatization of amino acids, which has been effectively performed with chloroformates. Although this method demonstrates good resolution and sensitivity, it requires manual derivatization procedure. It is a laborious method and fails to determine some amino acids (Thiele et al., 2008).

CE-MS

Capillary electrophoresis (CE) is a technique which uses an electric field to separate a mixed sample into its constituents (Lewis et al., 2013). After separation, quantitative determination will take place using mass spectrometry (MS). Poinot et al. (2010) extensively reviewed the application of CE for the analysis of amino acids in different food and beverage samples.

LC-MS

This method effectively separates the amino acids without any derivatization using liquid chromatography (LC). Confirmation of the identity and purity of peaks and quantification will take place with the interfaced MS (Armstrong et al., 2007).

ii. Factors affecting protein digestibility

Amaranth is a rich source of protein compared to other commonly utilized cereals such as maize, wheat, sorghum, barley and etc (Pedersen, 1987; Gamel et al., 2004). However, the presence of high amount of protein in this crop wouldn't guarantee that it has superior nutritional quality over the other cereals as the quality entirely depends on the extent to which the nutrients in it are available to the cells. In this regard, as one way of testifying the protein quality of this crop the protein digestibility could be used as an indicator.

Protein digestibility is essentially a measure of the susceptibility of a protein to proteolysis. A protein with high digestibility is potentially of better nutritional value than one of low digestibility (Duodu et al., 2003). This is because it would provide more amino acids for absorption after proteolysis. There are several determining factors to affect the protein digestibility of food proteins which could be divided into two categories: Exogenous factors and endogenous factors.

Exogenous factors

These refer to factors that arise from interaction of proteins with non-protein components like tannins, phytates, non-starch polysaccharides, starch, and lipids. The mechanism by which these exogenous factors interfere in the digestibility of protein could be either by involving in a chemical interaction with the protein and the resulting product may be indigestible or by forming a physical barrier and prevent access of proteases to the protein (Duodu et al., 2003). Several studies have reported that tannins are potential protein precipitants and they reduce protein digestibility and amino acid availability in animals fed tannin-containing cereals such as sorghum, and grain legumes such as field beans and fababean (Jansman et al., 1995; Duodu et al., 2003). Elkin et al. (1996) also conducted in vivo experiment to evaluate the effect of tannin on protein digestibility using 20 sorghum cultivars varying in tannin content. The report showed that there were significant overall inverse relationships between tannin content and the mean digestibility amino acids. Tannins could bind both dietary and endogenous proteins (such as digestive enzymes and proteins located at the luminal side of the intestinal tract) resulting in a significant reduction in protein digestibility and amino acid availability (Gilani et al., 2012).

Similarly, phytate has also been found to interact with protein and forms different complexes depending on the pH. At low pH (for example in the stomach), phytic acid forms electrostatic linkages with the basic arginine, lysine and histidine residues resulting in insoluble binary complexes. Above its isoelectric point, a protein carries a negative charge, and multivalent cation bridging (typically involving calcium) appears to be involved in the formation of a complex between phytate and proteins (Ravindran et al., 1999). Therefore, Phytate:cation:protein ternary complex would be expected to dominate at the higher pH found in the small intestine (Selle et al., 2000). Phytate can also negatively influence the activity of digestive enzymes, such as carboxypeptidases and aminopeptidases, by the chelation of mineral cofactors or interaction with the protein (either enzyme or substrate) (Gilani et al., 2012). Lothia et al. (1987) reported that addition of phytate to multienzyme proteolytic assay systems was shown to significantly (up to 25 %) inhibit in vitro digestion of casein. Phytate has also been reported to inhibit trypsin activity in some studies but not in all investigations

(Selle et al., 2000). Fermentation treatment of millets resulted in high percentage reduction in phytate content that could cause significant improvement in in vitro digestibility of millets (Antony and Chandra 1999; El Hag et al., 2002; Ali et al., 2003). However, there are so many biochemical changes occurring during fermentation as a result only phytic acid degradation might not be the sole contributor to the observed improvement in protein digestibility.

Interaction of protein with starch could also affect the protein digestibility mostly by physically blocking the action of enzymes on protein. Duodu et al. (2002) showed that treating cooked maize whole grain and endosperm flours with alpha-amylase prior to pepsin digestion led to an improvement in protein digestibility.

Proteins are also part of cell wall components of many cereals in the form of glycoprotein (Duodu et al., 2003). The glycoproteins are rich in hydroxyproline residues that could serve as an attachment point for arabinose oligosaccharide (Raz et al., 1991). The polypeptide-carbohydrate linkage is thought to be an *O*-glycosidic linkage in which the reducing terminus of the carbohydrate is attached to an –OH group on the polypeptide. Such structural glycoproteins are highly resistant to most proteases, especially when the oligoarabinose side chains are still attached and entirely influence the protein digestibility (Duodu et al., 2003).

Endogenous factors

These refer to factors that arise out of changes within the proteins themselves and do not involve interaction of the proteins with non-protein components. During food processing formation of disulfide bridge, which is dependent on the amount of cysteine present, may result in formation of enzymatically resistant protein polymers. Such protein crosslinks may bring about decreases in the digestibility and biological value of the food proteins (Duodu et al., 2003). Racemization (i.e. conversion of D form of amino acid to the L form) could improve the protein digestibility as the L-form of amino acid is better absorbed than the D-form (Liardon and Hurrell, 1983). A highly ordered structure of protein could also encounter distortion during food processing and influence the digestibility. Duodu et al. (2001) showed the changes in secondary structure of sorghum and maize after wet cooking and popping using FTIR and solid state C¹³ NMR spectroscopy. These changes in secondary structure

resulted in decreased protein digestibility although the effect of popping is less than that of wet cooking despite same structural changes were observed (Parker et al., 1999; Duodu et al., 2001).

2.5.10.3. Lipids

Lipids are classes of organic compounds including fatty acids and their derivatives, steroids, terpenes, carotenoids, and bile acids, which have in common a ready solubility in organic solvents such as diethyl ether, petroleum ether, hexane, benzene, chloroform, or methanol (Christie, 1982). Another definition by Kate (1986) says that those substances which are (a) insoluble in water; (b) soluble in organic solvents such as chloroform, ether or benzene; (c) contain long-chain hydrocarbon groups in their molecules; and (d) are present in or derived from living organisms. The U.S. Food and Drug Administration (FDA) has defined lipids as the sum of the components with lipid characteristics that are extracted by Association of Official Analytical Chemists (AOAC) methods or by reliable and appropriate procedures (Schmidt et al., 1995). There are different issues raised in all the definitions, however, a more precise working definition is difficult given the complexity and heterogeneity of lipids.

i. Classification of Lipids

Classification of lipid is possible based on physical properties at room temperature (oils are liquid and fats are solid), their polarity (polar and neutral lipids), their essentiality for humans (essential and nonessential fatty acids), or their structure (simple or complex). Neutral lipids include fatty acids, alcohols, glycerides, and sterols, whereas polar lipids include glycerophospholipids and glyceroglycolipids. The separation into polarity classes is rather arbitrary, as some short-chain fatty acids are very polar. A classification based on structure is, therefore, preferable.

Based on structure, lipids can be classified as derived, simple, or complex. The *derived lipids* include fatty acids and alcohols, which are the building blocks for the simple and complex lipids. *Simple lipids* are composed of fatty acids and alcohol components which include acylglycerols, ether acylglycerols, sterols, and their esters and wax esters. In general terms,

simple lipids can be hydrolyzed to two different components, usually an alcohol and an acid. Complex lipids include glycerophospholipids (phospholipids), glyceroglycolipids (glycolipids), and sphingolipids. These structures yield three or more different compounds on hydrolysis (O'Keefe 2002).

The lipid content of amaranth is by far better than most commonly utilized cereals and pseudo cereal counterparts. Pedersen et al. (1987) reported that the lipid content in amaranth can reach up to 10.2 g/100g. Other studies reported that different amaranth species/varieties have different levels of lipid and could range between 6.4-8.6 g/100g (Mustafa et al., 2011; Nascimento et al., 2014). Therefore amaranth could be used as a candidate crop for the oil industries. The high amount of oil in amaranth can be used to reduce the amount of added fat when amaranth flour is used as ingredient in baked products such as cookies and cakes, where fat plays an important role in texture and flavor (Segura-Nieto et al., 1994). Gamel et al. (2007) reported the fraction of lipids in two varieties of amaranth. Triacylglycerols (TAGs) and free fatty acids (FFAs) represent the major fraction (80.3-82.3%). The percentage of other fractions; diacylglycerols (DAGs), monoacylglycerols (MAGs) and phospholipids represent 5.1-6.5, 3.0-3.5, and 9.1-10.2%, respectively.

2.5.10.4. Fatty acids

The fatty acids constitute the obvious starting point in lipid structures. They could be classified as saturated vs unsaturated (based on the presence of double bond) and essential vs non essential (based on essentiality to human) (O'Keefe, 2002). Depending on the number of double bonds present, unsaturated fatty acids could be classified as monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs). Several reports showed that amaranth contains high percentage of unsaturated fatty acids reaching to 3-4-fold compared to the saturated fatty acids (Prakash et al., 1995; Gamel et al., 2007). The only two essential fatty acids for humans are alpha linolenic acid (omega-3-fatty acid) and linoleic acid (omega-6-fatty acid) which are also unsaturated fatty acids. These fatty acids must be ingested because the body requires them for good health but cannot synthesize (Whitney and Rolfes, 2008). Amaranth oil was found to contain 40-50% omega-3-fatty acid and less than 1% omega-6-fatty acid (Gamel et al., 2007). This shows that amaranth oil has several health benefits

associated with its high level of omega-3-fatty acid (Larsson et al., 2004; Lunn and Theobald, 2006).

2.5.10.5. Squalene

Squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene) is a biosynthetic precursor to all steroids. It is an intermediate in cholesterol biosynthesis and an important ingredient in skin cosmetics due to its photoprotective role and as a lubricant for computer disks due to its thermostability (He et al., 2002; Ryan et al., 2007). Squalene is a component of the unsaponifiable fraction of lipids and mainly found in shark liver oil and olive oil (Ryan et al., 2007).

Several studies have indicated that squalene has tremendous health benefits such as acting as cancer chemopreventive agent (Smith, 2000), as an antidote to reduce accidental drug-induced toxicities (Aguilera et al., 2005; Senthilkumar et al., 2006), and antioxidant and antitumor activity (Huang et al., 2009). The squalene content of 11 genotypes of amaranth was reported to be in the range of 4.2-6.14 (He et al., 2002) which is by far better than the content in other vegetable oils such as linseed, sesame, maize, barely and etc (Ryan et al., 2007). The method of oil extraction was also found to provide different amount of squalene. Oil extracted in three different methods: supercritical CO₂ extraction, chloroform/methanol mixture solvent extraction and cold-pressing resulted in different squalene content, the highest being for supercritical CO₂ extraction (6.95 g/100 g of oil) followed by organic solvents (6.00 g/100g of oil) and in cold-pressed oil (5.74 g/100 g of oil) (Czaplicki et al., 2012). In general amaranth has been found to be the only best source of squalene to be considered next to shark liver oil.

2.5.10.6. Dietary Carbohydrates

The dietary carbohydrates are a diverse group of substances with a range of chemical, physical and physiological properties.

i. Classification of Dietary Carbohydrates

Chemical Approach

The chemical approach classifies dietary carbohydrate based on their degree of polymerization (DP) or the number of sugar units. Accordingly, all dietary carbohydrates fall in to three categories: sugars (DP:1–2), oligosaccharides (short-chain carbohydrates) (DP:3–9) and polysaccharides (DP: ≥ 10) (Table 2.2).

Sugars comprise monosaccharides such as glucose and fructose, disaccharides such as sucrose, maltose and lactose, and polyols (sugar alcohols) such as mannitol, sorbitol and xylitol. Oligosaccharides are, malto-oligosaccharides (α -glucans), principally occurring from the hydrolysis of starch and non- α -glucans such as raffinose and stachyose (α -galactosides), fructo- and galactooligosaccharides and other oligosaccharides (Asp, 1996).

Polysaccharides may be divided into starch (α -1:4 and α -1:6 glucans) and nonstarch polysaccharides (NSPs), of which the major components are the polysaccharides of the plant cell wall such as cellulose, hemicellulose and pectin but also includes plant gums, mucilages and hydrocolloids (Cummings and Stephen, 2007).

Table 2.1 Classification of the principal dietary carbohydrates.

Major classes (DP)*	Sub-groups (type of monosaccharide and α or β bonds)	Physiology
Sugars (1-2)	-Monosaccharides: glucose, fructose and galactose	Absorbed from the small intestine Glucose and sucrose give rapid glycemic responses
	-Disaccharides: Sucrose, maltose, lactose and trehalose.	Absorbed Lactose is fermented in many populations
	-Sugar Alcohols: sorbitol, manitol and xylitol	Poorly absorbed and partly fermented
Oligosaccharides (3-10)	Malto-oligosaccharides (α -glucan): Maltodextrins	-Digestible: digested and absorbed from small intestine and give rapid glycemic response. -Resistant: pass in to the large intestine and may be fermented
	Other oligosaccharides (NDO) [†] : Raffinose, stachyose, fructo- and galactooligosaccharides	Fermented, some selectively stimulate growth of bifidobacteria in large bowl
Polysaccharides (\geq 10)	Starch (α -glucan): Amylose, amylopectin and modified starch	-Digestible: varying rates of digestion and glycemic responses -Resistant: Not absorbed in small bowl, may be fermented and affect large bowl function
	Non starch polysaccharides: Cellulose, hemicelluloses, pectin, arabinoxylans, β -glucan, gums and mucillages.	-Cell wall: contribute to regulation of carbohydrate digestion in small bowl, fermented mostly but dependent on cell wall structure, major determinant of large bowl function, provides physical structure to plant foods. -Non cell wall: fermented to a variable degree, varying effects on carbohydrate and lipid absorption in the large bowl

*DP: Degree of Polymerisation or the number of monosaccharide units which make up the molecule.

[†]NDO. Non-digestible oligosaccharides.

Source: Cummings et al. (1997)

Physiological Approach

Based on their physiological properties dietary carbohydrates could be classified as: digestible and non digestible, glycemic and non glycemic, soluble and insoluble etc (Asp, 1996; Cummings and Stepehn, 2007). According to the first classification both oligosaccharides and polysaccharides may be undigestible in the small intestine. Starch is the digestible carbohydrate in most diet but a fraction of the starch, called resistant starch (RS) escapes digestion by the small intestine and contributes to the undigestible carbohydrate fraction (Cummings and Stepehn, 2007). These undigestible carbohydrate fractions have paramount contribution in improving bacterial populations thereby intensifies their prebiotic effect. Moreover, the biochemical profiles and physiological effects due to the undigestible carbohydrates fractions is also improved (Mussatto and Mancilha, 2007; Asp, 1996).

Foods containing carbohydrate have a wide range of effects on blood glucose concentration during the time course of digestion (glycemic response), with some resulting in a rapid rise followed by a rapid fall in blood glucose concentration, and others resulting in a slow extended rise and a slow extended fall. Prolonging the time over which glucose is available for absorption in healthy individuals greatly reduces the postprandial glucose response (Jenkins et al., 1990).

Polysaccharides are more slowly absorbed than simple sugars as a result the effect on postprandial blood glucose level is different. Slowly digestible carbohydrates contribute to a very smooth increment in blood glucose level while the rapidly digestible fractions caused a radical increase in postprandial blood glucose level. For example, fructose, a monosaccharide, gives a very low glycemic response and sucrose has a lower glycemic response than the most easily available forms of starch (Asp, 1996). It is therefore recommended for diabetic patients to consume diets rich in slowly digestible starch fractions. The glycemic index of some commonly consumed foods compared with a reference white bread is listed in Table 2.2.

Table 2.2 Glycemic Index of common foods

Food Item	GI (White Bread = 100)	Food Item	GI (White Bread = 100)
Rice, white, low amylose	126	Orange juice	74
Baked potato	121	Green peas	68
Corn flakes	119	Oat bran bread	68
Rice cakes	117	Apple	52
Carrots	101	Chickpeas	47
Sucrose	92	Skimmed milk	46
Spaghetti (boiled)	83	Kidney beans	42
Popcorn	79	Fructose	32
Banana	76		

Source: Foster-Powell and Brand Miller (1995).

Moreover, the rate and duration of the glycemic response of carbohydrate containing foods depends on the extent of digestibility of the major carbohydrate component called starch (Singh et al., 2010). The digestibility could be influenced by structural properties of starch and the processes applied to starchy foods. Starch consists of two types of molecules: amylose (linear polymer of α -D-glucose units linked by α -1,4 glycosidic linkages) and amylopectin (branched polymer of α -D-glucose units linked by α -1,4 and α -1,6 glycosidic linkages) (Rooney and Pflugfelder, 1986) and the ratio of amylose to amylopectin is different for different starch sources (Table 2.3) that could have significant contribution to the variation in starch digestibility.

Table 2.3 Some Typical Amylose-Amylopectin Mass Ratios for Starches.

Starch Source	Amylose	Amylopectin
High amylose corn	50-85	15-50
Corn	26	74
Waxy corn	1	99
Wheat	25	75
Rice	17	83
Cassava	17	83
Potato	21	79

Source: Zobel and Stephen, 2006

Starch could be classified as rapidly digestible (RDS), slowly digestible (SDS) and resistant starch (RS) based on in vitro digestion by simulating stomach and intestinal conditions and measuring glucose release at different time interval (Englyst & Cummings, 1987; Englyst et al., 1992). Rapidly digestible starch is the fraction of the starch that is hydrolyzed in 20 minutes and slowly digestible starch is the fraction of the starch that is hydrolyzed in between 20 and 120 minutes. Whereas the fraction of the starch that is left undigested after 120 minutes is called resistant starch (RS).

ii. Factors affecting starch digestibility

There are several factors to affect the digestibility of starch. Amylose/amylopectin ratio, the type and arrangement of the crystalline structure, the average molecular weight of the components, food particle size, amylose-lipid complexes, the presence of other materials in the food matrix (eg: sugar, protein and etc) and the presence of enzyme inhibitors (Thorne et al., 1983; Tester et al., 2006; Sharma et al., 2008) significantly played a role in influencing the starch digestibility.

Raw starches high in amylopectin have been shown to digest more quickly than those high in amylose (Hu et al., 2004; Sang et al., 2008). This is because amylopectin is a much larger molecule, average molecular weight of 10^5 and 10^6 , than amylose, average molecular weight of 10^4 . Therefore, amylopectin has a much larger surface area per molecule than amylose, which makes it a preferable substrate for amylolytic attack (Singh et al., 2010). Starch granular size is also another contributing factor for the variation in digestibility. Kaur et al. (2007) reported that there exist significant differences among the starch digestibility values for native potato starch and separated small, medium and large granular size fractions. The report showed that potato starch containing high percentage of smaller granules had higher digestibility followed by medium and larger size granules. The lower susceptibility of the large granule starches to enzymatic hydrolysis has been suggested to be due to their smaller granule surface-to-volume ratio, resulting in a decreased access for an enzymatic attack (Bednar et al., 2001). The pattern of the granular structure of the starch in A, B and C-type crystallites also influences the susceptibility for an enzymatic attack. Biliaderis (1989) reported that foods containing A-type starches are more susceptible for enzymatic attack than

B and C-type starches. The longer chains in B and C-type starches form longer and more stable helices making them more resistant towards enzymatic hydrolysis.

The interaction between amylose and lipid resulted in changing the structure of amylose from spiral to helical structure producing a thermally stable and water-insoluble starch. The susceptibility for amylase attack of such a complex structure of starch is very low and thus decreases the starch digestibility (Singh et al., 2010). Crowe et al. (2000) reported that addition of lauric, myristic, palmitic and oleic acids reduced the enzymatic hydrolysis of amylose by 35%. The report also showed that there exists better interaction of lipid molecules with amylose than amylopectin. This is attributed to the presence of helical conformation in amylose which could aid to form inclusion complexes with small hydrophobic molecules.

The action of α -amylase occurs rapidly if the starch is gelatinized than it is in its raw form. However, the presence of high amount of sugar could lower the rate of gelatinization (Rooney and Pflugfelder, 1986) and resulted in lower starch digestibility than expected.

Starch digestibility could also be influenced by protein. Starch granules mainly exist embedded with protein matrix and thus blocks the action of α -amylase towards starch. Lichtenwalner et al. (1978) clearly showed the effect of protein matrix on in vitro starch hydrolysis on sorghum. According to the report, pronase (protein hydrolyzing enzyme) treatment significantly increased the rate of starch hydrolysis because pronase hydrolyzed the protein matrix and increased the surface area of the starch for amylase attack. Another report by Jenkins et al. (1987) showed that the occurrence of starch-protein interaction resulted in decreased glycemic response and reduced rate of digestion.

The presence of high amount of anti-nutrients such as phytic acid, tannins and polyphenols may also play a role in starch digestibility. Therefore, processing of cereals and legumes using techniques such as dehulling, soaking and germination may result in an enhancement of digestibility due to the loss of these antinutrients which normally decreases the starch digestibility by inhibiting the activity of α -amylase. The inhibition effect of phytic acid on α -amylase could be through interaction with amylase protein and/or binding with minerals such

as calcium which is known to catalyze amylase activity thereby lowers the starch digestibility (Alonso et al., 2000a; Rehman and Shah 2005).

2.5.10.7. Dietary Fiber

The definition of dietary fibers has been a long subject of debate during the past years and many definitions has been proposed (Spiller, 2001). However, recently a compromise has been reached by Codex Alimentarius Commission and defined dietary fibers as carbohydrate polymers with ten or more monomeric units which are not hydrolysed by the endogenous enzymes in the small intestine of humans (Codex Alimentarius Commission, 2009). According to this definition, dietary fiber belongs to three different categories: (1) edible carbohydrates polymers naturally occurring in the food as consumed, (2) carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health and (3) synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health.

Dietary fibers have variety of chemical structures and physical properties (Landberg, 2012) and based on their characteristics they are classified as: dietary fiber vs functional fiber, soluble vs insoluble fiber, fermentable vs non fermentable and viscous vs non viscous. All these different fibers exert different physiological effects (Slavin et al., 2009). Dietary fiber is intrinsic and intact to the plants, while functional fibers are isolated forms (IOM, 2001). Based on the chemical properties of the fiber sources and analytical processes, fiber could be classified as soluble (e.g., gums, pectins), and insoluble (e.g., cellulose, wheat bran, soyhulls). Depending on the degree of fermentability in the large intestine fiber could also be classified as fermentable and non fermentable. Rapidly fermented fiber sources provide substrates for short chain fatty acid (SCFA) production by microflora in the large bowel, while slowly or incompletely fermented fiber sources improve bowel health by promoting laxation, reducing transit time, and increasing stool weight (Edwards, 1995; Schneeman, 2001). The last categorization is based on the ability to thicken when mixed with fluids. Therefore, viscous dietary fibers thicken and prolong gastric emptying and overall nutrient absorption (Malkki, 2001). It is also well documented that dietary fiber is involved in disease prevention such as

lowering risk of heart diseases, diabetes, obesity, diverticulitis, constipation, hemorrhoids and appendicitis, renal cell carcinoma, absorption of toxins and etc (Kendall et al., 2010).

i. Physiological and health benefits of dietary fibers

Dietary fibre is widely recognized as beneficial for overall human health, and high fiber intake is associated with reduced risk for a number of chronic condition and diverse physiological functions. Some of these health benefits are to aid proper removal of stool, lowering the blood cholesterol and glucose levels, modification of gut microecology, weight management, preventing from colon cancer, GI disease etc.

a) Laxation

Insoluble fibers are considered gut-healthy fiber because they have a laxative effect and add bulk to the diet, helping prevent constipation. They are resistant to bacterial degradation and have a greater influence on stool output and colonic transit. The increased stool weight is due to the physical presence of the fiber itself, water held by the fiber, and increased bacterial mass from fermentation (Kurasawa et al., 2000). Apart from insoluble dietary fibers, short chain fatty acids produced during fermentation of soluble fibers also contribute to fecal bulk (Cummings, 1978; 1997). The efficacy of different fibers in terms of increased fecal weight per gram of administered fiber was reported by Cummings (2001). The report showed that wheat bran, fruit or vegetables, psyllium, cellulose, oats, corn, legumes, and pectin could increase the fecal weight by 5.4, 4.7, 4.0, 3.5, 3.4, 3.2, 2.2 and 1.2 g/g fiber, respectively.

b) Cholesterol lowering

High fiber intake is associated with a lower prevalence of coronary heart disease (Rimm et al., 1996). The mechanism for the cholesterol-lowering effects of soluble fiber is likely related to the ability of fiber to increase bile acid excretion. Bile acids are produced in the liver from cholesterol, and enter the small intestine, where they may be bound by fiber and eliminated in the feces. This effect might be a function of the viscous nature of fibers in solution. Increased

loss of bile acid requires the liver to use more cholesterol for bile acid synthesis, thus reducing the body's cholesterol pool (Topping, 1991; Trautwein et al., 1999). Ye et al. (2012) reported that consumption of whole grains, known for their high fiber content, resulted in decreasing cardiovascular disease by 21% than the control groups. It is therefore to be noted that high fiber containing diets have a significant impact in lowering blood cholesterol and triglyceride levels and keep the consumer from diseases related with high cholesterol and triglyceride level in the body.

c) Improved Glycemic and Insulinemic Response

Viscous fibres, such as psyllium, β -glucan, and pectin, may form a gel in the small intestine, which acts to delay nutrient absorption, thus slowing delivery of glucose into the bloodstream and reducing the need for insulin (Brennan et al., 2012). Several reports also showed that meals containing soluble fibers (dietary or functional) have resulted in reductions in postprandial increases in blood glucose and insulin (Brand-Miller et al., 2012; Scazzina et al., 2013). The slower stomach emptying may also affect blood sugar levels and have a beneficial effect on insulin sensitivity, which may help control diabetes. A meta analysis study by Post et al. (2012) showed that supplementation of fiber for patients with type 2 diabetes mellitus was found to reduce fasting blood glucose and glycosylated hemoglobin (HbA1c). A randomized controlled trial experiment conducted on 121 participants with type 2 DM also showed that low GI legumes were found to decrease the level of HbA1c than a high wheat fiber (Jenkins et al., 2012). Therefore increasing dietary fiber in the diet of patients with type 2 diabetes is very important and strongly recommended as a disease management strategy.

d) Prebiotic Effect and Short chain fatty acid production

Fermentable fibers have the ability to increase bacterial mass with some acting as prebiotics to promote health-promoting bacteria such as lactobacilli and bifidobacteria in the colon (Roberfroid, 2005). These prebiotics have a role of reducing the risk and severity of gastrointestinal infection and inflammation, including diarrhea, inflammatory bowel disease, and ulcerative colitis as well as bowel function disorders, including irritable bowel syndrome.

They also increase the bioavailability and uptake of minerals and data suggest that they reduce the risk of obesity by promoting satiety and weight loss (Brownawell et al., 2012). Fermentable fibers in the colon are able to produce short-chain fatty acids (SCFAs) mainly butyrate (Anderson et al., 2009; Irrazábal et al., 2014). A steady production and absorption of SCFAs in the gut lumen is associated with a reduced risk of colorectal adenoma to colorectal cancers (Wong et al., 2006).

A recent report by De Vadder et al. (2014) explicitly showed that short-chain fatty acids (SCFAs), propionate and butyrate, activate intestinal gluconeogenesis (IGN). This has been proved in normal and IGN deficient mice, with referring to the metabolic benefits on body weight and glucose control induced by SCFAs, that is absent in mice deficient for IGN, despite similar modifications in gut microbiota composition. Therefore, the study recommended that regulation of IGN is necessary for the metabolic benefits associated with SCFAs and soluble fiber.

e) Weight management

Soluble fibers have the ability to trap water and form a gel, which could slows down digestion. They delay the gastric emptying of the stomach and make the person to feel full and helps to control body weight. Anderson et al. (2009) reviewed that reports of different cross sectional and prospective cohort studies revealed a strong negative association between fiber intake and obesity. The cross-sectional studies indicated that men and women with the highest level of fiber consumption have a relative risk for obesity of 0.77 (95% CI, 0.68–0.87) compared to those with the lowest fiber intake level and the prospective cohort studies reported that women and men with the highest level of fiber consumption had lower rates of weight gain and less obesity than those with the lowest level of fiber intake, with relative risks of 0.70 (95% CI, 0.62–0.78).

f) Fiber and colon cancer

Colorectal cancer (CRC) is one of the leading causes of cancer related deaths worldwide. In the United States, approximately 1 in 20 people will experience a diagnosis of colorectal cancer (Vargas and Thompson, 2012). The etiology of CRC is multifactorial and has been linked to genetic mutations, diet, inflammatory processes, and gut microbiota (Irrazabal et al., 2014). Gut microecology could demonstrate significant modification and when the diet of the consumer is rich in soluble fiber resulting in production of SCFAs responsible for the prevention of colon cancer (Wong et al., 2006). Meta-analysis of 25 prospective studies found a 10% decrease in relative risk for developing colorectal cancer when consuming 10 g of total dietary fiber daily (Aune et al., 2011). The report also showed 38% reduction in relative risk among individuals consuming legumes (eg, beans, alfalfa, peas, lentils, soy, and peanuts) as a primary source of dietary fiber.

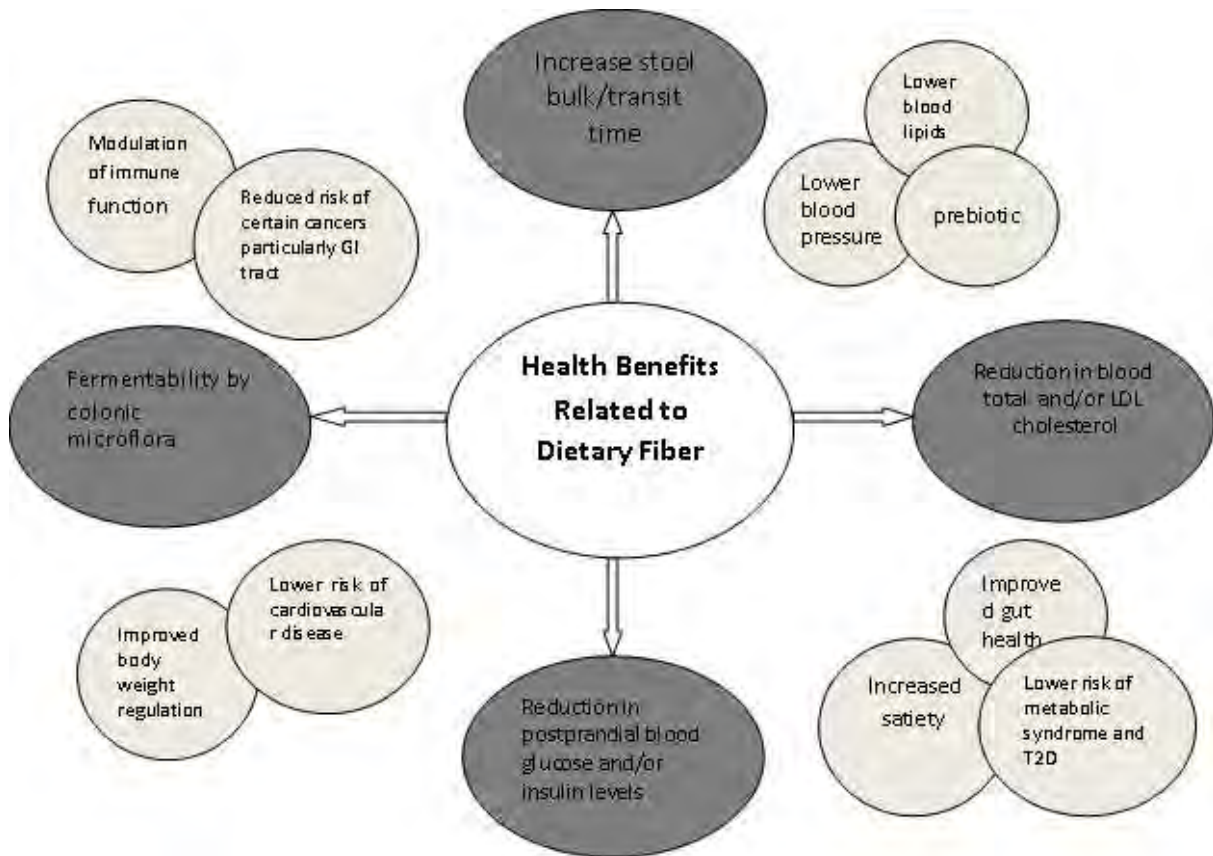


Figure 2.9 Health benefits of dietary fibers (Source: Reports of 2nd European BENE0 scientific symposium).

ii. Dietary fiber requirement

In the US, the Institute of Medicine recommends 25 g of fiber per day for women and 38 g for men (IOM, 2001). In Europe dietary recommendation postulate a daily intake of 25 g per day. However, the intake is far below the recommendations in both continents: 12-16 and 15-19 in US and Europe, respectively (Reports of 2nd European BENE0 scientific symposium). Dietary reference intake values of fiber through different life stage are shown on Table 2.4. The low availability of palatable, high fiber foods is one of the reasons for the consumers not to comply with the dietary guidelines. Moreover, some consumers perceive gas production as an uncomfortable side effect and restrict their dietary fiber consumption to avoid this. However, without gas production one cannot have the health benefits of fiber.

Table 2.4 Dietary reference intake for total fiber by life stage

Life stage	Adequate Intake (g/day)	
	Male	Female
1-3 y	19	19
4-8 y	25	25
9-13 y	31	26
14-18 y	38	26
19-30 y	38	25
31-50 y	38	25
51-70 y	30	21
>70 y	30	21
Pregnancy		28
Lactation		29

Source: Slavin (2005).

2.5.11. Mineral Absorption Inhibitors

Cereals, pulses and legume based commodities are rich and low-cost sources of nutrients for a large part of the World's population. But their nutritive value is limited by, the presence of absorption inhibitors such as phytates, tannins/polyphenols, dietary fibers etc.

2.5.11.1. Phytic acid/Phytates

Phytates represent a complex class of naturally occurring compounds that can significantly influence the functional and nutritional properties of foods (Maga, 1982). Phytate is the salt form of phytic acid and the later is composed of an inositol sugar, similar in structure to D-glucose, with six phosphate groups attached to each hydroxyl branch (Wodzinski & Ullah, 1996) (Figure 2.10). It is the major phosphorus storage compound in plant seeds accounting for up to 80% of the total phosphorus (Lopez et al., 2002) and it accumulates in the seeds during the ripening and maturation period (Loewus, 2002). Therefore, phytic acid is a common constituent of plant derived foods like cereals or legumes, which are the main staple food of people in developing countries. The daily intake of phytate for humans on vegetarian diets, on an average, is 2000–2600 mg whilst, for inhabitants of rural areas in developing countries, on mixed diets, it is 150–1400 mg (Reddy, 2002). Phytate accumulattion site in cereals and legumes is different (Table 2.5) but generally, 90% of phytate (or mineral salts of

phytic acid called phytins) are localized in the aleurone layer of many plants and 10% in the embryo (Lopez et al., 2002).

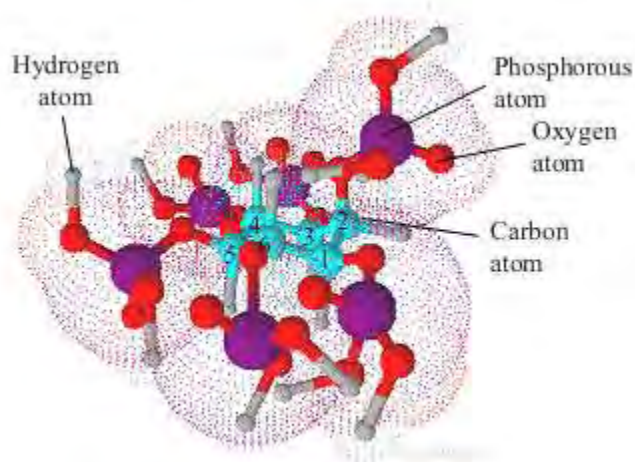


Figure 2.10 Structure of myo-inositol 1,2,3,4,5,6 hexakis[dihydrogen phosphahate] (Bohn et al., 2008)

Table 2.5 Distribution of phytate in morphological components of cereals and legumes

Cereal/legume	Morphological component	Phytate (%)	Distribution (%)
High lysine corn	Whole	0.96	-
	Endosperm	0.04	3.00
	Germ	5.72	88.90
	Hull	0.25	1.50
Soft wheat	Whole	1.14	-
	Endosperm	0.004	2.20
	Germ	3.91	12.90
	Aleurone layer (bran)	4.12	87.10
Brown rice	Whole	0.89	-
	Endosperm	0.01	1.20
	Germ	3.48	7.60
	Pericarp	3.37	80.00
Pearl millet	Whole	0.89	-
	Endosperm	0.32	-
	Germ	2.66	-
	Bran	0.99	-
Peas	Whole	0.79	-
	Cotyledon	0.78	88.70
	Germ	1.23	2.50
	Hull	0.01	0.10

(Source: Reddy, 2002)

The content of phytate in any plant food can vary depending on growing conditions such as season, soil profile, harvesting techniques, stage of maturation, species and genotype (Feil and Fossati, 1997; Coulibaly et al., 2011). Table 2.6 shows the content of phytic acid in major cereals, legumes and food products made from cereals and/or legumes.

Table 2.6 Phytate Contents of Cereals, legumes and their products.

Cereal/legume seed	Phytate (g/100g DM)	Cereal/legume seed	Phytate (g/100g DM)
<i>Cereal/cereal products</i>			
Wheat*	0.39–1.35	Pear millets (dehulled)*	0.3-0.52
Wheat bread [§]	0.32-0.73	Oat [¶]	0.88
Unleavened wheat bread [§]	0.32-1.06	Oat flakes [§]	0.84-1.21
Maize [§]	0.98-2.13	Oat porridge [§]	0.69-1.02
Maize bread [§]	0.43-0.82	Oat bran*	0.60-1.42
Unleavened maize bread [§]	1.22-1.93	Rye*	0.54-1.46
Barely*	0.38-1.16	Rye bread [§]	0.19-0.43
Sorghum [§]	0.59-1.18	Rice (polished, cooked) [§]	0.12-0.37
Sorghum (Low tannin)*	0.57	Rice (unpolished, cooked) [§]	1.27-2.16
Sorghum (High tannin)*	0.96	Rice (Basmati)*	0.06
Common millets*	0.50-0.70	Rice bran*	2.59-6.00
Teff [#]	1.54	Corn flakes [§]	0.04-0.15
Pearl millets*	0.18-0.99	Triticale [¶]	1.29
<i>Pseudo cereals</i>		<i>Legume/legume products</i>	
Buckwheat [¶]	1.42	Cowpea [¶]	0.66
quinoa [¶]	0.97	Cowpea (cooked) [§]	0.39-1.32
amaranth [¶]	1.39	Chickpea [¶]	0.48
<i>Infant cereals</i>		Chickpea (cooked) [§]	0.29-1.17
Infant cereal [§]	1.38	Soybean [¶]	1.40
Weaning foods [§]	0.06-0.70	Mungbean [¶]	0.83
Children's biscuits [§]	0.13	Fababeans (cooked) [§]	0.82-1.42
<i>Oil seeds</i>		Kidneybeans (cooked) [§]	0.83-1.34
Rapeseed [¶]	1.52	pea [¶]	0.63
Sunflower seed [¶]	1.52	lentil [¶]	1.15
Sesame seed (toasted) [§]	3.93-5.72	Lentil (cooked) [§]	0.21-1.01
		Peanuts [§]	0.92-1.97

Sources: *Reddy 2002, [§]Greiner and Konietzny, 2006, [¶]Egli 2002, [#]Baye, 2014

i. Adverse effects of phytate

Phytates have both beneficial and adverse effect as any food component. Some of the adverse effects of phytates are inhibiting mineral bioavailability, inhibition of starch and protein digestibility and hamper lipid utilization. While some of the beneficial effects are: prevent radical generation, act as antidiabetic agent, and prevent from coronary heart disease and renal lithiasis.

a) Inhibition of mineral bioavailability

Phytate is the major mineral absorption inhibitors present in many plant foods. It forms complexes with numerous divalent and trivalent metal cations. The phosphate groups of phytic acid are responsible for binding the metal cations such as iron, zinc, calcium, magnesium, manganese, copper and etc (Coulibaly et al., 2011). The inhibition action could depend on the pH of the intestine, the individual cation, the molar ratio of phytate to mineral and the presence of other componenets in the food such as mineral absorption inhibitors or enhancers (Vohra and Satyanarayana, 2003). Moreover, higher inositol phosphate were found to form strong association with the metal cations than lower inositol phosphates thereby lowers the mineral bioavailability (Lönnerdal et al., 1989; Sandberg et al., 1999).

b) Inhibition of starch digestibility

Several studies have shown that the presence of phytate in foods reduce the digestibility of starch (Thorne et al., 1983; Yoon et al., 1983; Thompson and Yoon, 1984; Knuckles and Betschart, 1987). This is due to the formation of phytate-starch complexes that reduces the solubility of starch which inturn adversely affect the digestibility and absorption of glucose. The glucose moiety of the starch could form linkage with phosphate groups of phytate thereby hinders the action of amylolytic enzymes to digest the starch (Noda and Sarker, 2014). In a study conducted by Lee et al. (2006) showed that diet supplemented with phytate resulted in reduced blood glucose levels of diabetic mice. Therefore, phytic acid addition to a meal could be used as an alternative strategy to manage blood glucose level in diabetes. However, low

starch digestibility will be a problem to infants and children due to their limited gastric capacity to ingest enough food to meet their dietary requirement.

c) Inhibition of protein digestibility

Phytate have been investigated to have a strong interaction with protein thereby lowers the protein digestibility. Knuckles et al. (1985) showed that digestibility of casein and bovine serum albumin significantly impacted by addition of phytate. The authors also showed that hydrolysis of the added phytate resulted in better protein digestibility. Another study by Carnovale et al. (1988) demonstrated that digestibility of lactalbumin, soybean protein and maize zein was inhibited by addition of phytate. Addition of phytase, an enzyme responsible to hydrolyze phytate, in broiler feedstuffs, on the other hand, was found to improve the protein digestibility and amino acid availability (Ravindran et al., 1999). The inhibition action of phytate against protein may be due to the non-specific nature of phytate–protein interactions and the chelation of calcium ions, which are essential for the activity of α -amylase (Kumar et al., 2010).

d) Effect on lipid metabolism

Alike other macronutrients, starch and protein, lipid metabolism is significantly influenced by the presence of phytate. This is due to the formation of “lipophytins” (i.e. phytate complexes with lipid and its derivatives) (Kumar et al., 2010). In vivo study conducted in mice by Lee et al. (2007) showed that the levels of serum and hepatic lipid levels decreased when phytate was supplemented in the diet of mice. The decreased levels of serum and hepatic lipid was also observed by an increase in total lipid, triacylglycerol, and total cholesterol levels in feces.

ii. Health Benefits of Phytic acid

a) Phytic acid as Antioxidant compound

Oxidation of food is a destructive process, causing substantial loss of nutritional value. Foods with high contents of unsaturated fatty acid and iron are more prone to undergo oxidation in

the presence of oxygen thereby produces undesirable flavour changes, discoloration, nutritional losses and microbiological spoilage (Kumar et al., 2010). The oxidation reaction can be minimized through the addition external anti-oxidant compounds or prevented by the existing antioxidant compound having the potential to quench radicals or prevent radical formation. Although many iron-chelating agents potentiate reactive oxygen formation and lipid peroxidation, phytic acid (abundant in edible legumes, cereals, and seeds) forms an iron chelate which greatly accelerates Fe^{2+} -mediated oxygen reduction yet blocks iron-driven hydroxyl radical generation and suppresses lipid peroxidation. As a result, phytate could be considered as a nontoxic natural food anti-oxidant (Graf et al., 1987). Moreover, phytic acid could also prevent browning and putrefaction of various fruits and vegetables by inhibiting the activity of polyphenol oxidase. Therefore, phytate may be used as a substitute for the present day food preservatives, many of which pose potential health hazards (Graf et al., 1987).

b) Phytic acid as an antidiabetic agent

Diabetes mellitus (DM) is a metabolic disorder caused by an absolute or relative lack of insulin. It is divided into 2 major categories: type 1 and type 2. These 2 types of diabetes have a distinct pathogenesis, but hyperglycemia and various life-threatening complications resulting from long-term hyperglycemia are their most common features (Bell, 2001). Effective blood glucose control is the key for preventing or reversing diabetic complications and improving the quality of life in diabetic patients. Lee et al. (2006) reported that supplementation of phytate at two levels 0.5% and 1.0% in diets of diabetic mice resulted in a remarkable decrease in fasting blood glucose level by 5% and 21%, respectively after 8 weeks of treatment compared with the control group. Non fasting blood glucose levels were also reduced by 8% and 15% at 0.5% and 1.0% phytate supplementation, respectively.

Another study by Kuppusamy et al. (2011) on type 2 diabetes mellitus (NIDDM) induced rats also demonstrated that supplementation of IP6 was found to significantly reduces the blood glucose levels. Moreover, the same study showed that the activity of carbohydrate degrading enzymes, α -glucosidase and α -amylase, was inhibited by IP6 in a dose dependent manner. It is

therefore clear that most of the animal studies showed that IP6 possess promising antidiabetic activity although the mechanism of action is not well known.

c) Phytate against coronary heart disease

Coronary heart disease is the leading cause of morbidity and mortality in many of the western countries (Jemal et al., 2005). Elevated plasma cholesterol or more specifically, elevated Low Density Lipoprotein cholesterol concentrations have been shown to be one of the risk factors. On the other hand people who consume more cereals, nuts and legume foods, the risk of coronary heart disease is low (Anderson and Hanna, 1999; Slavin et al., 2001; Anderson, 2004). This is due to the presence of high amount of dietary fiber or phytate as a component of fiber in many plant foods.

In vitro studies on animals have demonstrated that dietary phytate supplementation results in significant lowering of serum total cholesterol, triacylglycerol and low density lipoprotein cholesterol (Jariwalla et al., 1990; Lee et al., 2005). Hepatic total lipid and total cholesterol levels were also significantly reduced when the diet is supplemented with phytate (Lee et al., 2005). The etiology of coronary heart disease depends on the metabolic imbalance in regard to zinc and copper absorption (Klevay, 1975). The imbalance could be caused due to the amount and/or availability of zinc and copper. Phytate is known to preferentially complex with zinc than copper (Persson 1998) and therefore plays a significant role in lowering absorption of zinc without affecting the absorption of copper thereby affects the metabolic balance of zinc and copper.

d) Phytate against Renal Lithiasis

Renal lithiasis can be defined as the consequence of an alteration of the normal crystallization conditions of urine in the urinary tract (Grases et al., 2006). Renal lithiasis is a multifactorial problem which should be considered to be the risk factors related with urine composition and renal morphology. Epidemiological studies have shown that renal stone formation is related to dietary habits of a person. A study by Modlin (1967) on South African population that

constituted European and African origin population showed that renal calculi incidence in the European origin population is higher than the African origin population. The difference has been attributed to a different urine composition due to different dietary habits. The African origin population diet is abundant in whole wheat bread, corn, dry peas, fruits and vegetables while the European origin population consumes mainly refined foods of low phytate content. It has been demonstrated that phytate acts as a very effective inhibitor of heterogeneous nucleation of calcium oxalate (Grases and Costa-Bauzá, 1991; Grases et al., 1994), homogeneous nucleation of calcium phosphate (Grases et al., 2000) and crystalline growth of calcium oxalate (Grases and March, 1989). It is, therefore, necessary to incorporate phytate rich foods in the diet in order to prevent calcium oxalate crystallization and stone formation.

iii. Method of Analysis of phytate

Precipitation methods

Precipitation methods are based on the principle that phytate forms an insoluble stable complex with ferric ion in dilute acid. The phosphorus content in the precipitate can be determined after wet ashing or hydrolysis, giving a direct measure of the IP6 content. A certain molar ratio between iron and IP6 is necessary to have quantitative precipitation under this method. The content of IP6 could also be measured indirectly by quantifying the unprecipitated iron. For such type of measurement stoichiometric relationship between IP6 and unprecipitated iron is needed. The major limitations of precipitation methods is the lack of specificity to distinguish between IP6 from lower inositol phosphates, and are limited by the possible interference of phosphates especially in foods with high phosphate content (Skoglund and Sandberg, 2002).

Colorimetric Method

Colorimetric method for determination of phytate is based on the reaction between ferric chloride and sulfosalicylic acid, giving a pink-colored reagent with absorbance maximum at 500 nm. When IP6 is present, the iron becomes bound to the phosphate ester, therefore, unavailable to react with sulfosalicylic acid, resulting in a decrease in pink color intensity.

The decrease in absorbance is proportional to the concentration of IP6 present (Latta and Eskin, 1980). It was recognized that lower inositol phosphates and polyphenols could also complex with iron and therefore affect the precision of the measurement. As a result, Vaintraub and Lapteva (1988) proposed omission of purification step from the method used by Latta and Eskin (1988). Omitting the purification step was found to decrease the analysis time while the precision and sensitivity were increased.

Ion-exchange chromatography

It is a simple and low cost method to determine phytate for a large number of samples, though the analysis time is long. This technique was first introduced by Smith and Clarke (1952) and involves separation of inositol phosphates using a stepwise elution with HCl. Later, Harland and Oberleas (1977) proposed a modified method of anion exchange chromatography with step gradient elution for quantification of IP6. In this method the eluate is digested in order to measure inorganic phosphorus content and IP6 equivalent. Compared to precipitation methods, the values of IP6 obtained using ion exchange methods was higher and the possible reason for this was the presence of other interfering substances in the acid extract (Ellis and Morris, 1982). Addition of chelating agents like EDTA and adjusting the pH of the extract were proposed to bring comparable results in the two methods (Ellis and Morris, 1983).

The original method proposed by Harland and Oberleas (1977) was later modified and adopted as an AOAC method (Harland and Oberleas, 1986). However, it fails to accurately determine IP6 content due to interference from lower inositol phosphates (Phillippy and Johnston, 1985; 1988).

HPLC methods

HPLC methods are more sensitive and reproducible than the other techniques discussed so far for the simultaneous measurement of IP6 and lower inositol phosphates (Talamond, 2000). This method utilizes reversed phase octadecyl (C-18) stationary phases and aqueous potassium dihydrogen phosphate or sodium acetate mobile phases to separate IP6 from lower

inositols (Knuckles et al., 1982). The phytic acid was extracted using HCl and purification of the extract requires a strong anion exchange resin (Graf and Dintzis, 1982). Although this technique is time consuming, it selectively quantifies IP6 and lower inositols phosphates making it sensitive and reproducible technique for the analysis of phytate in most foods.

Enzymatic Method

This technique involves the sequential addition of phytase and alkaline phosphatase in to phytic acid extract. The phytase hydrolyzes *myo-inositol* hexaphosphate in to the lower inositol phosphates and the lower inositol phosphates further hydrolyzed in to *myo-inositol* and free phosphorus. The free phosphorus could be measured spectrophotometrically after the reaction with coloring agent (Megazyme phytic acid/Total phosphorus Assay, www.megazyme.com).

iv. Degradation of phytate

Degradation of phytate (*myo-inositol* hexakisphosphate, IP6) is an indispensable process to improve the nutritional quality of plant foods. This is because removal of phosphate groups from the inositol ring decreases the mineral binding strength of phytate which in turn resulted in improved mineral bioavailability (Sandberg et al., 1999). Moreover, the accessibility of other nutrients like protein, carbohydrates and lipids to the respective digesting enzymes namely protease, carbohydrase and lipase could also be enhanced.

Hydrolysis of IP6 may occur in the gastrointestinal tract prior to the intestinal site of absorption. Because most of the essential minerals and trace elements are absorbed in the duodenal or jejunal part of the small intestine, the site and degree of phytate degradation can affect the nutritional value of a high phytate diet (Holub, 1987). Moreover the efficiency of phytate degradation by endogenous enzymes, plant or microbial origin, depends on the source of the enzyme (Egli et al., 2002) and the medium of which the enzyme is working eg. pH and temperature, (Beal and Mehta, 1985; Silva and Trugo, 1996; Bergman et al., 2000). As a result selection and/or application of raw material having high phytase activity and

optimization of conditions for maximum phytic acid degradation during food processing is indispensable so as to improve the nutritional quality of plant foods.

a) Phytate degradation during food processing

The role of food processing techniques in improving the nutritional quality through phytic acid degradation is immense especially for people whose diet is mainly based on plant foods. This is because plant foods contain limited amount of important minerals which again are poorly available due to the presence of high amount of phytate. It is therefore usually important to apply processing mechanisms to decrease the content of phytate which then increases the availability/accessibility of important minerals like iron, zinc and calcium. Some of these biological processes are soaking, malting, fermentation and thermal treatment. The main working principle for many of those techniques is to improve the activity of endogenous enzyme.

Soaking

Soaking cereals and legumes promotes the transfer of phytate and other water soluble nutrients especially minerals to the media. Moreover, this process also enhances the action of naturally occurring endogenous phytases in cereals and legumes thereby resulting in a considerable amount of phytic acid degradation (Hotz and Gibson, 2001). Lestienne et al. (2005) observed that soaking of millet and soybean whole seeds for 24 h resulted in 29% and 23% reduction in phytic acid, respectively. The authors also reported that only 0.1 and 0.34% of the total phytate in millet and soybean, respectively, was transferred to the soaking media. The remaining percentage degradation was due to the action of the endogenous phytase. Another study by Liang et al. (2009) also reported that soaking brown and white rice in different soaking media resulted in significant reduction in phytic acid. However, the treatments also caused loss of tremendous amount of iron, zinc and calcium to the soaking media.

Malting/Germination

Malting is a process during which the whole grain is soaked usually in water and then germinated. During this process several enzymes including phytase are generated either through *de novo* synthesis or through activation of endogenous ones (Egli et al., 2002). In line with this many researchers reported that phytase activities of cereals and legumes are significantly improved after germination with a concomitant decrease in phytate content (Greiner et al., 2001; Centeno et al., 2003). For a cereal or legume to germinate, soaking is the preliminary step to be practiced. Therefore, many of the changes that occur during soaking especially loss of water soluble nutrients tends to affect the nutritional quality of malted grains.

On the other hand, malting could be considered as a means to concentrate enzymes that originates from the grain itself or from indigenous microbes present during the process (Laitila et al., 2006). The enzymes hydrolyses mineral absorption inhibitors thereby improves mineral bioavailability. Human absorption studies using radionuclide techniques by Larsson et al. (1996) on the absorption of iron and zinc from oat porridge made of untreated flour compared to that from oat porridge made from malted flour, with a phytate reduction of 77%, showed that absorption of iron particularly zinc was significantly improved from the porridge made from malted flour. Therefore, the application of malted grain during food formulation especially for complementary foods has paramount contribution in improving mineral bioavailability through degradation of phytate. Moreover, the energy and nutrient density of gruel prepared after adding flour of malted grains could be improved (Traore et al., 2004).

Fermentation

Fermentation has been used as a means of improving the keeping quality of food (Hotz and Gibson, 2007). It is an old method of food processing and preservation where both microbial and enzymatic actions are playing to achieve prolonged shelf life of the food. Moreover, it has been used as means to improve the sensory quality and acceptability of many raw materials making them preferred over the non fermented forms (Holzapfel, 2002). Fermentation can

induce phytate hydrolysis via the action of either endogenous or microbial phytase enzymes, which hydrolyze phytate to lower inositol phosphates (Hotz and Gibson, 2007). Bacterial production of organic acids such as citric, malic and lactic acid create favorable conditions for cereal phytase activity to experience higher phytic acid degradation. Moreover, the absorption of zinc and iron could also be enhanced via the formation of soluble ligands (Teucher et al., 2004). Larsson and Sandberg (1991) found that in oat and rye bran bread, made with 10% sourdough having pH 4.6, a 96–97% reduction of phytate occurred. Wang et al. (2007) also showed that fermentation of soybean, cowpea and ground bean resulted in degradation of phytic acid by 30.7%, 32.6% and 29.1%, respectively. Another study by Liang et al. (2008) showed that fermentation caused about 56-96% phytic acid degradation in brown rice. Combination of treatment such as germination and lactic acid fermentation of white sorghum and maize gruels can yield an almost complete degradation of phytate (Svanberg et al., 1993).

Thermal treatment: Cooking and Extrusion

Phytate, being a heat stable component in plant foodstuffs, is not easily degraded whilst cooking or extrusion. However, the intrinsic plant phytase is thermolabile and thus prolonged exposure to high temperature may lead to the inactivation of endogenous enzyme. Therefore, to improve phytate dephosphorylation during cooking, plants with heat-stable phytases or addition of exogenous heat-stable phytases has been recommended (Kumar et al., 2010). Gualberto et al. (1997) reported that extrusion cooking didn't affect the phytic acid content of cereal brans. On the other hand, Repo-Carrasco-Valencia et al. (2009) showed that phytic acid content of amaranth decreased during extrusion. The conditions such as moisture content, and applied temperature may have resulted in such differences.

b) Enzymatic degradation of phytate

Phytase is an enzyme that hydrolyses phytic acid to myo-inositol and phosphoric acid in a stepwise manner forming myo-inositol phosphate intermediates and free phosphorus as a final product (Figure 2.11) (Wodzinski and Ulla, 1996). The enzyme is chemically named as myo-inositol (1,2,3,4,5,6) hexakisphosphate phosphohydrolase. In addition to releasing the

phosphorus in plant based diets, phytases improves the availability of minerals such as calcium, iron, zinc and magnesium, protein and lipid to the body. Thus, by releasing bound phosphorus in feed ingredients of vegetable origin, phytase makes more phosphorus available for bone growth and protects the environment against phosphorus pollution (Baruah et al., 2007). Ruminants are not able to produce phytase which is responsible to degrade phytate. As a result, the enzyme has long been utilized as a feed additive for animal nutrition.

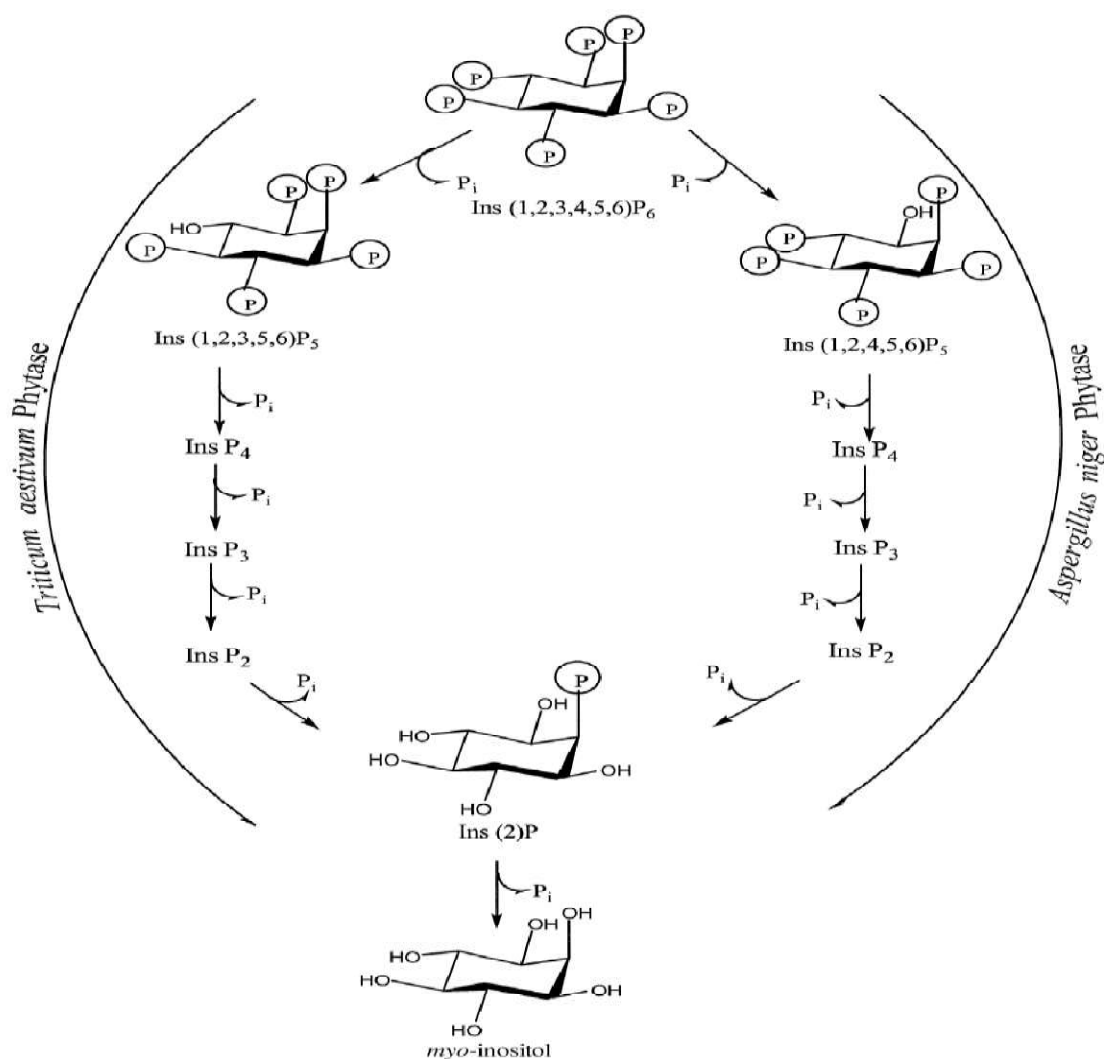


Figure 2.11 Hydrolysis of phytic acid by plant and fungal phytases to inositol and phosphoric acid by phytase (Source: Wodzinski and Ulla, 1996)

v. **Classification of phytase**

Phytase has been categorised on two bases, depending on the site where the hydrolysis of the phytate molecule is initiated and on the pH of activity. The International Union of Pure and Applied Chemistry and the International Union of Biochemistry (IUPACIUB) acknowledged that phytase falls into two categories, depending on the site where the hydrolysis of the phytate molecule is initiated (Selle et al., 2007). These are 3-phytase (EC 3.1.3.8 or myo-inositol hexakisphosphate 3-phosphohydrolase) and 6-phytase (EC 3.1.3.26 or myo-inositol hexakisphosphate 6-phosphohydrolase).

The 3-phytase hydrolyzes the ester bond at the 3 position (D-3) of myo-inositol(1,2,3,4,5,6)hexakisphosphate to D-myo-inositol (1,2,4,5,6)pentakisphosphate and orthophosphate and a 6-phytase first hydrolyzes the 6-position (L-6) of myo-inositol(1,2,3,4,5,6)hexakisphosphate to D-myo-inositol (1,2,3,4,5) pentakisphosphate and orthophosphate (Wodzinski and Ulla 1996). The 3-phytase is typically found in microorganisms, while 6-phytase is usually present in the seeds of higher plants (Centeno et al., 2001). The phosphate group released due to the action of phytase in the step wise degradation of phytate combines with molybdate reagent to form phosphomolybdenum blue that can be detected colorimetrically (Vohra and Satyanarayana, 2003).

Phytases can also be categorised into two major classes based on their optimum pH: the histidine acid phosphatases and alkaline phytases. The former show the optimum activity at pH around 5.0 whilst the latter are more pronounced at pH near to 8.0 (Baruah et al., 2007; Cao et al., 2007). However, most of the so far investigated phytate-degrading enzymes belong to the acidic type that could work at an optimal pH of 5.0 and their optimal pH ranges from 4.5 to 6.0 (Konietzny and Greiner, 2002). Phytate-degrading enzymes are widespread in nature, occurring in plants, micro-organisms, as well as in some animal tissues (Konietzny and Greiner, 2002). The activity of plant phytases especially cereals and legumes showed wide variation and generally the phytase activity of cereals has been shown to be higher than that of legumes (Table 2.7).

Table 2.7 Phytase activity of common cereals and legumes

Grains and seeds common name	Botanical name	Phytase activity (PU/g dry matter)*	Grains and seeds common name	Botanical name	Phytase activity (PU/g dry matter)*
Cereals			Legumes		
Barley	<i>Hordeum vulgare</i>	1.83 ± 0.05	Blackeyed bean	<i>Vigna unguiculata</i>	0.39 ± 0.00
Maize	<i>Zea mays</i>	0.13 ± 0.00	Chickpea	<i>Cicer arietinum</i>	0.25 ± 0.02
Millet	<i>Pennisetum typhoides</i>	0.24 ± 0.01	Cowpea	<i>Vigna niebe</i>	0.40 ± 0.01
Oat	<i>Avena sativa</i>	0.14 ± 0.00	Dwarf bean	<i>Phaseolus vulgaris nana</i>	0.28 ± 0.01
Rice	<i>Oryza sativa</i>	0.19 ± 0.00	Lentil	<i>Lens culinaris</i>	0.26 ± 0.01
Rye	<i>Secale cereale</i>	6.92 ± 0.41	Lucerne	<i>Medicago sativa</i>	0.81 ± 0.08
Sorghum	<i>Sorghum sudanensis</i>	0.11 ± 0.00	Lupin	<i>Lupinus albus</i>	0.24 ± 0.02
Sweet maize	<i>Zea mays saccharata</i>	0.38 ± 0.01	Mungbean	<i>Phaseolus aureus or Vigna radiata</i>	0.27 ± 0.00
Triticale	<i>Triticosecale</i>	4.82 ± 0.04	Pea	<i>Pisum sativum</i>	0.20 ± 0.01
Wheat	<i>Triticum aestivum</i>	3.08 ± 0.17	Soybean	<i>Glycine max</i>	0.34 ± 0.01
	Pseudocereals		White bean	<i>Phaseolus vulgaris</i>	0.23 ± 0.01
Amaranth	<i>Amaranthaceae</i>	1.27 ± 0.02		Oil seeds	
Buckwheat	<i>Fagopyrum esculentum</i>	2.90 ± 0.17	Rapeseed	<i>Brassica napus oleifera</i>	0.30 ± 0.01
Quinoa	<i>Chenopodium quinoa</i>	0.62 ± 0.02	Sunflower seed	<i>Helianthus annuus</i>	0.13 ± 0.01

*phytase unit (PU) equivalent to the enzymatic activity which liberates 1 µmol inorganic phosphate per min.

Source: Adapted from Egli et al. (2002)

vi. Applications of Isolated Phytases during Food Processing

Addition of phytases to a food has been shown to enhance the bioavailability of mineral and trace elements. Moreover, addition of phytase during food processing was reported to affect economy of the production process as well as yield and quality of the final products. Technical improvements by adding phytase during food processing have been reported for bread making, production of plant protein isolates, corn wet milling and the fractionation of

cereal bran (Greiner and Konietzny, 2006). However, the utilization of phytases for human food application was limited in that many of the isolated phytases have been originated from genetically modified microorganisms.

2.5.11.2. Polyphenols

Polyphenols are a group of chemical substances found in plants characterized by the presence of more than one phenol unit or building block per molecule (Landete, 2012). They are secondary metabolites widely distributed in the plant kingdom. They are divided into several classes, i.e. phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), flavonoids (flavonols, flavones, flavanols, flavanones, isoflavones, proanthocyanidins), stilbenes, and lignans, which are distributed in plants and food of plant origin (Manach et al., 2004, 2005). Major food sources include berries, chocolate, coffee, tea, grapes, olives and other fruits and vegetables (El Gharras, 2009).

Polyphenols are metal chelaters such as iron and zinc and reduce their absorption (Mukherjee et al., 2009). The prevalence of micronutrient deficiency especially iron, zinc and calcium in developing countries whose diets are mainly plant based which are identified as poor sources of micronutrients, could be exacerbated due to the high intake of dietary polyphenols in those plant based diets. Moreover, polyphenols/tannins were also reported to complex with proteins and inhibit the action of digestive enzymes thereby reduce protein digestibility (Waghorn et al., 1987). However, in the contemporary world polyphenols are much more friends than foe as they have diverse health benefits such as antimicrobial, antiviral, anti-inflammatory, antitumor, anticancer and antioxidative action (Lule and Xia, 2005; Landete et al., 2012).

2.5.12. Determination of antioxidant activity

Both phytic acid and polyphenols which were claimed to affect mineral bioavailability and macronutrient digestibility has also been reported to have paramount health promoting effect mainly through scavenging free radicals or inhibiting generation of free radicals by quenching the radical initiators (Rice-Evans, 2001; Minihane and Rimbach, 2002). Through their antioxidant action both polyphenols and phytic acid could prevent the development of

coronary heart disease and cancer (Lule and Xia, 2005; Kumar et al., 2010; Kruger et al., 2014). The antioxidant action of food bioactives will not solely depend on the content of polyphenols and phytic acid but the contribution of other dietary antioxidant compounds such as peptides and amino acids (Wu et al., 2003), vitamins, minerals and etc (Figure 2.12) should also be considered and the final antioxidant activity will be the cumulative action of all those bioactive compounds.

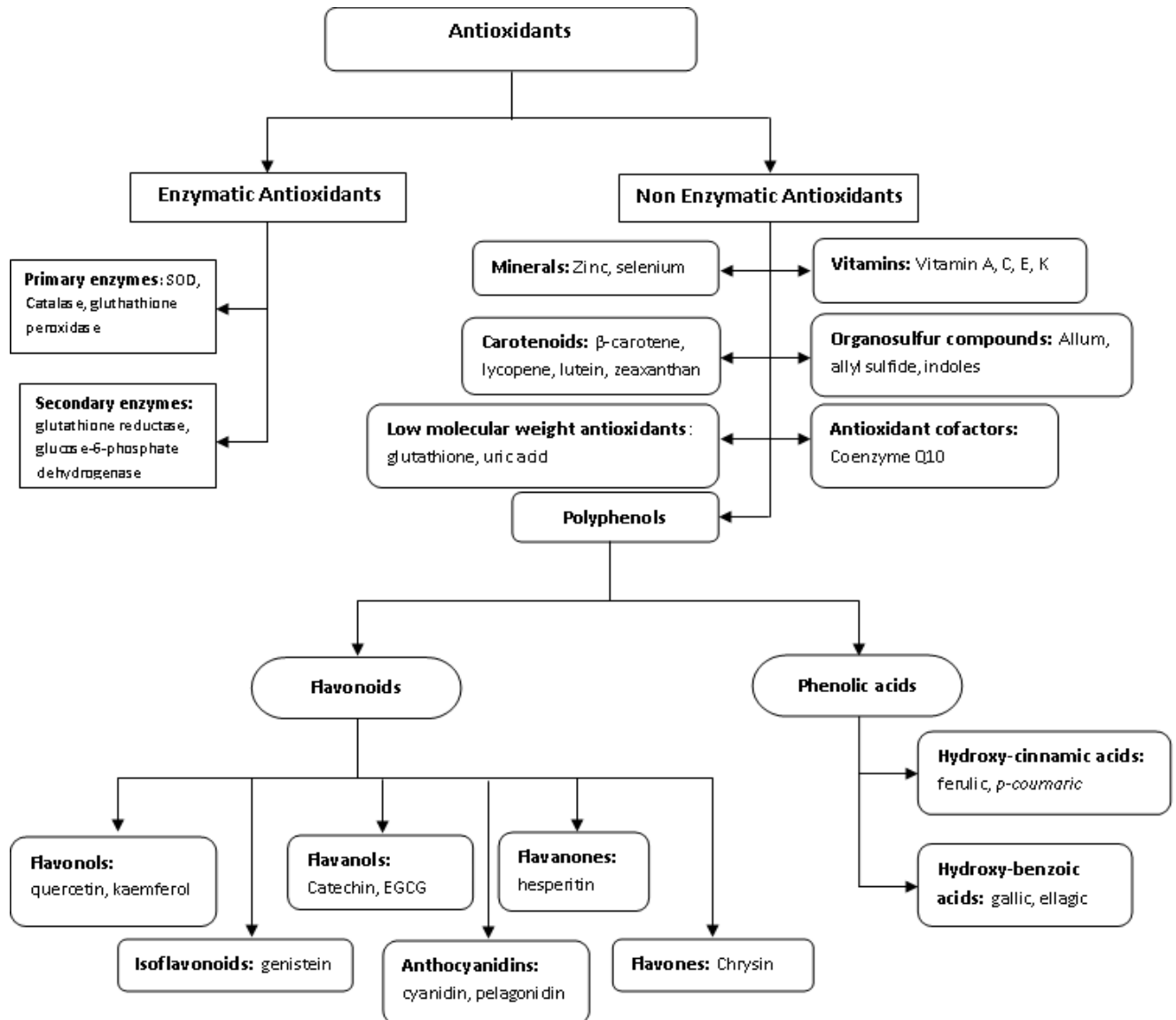


Figure 2.12 Classification of antioxidants (source: Shalaby and Shanab, 2013).

Antioxidant activity caused by food bioactives is mainly determined by chemical methods primarily through: (I) hydrogen atom transfer methods (HAT) or (II) electron transfer methods (ET) (Prior et al., 2005; Badarinath et al., 2010). 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging, oxygen radical absorbance capacity (ORAC), lipid peroxidation inhibition capacity, and total radical-trapping antioxidant parameter (TRAP) are the most commonly used hydrogen atom transfer methodologies, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, Trolox equivalent antioxidant capacity (TEAC), and Ferric Reducing Antioxidant Power (FRAP) are the main ET tests (Badarinath et al., 2010). The mechanism of actions of two of the commonly used methodologies is described below.

2.5.12.1. Mechanism of action of antioxidant in ABTS Assay

The mechanism of action of ABTS assay is based on the reaction between $\text{ABTS}^{\bullet+}$ radical cation, which is generated when ABTS is allowed to react with potassium persulphate, and antioxidant molecules in the test foods. $\text{ABTS}^{\bullet+}$ chromophore has a blue/green color having absorption maximum at four wavelengths: 415nm, 645 nm, 734 nm and 815 nm (Re et al., 1999). However, absorption maximum at 415nm and 734nm has been selected as the preferred measurement wavelength due to the lower degree of interference (Prior et al., 2005).

Addition of antioxidants to the pre-formed radical cation reduces the cation to ABTS depending on the antioxidant activity, the concentration of the antioxidant and the duration of the reaction. Therefore the blue/green color of the radical fades and causes reduction in absorbance (Re et al., 1999). $\text{ABTS}^{\bullet+}$ reacts rapidly with antioxidants, typically within 30 min. It can be used over a wide pH range and can be used to study effects of pH on antioxidant mechanisms. Moreover, the radical cation is soluble in both aqueous and organic solvents and is not affected by ionic strength, so can be used in multiple media to determine both hydrophilic and lipophilic antioxidant capacities of food extracts and body fluid (Prior et al., 2005). The radical scavenging activity assessed by this method has usually been expressed as trolox equivalent antioxidant activity as trolox is the commonly used standard (Thaipong et al., 2006).

2.5.12.2. Mechanism of action of antioxidant in DPPH Assay

DPPH is a free radical stable at room temperature which has unpaired electron at one atom of nitrogen bridge (Eklund et al., 2005). Unlike other radical ions, DPPH is not affected by certain side reactions, such as metal ion chelation and enzyme inhibition making it a preferred radical for antioxidant determination. Methanolic solution of DPPH produces purple color having an absorption maximum at 517nm. Organic solvent extract of the test food is mixed with DPPH solution and the antioxidant molecule quenches the DPPH radical by electron donation and convert them to a colorless product (i.e., 2, 2-diphenyl-1-hydrazine, or a substituted analogous hydrazine) resulting in a decrease in absorbance at 515 with concomitant color change from purple to light yellow (Prior et al., 2005). The rate at which the color of DPPH fades depends on the strength/amount of antioxidant molecule present in the test food.

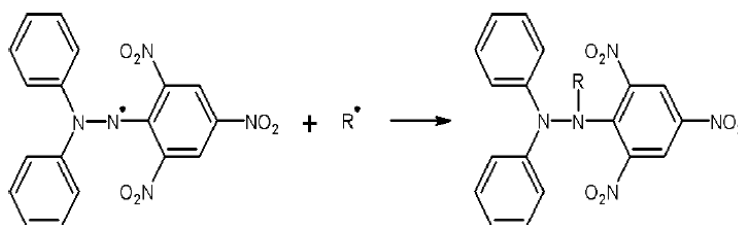


Figure 2.13 Mode of action of DPPH radical with antiradical compound (RH) (source: Shalaby and Shanab, 2013).

2.5.13. Complementary feeding strategies

2.5.13.1. Complementary food in developing countries

Complementary foods could be defined as those foods which are appropriately timed, nutritionally adequate and hygienically prepared foods that are given to infants along with continued breastfeeding from six months of age (Mensah et al., 1991). Complementary foods can especially be prepared for the infants or can be the same foods available for family members, modified in order to meet the eating skills and needs of the infant (WHO, 1998).

The complementary foods are usually prepared from cereals, legumes and different fruits and vegetables. It should be nutritionally dense and safe for the child. Moreover, it must provide about 356, 479 and 772 kcal/d for an average breast fed children aged 6–8 months, 9–11 months and 12–23 months, respectively based on on the estimated average total energy requirement plus 25% reported in a US longitudinal studies of children (Dewey and Brown 2003). Besides, the complementary food should be very rich in the major micronutrients such as iron, zinc, calcium, vitamin A, iodine and others as the deficiency of these nutrients causes serious nutrition related diseases (Dewey and Brown, 2003) and affects the mental, motor and behavioral developments of the child (Shafir et al., 2008, Grantham-McGregor, 1999, Grantham-McGregor, 1995).

Infants and young children are at increased risk of developing malnutrition from about six months of age onwards. This is because after six months breast milk will no longer be sufficient to meet all nutritional requirements and the baby should start taking complementary foods although these complementary foods may not have high nutritional quality and safety than the breast milk (Daelmans et al., 2003). Therefore, if children are not properly fed with a complementary food which is rich in those major WHO problem nutrients such as protein, Fe, Zn, Ca, vitamin A, vitamin B6 and etc, the baby is susceptible for being malnourished. Thus, although the causes for malnutrition are diverse, inappropriate or inadequate complementary feeding takes the lion share to affect the growth and development of infants and young children in developing countries (WHO, 1998). Therefore feeding children with an appropriate complementary food that could meet their energy and nutrient needs is crucial to alleviate malnutrition, child mortality and morbidity.

2.5.13.2. Characteristics of Complementary Food

Since the period of complementary feeding is the time where malnutrition starts in many children, the food to be provided should have the following characteristics: (1) it should be rich in calories and adequate in good-quality protein, vitamins, and minerals, (2) the food, when stirred with cold or warm water or milk, should form a slurry or semisolid mass of soft

consistency, enabling the child to swallow it easily, (3) the prepared food should have low dietary bulk (low fiber content), (4) the food should be precooked and predigested or processed in such a way that it needs minimum preparation prior to feeding and is easily digested by the child, (5) the food should have low content of antinutritional factors (6) it is advisable not to add artificial colors and flavors to weaning foods, and the composition of the food must follow the guidelines and standards recommended by WHO (Sajilata et al., 2002; Dewey and Brown, 2003; Pelto et al., 2003, Obiolephehai, 2003).

2.5.13.3. Amount and Frequency of Complementary Feeding

The amount and frequency of complementary food given to the baby will depend on the volume of breast milk, the energy density of the food and the age and body weight of the baby (WHO, 1998). Accordingly, the global consultation on complementary feeding held in 2003 recommended that assuming a diet with energy density of 0.8 kcal/g and low breast milk intake, infants aged 6-8 months, 9-11 months and 12-24 months be given three, three and four meals per day, respectively with the addition of one or two nutritious snacks (Daelmans et al., 2003). Therefore, depending on the energy density, the frequency of feeding could increase or decrease in accordance with the recommendation by the global consultation. Table 2.8 below could also show how the frequency of complementary feeding varies based on age and amount of breast milk intake.

Islam et al., (2008) also reported that the energy density and feeding frequency of the complementary foods could affect the total energy intake and breast milk consumption. According to the report, the amounts of complementary foods consumed were inversely related to their energy density and breast milk intake. The total energy intake (EI) (kcal/d) increased when the density and frequency of the complementary food increased. As a result it is recommended that appropriate combinations of these dietary factors (energy density, feeding frequency, breast milk intake) should be investigated.

Table 2.8 Minimum dietary energy density required to attain the level of energy needed from complementary foods in one to five meals per day, according to age group and level (low, average, or high) of breastmilk energy intake (BME)*

	6-8 mo			9-11 mo			12-23 mo		
	BME			BME			BME		
	low	average	high	low	average	high	low	average	high
Total energy required + 2SD (kcal/day) [†]	769	769	769	858	858	858	1118	1118	1118
Energy from breast milk (kcal/day)	217	413	609	157	379	601	90	346	602
Energy required from complementary food (kcal/day) [‡]	552	356	160	701	479	257	1028	772	516
Estimated gastric capacity, well nourished (g) [*]	249	249	249	285	285	285	345	345	345
Minimum Energy Density (kcal/g)									
1 meal/day	2.22	1.43	0.64	2.46	1.68	0.90	2.98	2.24	1.50
2 meals/day	1.11	0.71	0.32	1.23	0.84	0.45	1.49	1.12	0.75
3 meals/day	0.74	0.48	0.21	0.82	0.56	0.30	0.99	0.75	0.50
4 meals/day	0.56	0.36	0.16	0.61	0.42	0.23	0.74	0.56	0.37
5 meals/day	0.44	0.29	0.13	0.49	0.34	0.18	0.60	0.45	0.30

*Assumed gastric capacity: 30 g/kg reference body weight. BW of well nourished children is 8.3kg, 9.5kg and 11.5kg in three ascending age groups.

[†]Total energy requirement is based on new US longitudinal data averages plus 25% (2SD).

[‡]Energy needs from complementary foods set at total energy requirement + 2SD (i.e, +25%) minus estimated energy intake from breast milk.

Source: Dewey and Brown (2003)

2.5.13.4. Complementary Feeding Age

The World Health Organization (WHO) forwarded a recommendation that infants should be exclusively breastfed for the first 6 months of life with the introduction of complementary foods thereafter and continued breastfeeding for the first 2 years (WHO, 2003). This global recommendation was made after a series of expert consultations by comparing infant and maternal outcomes of exclusive breastfeeding for 3-4 mo versus 6 mo. The report showed that infants exclusively breastfed for 6 mo experienced less morbidity from gastrointestinal infection and showed no deficits in growth. However, exclusive breastfeeding until 6 months can lead to iron deficiency in those babies where the maternal iron status and infant endogenous stores are not optimal. Thus it is advisable that infants should be managed individually in order not to ignore insufficient growth or other adverse outcomes.

In many developing countries where complementary foods are of lesser nutritional quality than breast milk, exclusive breast feeding until the age of 6 mo is highly recommended taking care of those infants who requires individual treatment. Moreover, the hygienic condition of the food is usually not good for the baby as a result it is advisable to breastfeed the baby even after six month. Despite all the advantages that breastfeeding provides, a recent report showed that many countries failed to comply with the recommendation and the breastfeeding rate is below 50% in many regions of the world (Table 2.9).

Table 2.9 Breastfeeding rates (%) in the developing world by region (UNICEF, 2014)

Region	Exclusive breast fed (< 6 months)	Breastfeeding at age 2 years
Sub-Saharan Africa [¶]	36	50
Eastern and Southern Africa [¥]	52	61
West and Central Africa [§]	25	44
Middle East and North Africa [§]	-	-
South Asia ⁺	49	78
East Asia and Pacific [‡]	30	51
Latin America and Caribbean [#]	39	-
CEE/CIS ^{§§}	-	-
Least developed countries*	48	64
World	38	58**

[¶]**Sub-Saharan Africa** includes Eastern and Southern Africa; West and Central Africa; Djibouti and Sudan.

[¥]**Eastern and Southern Africa** includes Angola; Botswana; Burundi; Comoros; Eritrea; Ethiopia; Kenya; Lesotho; Madagascar; Malawi; Mauritius; Mozambique; Namibia; Rwanda; Seychelles; Somalia; South Africa; South Sudan; Swaziland; Uganda; United Republic of Tanzania; Zambia; Zimbabwe. [§]**West and Central Africa** includes Benin; Burkina Faso; Cabo Verde; Cameroon; Central African Republic; Chad; Congo; Côte d'Ivoire; Democratic Republic of the Congo; Equatorial Guinea; Gabon; Gambia; Ghana; Guinea; Guinea-Bissau; Liberia; Mali; Mauritania; Niger; Nigeria; Sao Tome and Principe; Senegal; Sierra Leone; Togo. [§]**Middle East and North Africa** includes Algeria; Bahrain; Djibouti; Egypt; Iran (Islamic Republic of); Iraq; Jordan; Kuwait; Lebanon; Libya; Morocco; Oman; Qatar; Saudi Arabia; State of Palestine; Sudan; Syrian Arab Republic; Tunisia; United Arab Emirates; Yemen.

⁺**South Asia** includes Afghanistan; Bangladesh; Bhutan; India; Maldives; Nepal; Pakistan; Sri Lanka. [‡]**East Asia and Pacific** includes Brunei Darussalam; Cambodia; China; Cook Islands; Democratic People's Republic of Korea; Fiji; Indonesia; Kiribati; Lao People's Democratic Republic; Malaysia; Marshall Islands; Micronesia (Federated States of); Mongolia; Myanmar; Nauru; Niue; Palau; Papua New Guinea; Philippines; Republic of Korea; Samoa; Singapore; Solomon Islands; Thailand; Timor-Leste; Tonga; Tuvalu; Vanuatu; Viet Nam. [#]**Latin America and Caribbean** includes Antigua and Barbuda; Argentina; Bahamas; Barbados; Belize; Bolivia (Plurinational State of); Brazil; Chile; Colombia; Costa Rica; Cuba; Dominica; Dominican Republic; Ecuador; El Salvador; Grenada; Guatemala; Guyana; Haiti; Honduras; Jamaica; Mexico; Nicaragua; Panama; Paraguay; Peru; Saint Kitts and Nevis; Saint Lucia; Saint Vincent and the Grenadines; Suriname; Trinidad and Tobago; Uruguay; Venezuela (Bolivarian Republic of).

^{§§}Central Eastern Europe/Commonwealth of Independent States (**CEE/CIS**) includes Albania; Armenia; Azerbaijan; Belarus; Bosnia and Herzegovina; Bulgaria; Croatia; Georgia; Kazakhstan; Kyrgyzstan; Montenegro; Republic of Moldova; Romania; Russian Federation; Serbia; Tajikistan; the former Yugoslav Republic of Macedonia; Turkey; Turkmenistan; Ukraine; Uzbekistan. ***Least developed countries** includes Afghanistan; Angola; Bangladesh; Benin; Bhutan; Burkina Faso; Burundi; Cambodia; Central African Republic; Chad; Comoros; Democratic Republic of the Congo; Djibouti; Equatorial Guinea; Eritrea; Ethiopia; Gambia; Guinea; Guinea-Bissau; Haiti; Kiribati; Lao People's Democratic Republic; Lesotho; Liberia; Madagascar; Malawi; Mali; Mauritania; Mozambique; Myanmar; Nepal; Niger; Rwanda; Samoa; Sao Tome and Principe; Senegal; Sierra Leone; Solomon Islands; Somalia; South Sudan; Sudan; Timor-Leste; Togo; Tuvalu; Uganda; United Republic of Tanzania; Vanuatu; Yemen; Zambia

**Excludes china

3. Chapter Three-Results and Discussion

3.1. Nutritional qualities of three types of *Amaranthus caudatus* grains cultivated in Ethiopia and the effect of popping and fermentation

3.1.1. Introduction

The species *Amaranthus* belongs to the family of Amaranthaceae and believed to have originated from Central and South America (Gamel et al., 2006). It produces a large amount of biomass in a short period of time (Kauffman and Weber, 1990) and therefore has the potential to contribute to a substantial increase in world food production. Amaranth is a pseudocereal with excellent nutritional and functional properties, capable of withstanding extreme climate and soil conditions (Capriles et al., 2008). Studies done so far indicated that the crop has excellent nutritional profile with high level of protein, minerals, and fat as compared to the common utilized cereals (Mustafa et al., 2011; Segura-Nieto et al., 1994; Pedersen et al., 1987). Moreover, it has excellent amino acid and fatty acid profiles with high amount of lysine and high percentage of unsaturated fatty acids (Martirosyan et al., 2007; Kunyanga et al., 2012).

The seeds of amaranth can be subjected to several treatments such as popping, toasting, cooking, roasting, flaking, extruding or grinding to be consumed as suspensions with water or milk or to be mixed with other flours for bread and pasta making with improved technological quality (Capriles et al., 2008; Escudero et al., 2004). Despite the good nutritional profile with respect to its micronutrients, amino acid and fatty acid profile; its nutritive value is limited by the presence of high level of mineral absorption inhibitors especially phytate content ranging from 0.52 to 2.24g/100g in the different species studied so far (Lorenz and Wright, 1984; Pedersen et al., 1987). According to Coulibaly et al. (2011), growing conditions such as season, soil profile, harvesting techniques, stage of maturation, species and genotype are the main possible factors for the existing variations in phytate contents of crops. In another study, Ahn et al. (2010) indicated that phytate content is significantly influenced by variety, location, growing year, and storage conditions. In line with this, some researchers also documented levels of other potential mineral absorption inhibitors such as tannins (Lorenz and Wright,

1984; Teutonico and Knorr, 1985; Mustafa et al., 2011) in different species of amaranth growing in different countries.

In Ethiopia amaranth can grow nearly all over the country yet the crop is underutilized in many parts of the country. In the Southern Nations Nationalities and Peoples (SNNP) region, especially in Bench Majji Zone, a particular ethnic group called Me'etit cultivates the crop and utilizes it in different forms. The seed could be popped and milled to prepare thin porridge called "Atmit", thick porridge called "Genfo" or mixed with cold water to prepare an instant drink called "Besso" or fermented to prepare a drink called "Shamita/Borde". The raw seeds could also be milled, mixed with other cereal flours like maize and sorghum and then fermented for 3-4 h and 2-3 days to prepare unleavened bread called "Kita" and "Injera", a traditional pancake, respectively. Moreover, Amaranth could also be used to prepare alcoholic drink called "Areke" after malting the seeds.

Although amaranth has multiple food uses in the country that could contribute to ensure food security, there is limited information on the nutritive value and mineral absorption inhibitors of amaranth grain adapted and grown under the agro ecological conditions of Ethiopia. Therefore, the aim of the present study was to determine the proximate composition, minerals and mineral absorption inhibitors (phytic acid and iron binding polyphenols) of three different types of *Amaranthus caudatus* grains cultivated in SNNP region, Ethiopia. Moreover, the effect of commonly used processing methods, prior to the consumption of amaranth in Ethiopia, popping and fermentation, on degradation of mineral absorption inhibitors and on overall nutritive value was studied.

3.1.2. Materials

3.1.2.1. Description of sampling areas

Bench Maji is one of the Zones of the Ethiopian Southern Nations, Nationalities and Peoples Region (SNNPR). Bench Maji is bordered on the south by the Ilemi Triangle, on the west by South Sudan, on the northwest by the Gambela Region, on the north by Sheka, on the northeast by Keffa, and on the

east by Debub Omo. The Omo River defines much of its eastern border with Debub Omo. The administrative center of Bench Maji is Mizan Teferi.

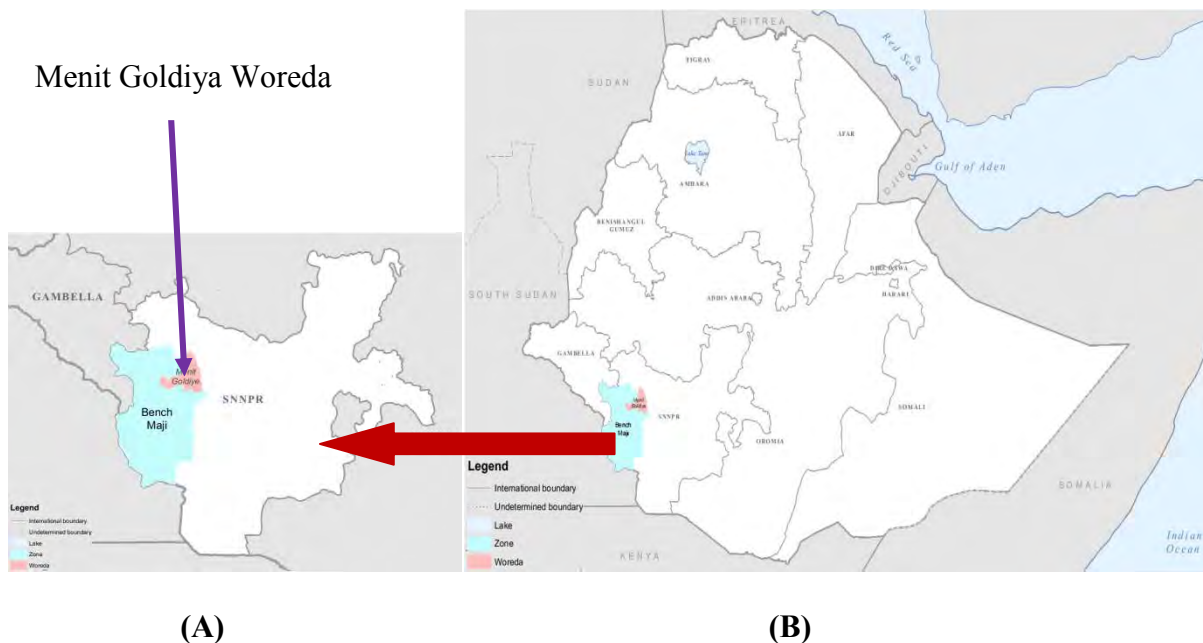


Figure 3.1.1 Map of SNNPR (A) and Map of Ethiopia (B)

3.1.2.2. Sample collection

Three different types of *Amaranthus caudatus* grains, white, red and brown in color, were purchased from six farmers living in Chat Kebelle, Bench Majji Zone, Southern Nations, Nationalities and Peoples region, Ethiopia in October 2011. The grains of the six origins were sorted to remove immature seeds, cleaned and washed to remove sand and soil. The washed seeds were sun dried and equal amount of the six samples were mixed to prepare a composite sample for each amaranth types.



White Amaranth

Red Amaranth

Brown Amaranth

Figure 3.1.2 The three different *Amaranthus caudatus* grains.

3.1.3. Methods

3.1.3.1. Grain Characterization

The amaranth grains were counted, weighed and weight expressed in grams. The thousand kernel weight (TKW) was determined by weighing thousand seeds. The bulk density was calculated as weight of sample per unit volume (g/mL). The apparent bulk volume of the raw and popped seeds was measured with a 20 mL-graduated cylinder and was expressed as the expansion ratio (the volume of popped seeds/the volume of equal weight of raw seeds). The popping yield was calculated from the difference in weight of popped and un-popped grain (i.e. [wt of popped-wt of un-popped]*100/total weight).

3.1.3.2. Sample preparation

i. Preparation of raw amaranth flour

The composite samples for each type of amaranth grain were milled, sieved using 0.425 mm sieve and stored at 4 °C until further analysis.

ii. Popping

Cleaned and sun dried amaranth grains were popped as described in Amare et al. (2015). The popped grains were milled to pass a 0.425 mm sieve and stored in polyethylene bags at 4 °C for further analysis.

iii. Fermentation

Natural fermentation was carried out according to the method described by Ibrahim et al. (2005) with modification. Briefly, 250 g of amaranth flour was mixed with 500 ml distilled water in a 600 mL beaker and then left to ferment for 48 h at room temperature (22±2 °C). Thereafter, the sample was mixed with a glass rod and transferred to three aluminum dishes (30 cm diameter each) and dried in a hot oven (Heraeus UT 5042, Germany) at 50 °C for 20

h. The dried sample was then ground to pass a 0.425 mm screen and stored in polyethylene bags at 4 °C for further analysis.

3.1.3.3. Proximate Composition Analysis

The proximate composition was analyzed using AOAC procedures (AOAC 2000) and results were expressed as g/100 g dry matter

i. Dry matter content

Dry matter content was determined using AOAC (2000) method number 925.05. The sample dish was dried at 130 °C for one hour and placed in a desiccator for about 15-20 minutes to cool. This was weighed (M_1). Five gram of the sample was weighed into the moisture dish (M_2) and dried in an oven at 105 °C to a constant weight (M_3). The measurement was made in triplicates and the percent dry matter was determined using the following equation:

$$\text{Dry matter content (\%)} = \left(\frac{M_3 - M_1}{M_2 - M_1} \right) \times 100 \dots \dots \dots (1)$$

$$\% \text{ moisture} = 100 - \% \text{ dry matter}$$

ii. Crude protein

Crude protein contents of samples were determined according to the association of Official Analytical Chemists (AOAC) standard method 979.09 (AOAC 2000). Briefly, approximately 500 mg of dried sample was weighed and placed in Kjeldahl digestion tube in triplicate. To this, 6 mL of conc. H_2SO_4 was added. Then 3.5 mL hydrogen peroxide followed by 3 g of a mixture of $CuSO_4$ and K_2SO_4 (1:15 w/w) was added as a catalyst and digested at 370 °C until the solution turns to light green color. The digest was cooled and placed in an automatic distillation apparatus. During this step water was first added to the solution followed by 35% sodium hydroxide solution to neutralize the system. When distillation proceeds the vapor was captured by 2% boric acid solution in another flask. This solution was finally titrated by 0.1M HCl to determine the total nitrogen content and bromocresol green was used as an indicator. Reagent blanks were also run side by side and the nitrogen content was subtracted from sample nitrogen. Percent nitrogen (% N) was calculated using the following equation:

$$\% N = \frac{(V_s - V_b) * N * 14.007}{\text{gram of sample}} * 100 \dots\dots\dots (2)$$

Where; Vs-Volume of acid consumed by the sample, Vb-Volume acid consumed by the blank, N-Normality of HCl.

Crude protein content was determined by multiplying % nitrogen using a conversion factor of 5.85 (Berghofer & Schoenlechner 2002).

$$\text{Crude protein} \left(\frac{\text{g}}{100\text{g}} \text{DM} \right) = \frac{\% N * 5.85}{\text{DM}} \dots\dots\dots (3)$$

iii. Crude Fat

The amount of crude fat (includes fats, phospholipids, sterols, fatty acids, carotenoids, pigments, chlorophyll, etc.) was determined by the Soxhlet extraction method according to AOAC, 920.39, 2000. Exactly 2.5 g of sample was weighed into an extraction thimble and covered with fat free cotton. Then 60 mL of diethyl ether (Sigma-Aldrich, USA) was added to pre weighed, cleaned and dried receiving flask and fitted into the apparatus. Water and heater was turned on to start extraction for 4 h. Then, the flask was dried in an oven at about 70 °C for 30 minutes to remove the solvent and cooled in a dessicator and weighed. The percent crude fat was determined by using the following formula:

$$\text{Crude Fat} \left(\frac{\text{g}}{100\text{g}} \text{DM} \right) = \frac{\text{Extracted Fat(g)}}{\text{Sample weight(g)} * \text{DM}} * 100 \dots\dots\dots (4)$$

iv. Ash

The ash content was determined after the removal of organic matter by dry ashing according to (AOAC 923.03, 2000). Accurately weighed sample (5 g) was placed into a pre-dried crucible and charred in hot plate under the hood. The charred sample was placed in muffle furnace. The furnace was closed and ignited at 550 °C for 5 h until the sample became white/gray. The crucibles were cooled in a desiccator and weighed. The ash content was calculated using the following equation:

$$\text{Ash} \left(\frac{\text{g}}{100\text{g}} \text{DM} \right) = \frac{(\text{Weight after ashing}) - (\text{weight of crucible})}{\text{weight of sample} * \text{DM}} \times 100 \dots \dots \dots (5)$$

v. ***Neutral detergent and acid detergent fiber Determination***

ADF and NDF contents were determined according to the gravimetric method of Van Soest (1963) using a Fibertec 1020 (Foss, Hillerod, Denmark).

a. **Preparation of ADF reagent**

Cetyltrimethylammonium bromide (30 g) was weighed and 1416 mL of water was added. The mixture was dissolved on the hot plate with stirring followed by addition of 84 mL of 96% H₂SO₄.

b. **ADF Determination**

About 0.5 g of celite was placed in a sintered glass and weighed. To this, 1 g of sample was weighed. The crucible was placed in an Erlenmeyer flask fitted with a vacuum pump for defatting the sample using petroleum ether. The crucible was placed on the Fibertec for the enzymatic hydrolysis step. Fifty milliliter of hot water was added to each sample followed by 200 µL of thermostable alpha amylase and heated for 30 minutes. Fifty milliliters of ADF reagent and 2 drops of octanol was added and heated to boiling for 1 h. After the extraction was completed, rinse the residue with distilled water 3 times (40 mL each). The sample was washed 2 times using 10 mL acetone in a vacuum pump and the crucible was placed in an oven at 105 °C overnight. The crucible was allowed to cool and weighed (W1). The weighed crucible was placed in a muffle furnace at 500 °C for 3 h and weighed after cooling in a desiccator (W2). The amount of ADF (mg/100g DM) was calculated using the equation below

$$\text{ADF} \left(\frac{\text{mg}}{100\text{g}} \text{DM} \right) = \frac{W1 - W2}{W_{\text{Sample}} * \text{DM}} * 100 \dots \dots \dots (6)$$

Where, W1-weight of sample and crucible after oven drying and W2-weight of sample and crucible after ashing, DM-dry matter content of the sample.

c. Preparation of NDF reagent

1. Forty gram of sodium lauryl sulfate was dissolved in 500 ml distilled water using magnetic bar, then 13.32 ml of ethoxyethanol.2H₂O was added.
2. EDTA (24.80 g) and sodium borate decahydrate (9.08 g) was dissolved in 400 mL distilled H₂O while heating in a magnetic stirrer.
3. Na₂HPO₄ (6.08 g) was dissolved in 100 mL distilled water with a magnetic bar.

All the the above three solutions were transferred into a 1500 mL Erlenmeyer flask, adjusted to the mark with distilled H₂O and thourghoul mixed by magnetic stirring.

d. NDF Determination

About 0.5 g of celite was transferred into a sintered crucible and the weight of the crucible with celite was recorded. About 0.4 g of sample was weighed in to the crucible with celite. The sample was deffated using petroleum ether in a vacuum pump. The crucible was fitted in to the Fibertech and 50 ml of boiled distilled water and 200 µL of thermostable alpha amylase was added to each sample and heated for 30 minutes. Fifty milliliters of NDF reagent, 0.5 g sodium sulfite and 2 drops of octanol was added to each sample and extraction proceeds for about 1 h. After extraction each sample was rinsed 3 times using distilled water while it was in the Fiber tech instrument. The crucibles were placed in an Erlenmeyer flask fitted with vacuum pump and rinsed 2 times with acetone. The crucibles were allowed to dry in an oven at 105 °C overnight, cooled and weighed (W1). The weighed crucible was placed in a muffle furnace at 500 °C for 3 h and weighed after cooling in a desiccator (W2). The amount of ADF (mg/100g DM) was calculated using the equation below

$$\text{NDF} \left(\frac{\text{mg}}{100\text{g}} \text{DM} \right) = \frac{W1 - W2}{W_{\text{Sample}} * \text{DM}} * 100 \dots \dots \dots (7)$$

Where, W1-weight of sample and crucible after oven drying and W2-weight of sample and crucible after ashing, DM-dry matter of the sample.

vi. Crude carbohydrate

Available carbohydrates were determined by difference (i.e. subtracting the sum of protein, fat, ash, fiber and moisture content from 100) and the result was expressed as g/100g DM (FAO 2003).

$$\begin{aligned} \text{Available Carbohydrate} \left(\frac{\text{g}}{100\text{g DM}} \right) \\ = 100 - (\text{weighting (protein+ fat + moisture+ ash) in 100g of food}) \end{aligned}$$

3.1.3.4. Mineral Analysis

i. Digestion

Mineral and trace elements sample digestions were undertaken using a closed-vessel microwave digestion system, Milestone ETHOS 1 Series; EuroFIR Method indicator MI 1196. Sample powder was weighted (0.5 g) to proper Teflon digestion vessels. A mixture of concentrated nitric acid (4 mL), hydrogen peroxide (1 mL) and deionised water (3 mL) was carefully added, and vessels were properly closed and introduced into the microwave oven. A micro-wave program was established and optimised. Vessels were there-after cooled to room temperature and digested samples were diluted up to 25 mL with deionised water, for subsequent determination of minerals and trace elements. To assess possible contamination, blank solutions were prepared containing the same reagents and using the same procedure as the samples and standards.

ii. Determination of Iron (Fe), Zinc (Zn), Calcium (Ca) and Magnesium (Mg) by Atomic Absorption Spectrometry

Principle: In this technique the atoms of an element are vaporized and atomized in the flame. The atoms then absorb the light at a characteristic wavelength. The source of the light is a hollow cathode lamp, which is made up of the same element, which has to be determined. The lamp produces radiation of an appropriate wavelength, which while passing through the flame is absorbed by the free atoms of the sample. The absorbed energy is measured by a photo-

detector read-out system. The amount of energy absorbed is proportional to the concentration of the element in the sample.

Procedure: The digested sample was analyzed for mineral contents by Atomic Absorption Spectrophotometer (AA800, Perkin Elmer, Les Ulis, France). Different electrode lamps were used for each mineral. The equipment was run for standard solutions of each mineral before and during determination to check that it was working properly. For the determination of Ca, 1.0 mL 10% lanthanum chloride solution was added to the original solution to avoid interference of Mg on Ca determination.

The concentrations of minerals recorded in terms of “ppm” were converted to milligrams of the minerals per 100 g of dry matter (mg/100 DM) of the minerals by using the formula below:

$$\text{Conc} \left(\frac{\text{mg}}{100\text{g}} \text{DM} \right) = \frac{\text{Conc (ppm)} * \text{dilution volume} * \text{dilution factor} * 10}{\text{Weight of sample(g)} * \text{Dry matter content}} \dots \dots \dots (8)$$

3.1.3.5. Determination of Antinutrients

i. Phytic acid Measurement (HPAEC method)

myo-inositol hexakisphosphate (IP6) content was determined using the method of Talamond et al. (1998) modified as in Hama et al. (2011). Briefly 100 mg of the dried sample was extracted by using 5 mL of 0.5M HCl in a boiling water bath for 6 minutes. The mixture was cooled in an ice bath and centrifuged at 4500 g for 20 minutes at 4 °C. The supernatant was filtered using 0.2 µm membrane filter before drying in a Speedvac (JOUAN, Saint Herblain, France). After drying, the sample was reconstituted with 2 mL of MilliQ water and injected into a high-performance anion-exchange chromatography using an AS-11 precolumn and column kit (Dionex, Sunnyvale, USA) for analysis with conductimetric detector. The HPLC conditions were: mobile phase 200 mM NaOH and HPLC grade water with gradient elution (15 to 40% of 200 mM NaOH for 8 minutes, 40% 200 mM NaOH for 1 minute and 15 % 200 mM NaOH for 10 minutes), flow rate: 1ml/min, column temperature: 30 °C, injection volume: 50 µL. Standard phytic acid solutions were also prepared from sodium phytate (Sigma) to develop a calibration curve. Results were expressed in gram per 100 g DM.

ii. Iron binding Polyphenol Measurement

Iron-binding polyphenols (galloyl and catechol groups) were analyzed using the method of Brune, Hallberg, & Skanberg (1991). Briefly, to 50 mg of sample, 5 mL of 50% dimethylformamide (Carlo Erba) in 0.1N acetate buffer (pH 4.4) was added and the mixture was incubated for 16 h at 28 °C in a shaking water bath at 160 rpm. The solution was filtered using Whatman GF/A filter paper. From the supernatant, 0.5 mL was taken and mixed with 2 ml FAS (ferric ammonium sulfate) reagent containing 50% urea (Merck) in 0.1M acetate buffer (pH 4.4), 1% gum arabic (sigma) and 5% ferric ammonium sulfate (Riedel de Haen) in 1M HCl. The mixture was incubated for 15 minutes at 30 °C and the absorbance of the colored complexes was measured at 680 nm and 578 nm, corresponding to the absorption maxima of Fe-galloyl and Fe-catechol complexes, respectively against a reagent blank consisting of 0.5 mL 50% DMF and 2 ml of FAS-reagent. A food blank was also made by mixing 0.5 mL of 50% DMF and 2 ml of sample extract and the absorbance was subtracted. The concentration of galloyl groups (expressed as tannic acid equivalents) and catechol groups (expressed as catechin equivalents) were calculated from standard curves for catechin (sigma) and tannic acid (Merck) at the two wavelengths. All analysis were done in triplicate.

3.1.3.6. Estimation of mineral bioavailability

i. Molar ratios

Molar ratios of phytic acid to minerals are the most frequently used method to estimate mineral bioavailability. The desirable molar ratios suggested for optimal absorption for adult diets are less than 1.0 or preferably less than 0.4 for phytic acid:iron (Hurrell, 2004), less than 0.17 for phytic acid:calcium (Umata et al., 2005), and less than 15 for phytic acid:zinc (Gibson, 2006).

3.1.3.7. Quality assurance and quality control

In order to validate the accuracy of the analytical methods for protein, fat, ash and minerals three certified reference materials from the Institute for Reference Materials and

Measurements (EU-IRMM), namely BCR-381 (rye flour), BCR-191 (brown bread) and BCR-679 (white cabbage) were used. The measurements were conducted based on the above procedure and the data was compared with the certified reference values.

3.1.4. Statistical Analysis

Data were submitted to Statgraphics plus 5.1 (Statpoint, Warrenton, USA) software and analyzed using analysis of variance (ANOVA) to determine significant differences among the processing methods. Duncan multiple range test was used to compare the means. Pearson correlation coefficient (R^2) and p-value were used to show correlations and their significance. Differences were considered significant when $p < 0.05$.

3.1.5. Results and discussion

3.1.5.1. Analytical data quality assurance

Quality assurance results for proximate and mineral analysis of amaranth are presented in Table 3.1.1. The result indicated that the measured values for the selected nutrients were in good agreement, i.e. within the certified range or $< \pm 10$ of the certified values of the three reference materials which proves the validity of the methods.

Table 3.1. 1 Quality assurance results for nutrient analysis of amaranth grain

Parameter	Units	Method of analysis	Reference material	Certified value	Analyzed values	Recovery, %
<i>Proximate</i>						
Protein	g/100g	Kjeldahl	BCR 381	1.56 ± 0.01	1.48 ± 0.06	95
Fat		Soxhlet extraction	(rye flour)	1.36 ± 0.16	1.25 ± 0.05	92
Ash		Ignition on muffle furnace		1.08 ± 0.11	1.09 ± 0.04	101
<i>Mineral</i>						
Iron	mg/100g		BCR 191	4.07 ± 0.23	3.90 ± 0.38	96
Zinc			(Brown bread)	1.95 ± 0.05	1.99 ± 0.04	102
Calcium		Microwave digestion and flame AAS		41.00	39.70 ± 0.82	97
Iron		determination	BCR 679	7.97 ± 0.27	7.55 ± 0.18	95
Zinc			(White cabbage)	5.5 ± 0.25	5.72 ± 0.01	104
Calcium				776.8 ± 65.5	747.8 ± 1.2	96

3.1.5.2. Grain Characterization

The grains of the three different types of amaranth were differing in shape. The white and red types were flattened and the brown one had a lenticular shape. Popping yield was also differing, 97% for the white and red colored grain and 82% for the brown colored grain. Thousand kernel weight (TKW) ranged from 0.53 to 0.68 g which falls in the range reported by Kaur et al. (2010). The expansion volume during popping for the white amaranth (40 mL) was less than that of the red (46 mL) and brown (47 mL) amaranth while the expansion ratio for white, red and brown amaranth types was 4.1, 4.5 and 3.5, respectively. The bulk density is almost identical for all the three amaranth grains (0.77-0.79 g/mL).

3.1.5.3. Proximate composition of raw amaranth grains

Table 3.1.2 shows the proximate composition of three different types of *Amaranthus caudatus* grain. The protein and fat content of the raw amaranth falls in the range of 14.0-15.5 and 7.5 to 7.7 g/100 g DM, respectively. The results were in agreement with previous reports by Kaur et al. (2010) and He et al. (2002). The fat content in amaranth grain was superior to most conventional cereals such as wheat, maize and teff (Forsido et al., 2013). The available carbohydrate content of brown amaranth was less than that of white and red colored amaranth (Table 3.1.2). This is associated with the high level of dietary fiber in the brown amaranth as all the three amaranth types contain comparable quantities of other nutrients.

Table 3.1.2 Proximate composition of three types of raw *Amaranthus caudatus* grains*

Sample	DM	Protein	Fat	Available Carbohydrate (g/100g DM)	Ash	ADF	NDF
White amaranth	89.23 ± 0.10	13.88 ± 0.16	7.64 ± 0.05	68.41 ± 0.35	2.99 ± 0.08	5.49 ± 0.14	7.08 ± 0.36
Red amaranth	89.14 ± 0.09	15.15 ± 0.08	7.54 ± 0.06	67.15 ± 0.35	2.57 ± 0.02	5.99 ± 0.16	7.59 ± 0.41
Brown amaranth	88.88 ± 0.57	15.53 ± 0.24	7.72 ± 0.03	59.50 ± 1.98	3.10 ± 0.16	14.15 ± 1.89	14.79 ± 0.14

*values are mean of triplicates ± SD, DM-dry matter, ADF-Acid detergent fiber, NDF-Neutral detergent fiber

The amount of ash in the three types of amaranth whole seeds was in the range between 2.6 to 3.1, the maximum being for the brown colored amaranth and the minimum for red colored amaranth. This result fell in the range reported by Kaur et al. (2010). The content of acid

detergent fiber (ADF) was highest for brown amaranth (14.2 g/100 DM) followed by the red (6.0 g/100g DM) and white (5.5 g/100g DM) (Table 3.1.2). The content of neutral detergent fiber (NDF) was about twice higher in brown amaranth than in the two others (Table 3.1.2). Colored amaranth is known to have high amount of dietary fiber (Pedersen, 1987). The results of NDF in raw samples were also in agreement with the report by Mustafa et al. (2011) and higher than the amount of insoluble dietary fiber present in *Amaranthus hypochondriacus* reported by Czerwinski et al. (2004). The difference between ADF and NDF was comparable in white and red amaranth but lower in brown amaranth. This shows that brown amaranth could have lower amount of lignin, which is included during NDF determination, than the other two types of amaranth.

3.1.5.4. Effect of popping and fermentation on proximate composition

The effect of popping and fermentation on the amount of dry matter (DM), protein, fat, carbohydrate, ash and dietary fiber (ADF and NDF) of three types of amaranth is shown in Table 3.1.3. Popping resulted in a significant decrease ($p < 0.05$) in protein content from 14.9 to 14.3 g/100g DM. The decrease in protein content might be attributed to volatilization of nitrogen containing compounds. On the other hand, fermentation brought a significant increment in protein content by 2.7% at $p < 0.05$. This is attributed to the loss of dry matter mainly carbohydrates (Svanberg and Lorii, 1997). Because the fermentation is spontaneous i.e. without adding yeast or bacteria, no huge difference in protein was expected (Kazana and Fields, 1981; Hamad and Fields, 1979). The fat content was significantly increased ($p < 0.05$) from 7.6 g/100g DM to 8.5 and 9.2 g/100g DM during popping and fermentation, respectively. The increase in fat content during fermentation might be due to the presence of some yeast strains which produce fat (Khetarpaul and Chauhan, 1989). The improvement observed after popping could be due to the partial removal of pericarp that is poor in fat, leading to concentrating the fat content.

Table 3.1.3 Effect of processing on mean proximate composition of the three types of *Amaranthus caudatus* grains*

Treatment	Protein	lipid	Carbohydrate	Ash	ADF	NDF
(g/100 g DM)						
Raw	14.9 ± 0.8b	7.6 ± 0.1c	65.0 ± 4.3	2.9 ± 0.3b	8.8 ± 4.5b	9.6 ± 3.6b
Popped	14.3 ± 0.5c	8.5 ± 0.6b	57.9 ± 5.5	3.2 ± 0.4a	10.1 ± 5.6a	16.2 ± 4.7a
Fermented	15.3 ± 0.9a	9.2 ± 0.2a	63.0 ± 4.8	3.3 ± 0.6a	8.6 ± 4.6b	9.3 ± 3.5b

*values are means of nine measurements ± SD and means followed by different letters in the same column are significantly different at $p < 0.05$

The ash content exhibited a statistically significant ($p < 0.05$) increase during popping and fermentation by 10 and 14 %, respectively and this is associated with loss of dry matter allowing the minerals to concentrate (Svanberg and Lorii, 1997). The ADF and NDF values of samples increased significantly after popping from 8.8 to 10.1 and 9.6 to 16.2 g/100g DM ($P < 0.05$), respectively. Ramulu and Rao (1997) also observed increased levels of insoluble dietary fiber during heat treatment of cereals. But the effect of fermentation on both ADF and NDF is not noticeable.

3.1.5.5. Mineral contents of raw amaranth grains

The content of total iron, calcium and magnesium was highest in brown colored amaranth (Table 3.1.4). Zinc contents in the three types of amaranth were lower than that reported by Mustafa et al. (2011) and Nascimento et al. (2014). The iron content in raw samples exhibited huge variation ranging between 12.29-21.15 mg/100g DM and fell in the range reported by Mustafa et al. (2011) but higher than that reported by Nascimento et al. (2014). Calcium content also exhibited huge variation among the three types of amaranth (102-215 mg/100 g DM in raw samples). Generally, brown amaranth had highest mineral contents, particularly iron and calcium which were nearly twice those measured in the white and red amaranth. The result was not far from the reports by Pedersen et al. (1987) and Mustafa et al. (2011). The amount of Mg was in the range of 292-340 mg/100 g DM for raw samples and fell in the range reported by Mustafa et al. (2011) and higher than that reported by Nascimento et al. (2014).

Table 3.1.4 Mineral content of three types of raw *Amaranthus caudatus* grains*

Sample	Fe	Zn	Ca	Mg
(mg/100 g DM)				
White amaranth	13.06 ± 1.79	3.40 ± 0.42	122 ± 2	292 ± 33
Red amaranth	12.29 ± 0.51	2.73 ± 0.08	102 ± 5	301 ± 10
Brown amaranth	21.15 ± 1.4	3.43 ± 0.21	215 ± 2	340 ± 8

*values are mean of triplicates ± SD

3.1.5.6. Effect of popping and fermentation on mineral contents

Popping significantly reduced ($p < 0.05$) the content of iron and calcium by 31 and 8%, respectively. This decrease in iron content could be attributed to the loss of pericarp during popping as more than 66% of total minerals are found in the bran and germ fractions in amaranth (Berghofer and Schoenlechner, 2002). On the other hand, no significant effect ($p < 0.05$) was observed on the content of zinc and magnesium due to popping (Table 3.1.5). It may therefore be concluded that the outer layer of amaranth grain contains high amount of iron and calcium. On contrary, fermentation brought significant increase ($p < 0.05$) on the content of iron from 15.50 to 17.65 mg/100g DM and magnesium from 311 to 330 mg/100g DM but no significant change was observed on zinc and calcium (Table 3.1.5).

Table 3.1.5 Effect of processing on mean mineral content of three types of *Amaranthus caudatus* grains*

Sample	Fe	Zn	Ca	Mg
(mg/100 g DM)				
Raw	15.50 ± 4.4b	3.19 ± 0.4a	147 ± 52a	311 ± 28b
Popped	10.67 ± 0.78c	3.24 ± 0.4a	135 ± 46b	327 ± 32ab
Fermented	17.65 ± 4.9a	3.34 ± 0.5a	146 ± 51a	330 ± 20a

*values are mean of nine measurements ± SD and means followed by different letters in the same column are significantly different at $p < 0.05$

3.1.5.7. Mineral absorption inhibitors in raw amaranth samples and the effect of processing

Table 3.1.6 presents myo-inositol hexakisphosphate (IP6) content in raw amaranth samples. The amount obtained in all the three types of amaranth was higher than that present in other

cereals such as wheat, maize, buckwheat and rye (Egli et al., 2003). Such a high content of phytate probably strongly impairs the mineral bioavailability of the studied amaranth types and their products which then limits utilization of the crop to a wider consumer groups. In addition to phytate, polyphenols are also responsible for lowering mineral bioavailability (Brune et al., 1991; Khokhar and Owusu Apenten, 2003). Therefore, polyphenols particularly iron binding polyphenols (galloyls and catechols) were measured in all raw samples (Table 3.1.6). As was expected, the content of galloyl and catechol was highest for raw brown colored amaranth compared to raw red and raw white colored amaranth. Pigmented substances are known to have high amount of phenolic compounds (Manach et al., 2004) and this was also confirmed by the results of total polyphenol in the three differently colored amaranth in this study (See section 3.6).

Table 3.1.6 Mineral absorption inhibitors in raw *Amaranthus caudatus* grains*

Samples	IP6 (g/100g DM)	Galloyl (mg TAE/100g DM)	Catechol (mg CE/100g DM)
White Amaranth	2.20 ± 0.15	93 ± 11	ND
Red Amaranth	1.85 ± 0.25	100 ± 15	24 ± 16
Brown Amaranth	2.09 ± 0.15	143 ± 10	54 ± 21

*values are mean of triplicates ± SD; ND-not detected, IP6-*myo*-inositol hexakisphosphate

Substantial reduction in IP6 occurred during popping and fermentation by 39 and 77%, respectively. For popping, this might partly be due to the partial removal of pericarp during popping as phytates are concentrated in the bran of most cereals (Hama et al., 2011). Moreover, popping could also cause a decrease in phytic acid in the same way that roasting and autoclaving decreased the content in chickpea (Hussain et al. 1989). Repo-Carrasco-Valencia et al. (2009) also reported that extrusion cooking decreased the content of phytic acid in two varieties of *Amaranthus caudatus* by about 20-26%. Concerning fermentation, the decrease is noticeable and could be due to the activation of endogenous phytase or due to the action of exogenous phytase from fermentation microorganisms (yeast and lactic acid bacteria) when a favorable pH is created (Baye et al., 2013).

Popping did not exhibit a significant effect on the content of both galloyl and catechol. On the other hand, fermentation had a significant effect in decreasing the contents of both galloyl and catechol, by 96 and 100%, respectively (Table 3.1.7). This result is in agreement with the reports of Mouquet-Rivier et al. (2008). An increased activity of polyphenol oxidase during fermentation could be the contributing factor to the polymerization of polyphenols making them less extractable.

Table 3.1.7 Effect of processing on mean mineral absorption inhibitors of three types of *Amaranthus caudatus* grain

Treatment	IP6 (g/100g DM)	Galloyl (mg TAE/100g DM)	Catechol (mg CE/100g DM)
Raw	2.05 ± 0.23c	112.00 ± 25.66b	26.11 ± 1.31b
Popped	1.25 ± 0.26b	104.56 ± 32.94b	24.78 ± 2.01b
Fermented	0.47 ± 0.31a	4.78 ± 22.06a	0.00 ± 0.00a

*values are mean of nine measurements ± SD and means followed by different letters in the same column are significantly different at $p < 0.05$, IP6- myo-inositol hexakisphosphate.

3.1.5.8. Estimation of mineral bioavailability using molar ratio

The molar ratios of IP6:Fe for the three amaranth types under different processing is shown in Figure 3.1.3A. The result showed that IP6:Fe molar ratio was highest for white amaranth followed by red and brown amaranth. The result also showed that popping and fermentation brought a marked decrease in the IP6:Fe molar ratio. However, only fermentation in the brown amaranth enabled achievement of the recommended IP6:Fe ratio of < 1 . The fermented samples of the two other types should be consumed with mineral absorption enhancers in which case higher IP6:Fe molar ratio could be entertained to achieve better mineral bioavailability (Hurrell, 2004).

The effect of popping and fermentation was also significant in reducing the molar ratios of IP6:Zn in all the three types of amaranth (Figure 3.1.3B). However, only the fermented brown samples met the cut off level (IP6:Zn < 15). It has been observed that high levels of Ca exacerbate the inhibitory effect of phytic acid on Zn absorption in humans by forming a Ca–Zn–IP6 complex in the intestine, a more insoluble complex than phytate complexes formed with either ion alone (Cossack and Prasad, 1983; Fordyce et al., 1987). Thus the use of

IP6*Ca/Zn has been suggested as a better indicator of Zn bioavailability and if the value is above 200, the deleterious effect of Ca on Zn absorption is expected (Hemalatha et al. 2007). As seen in Figure 3.1.3D, both popping and fermentation decreased the value of IP6*Ca/Zn significantly and all, except the raw brown amaranth, had molar ratio above 200 due to the relatively high amount of calcium in brown amaranth (Table 3.1.2). The molar ratio of IP6:Ca was also decreased by 21-51% and 61-93% during popping and fermentation, respectively. However, only fermented brown amaranth is able to meet the recommendation (Fig 3.1.3C).

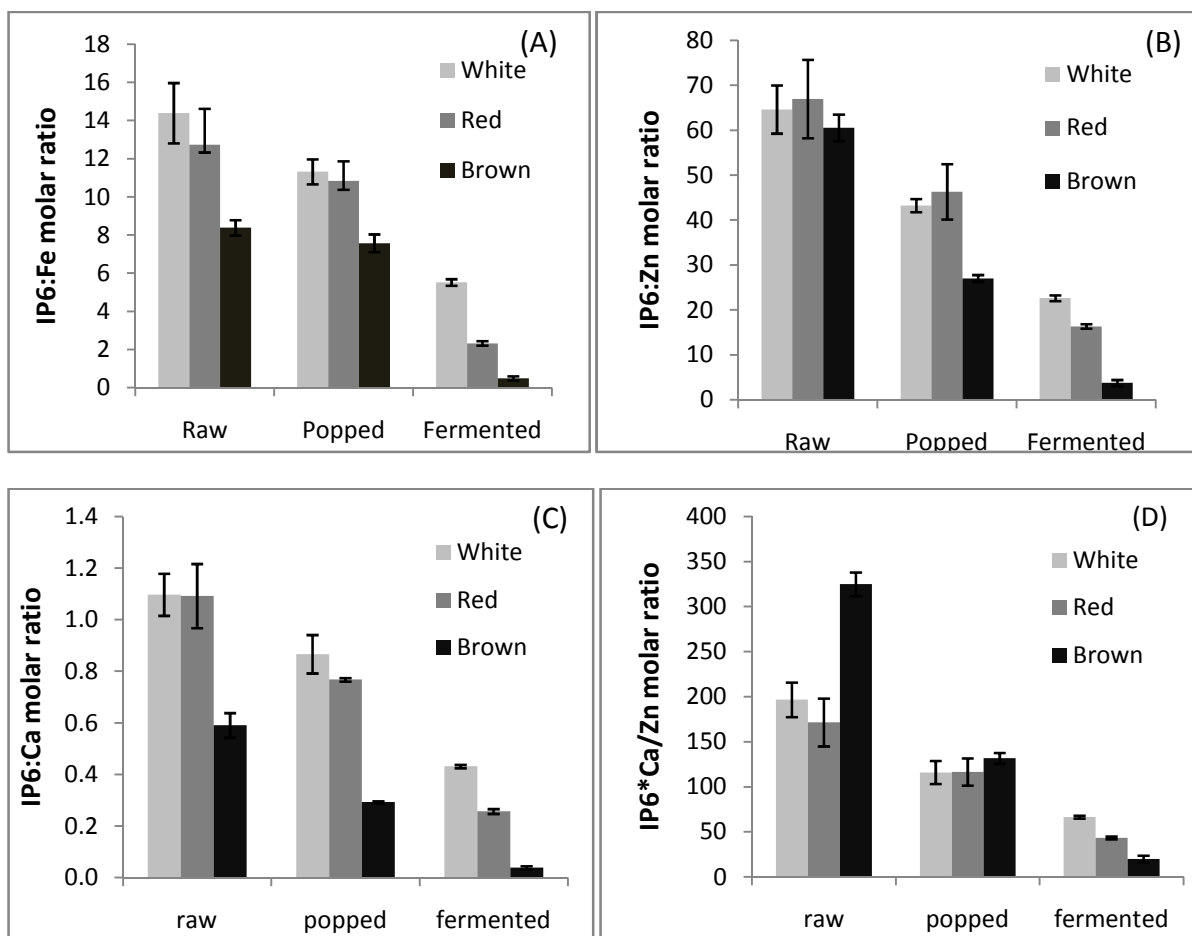


Figure 3.1.3 Effect of processing on molar ratios of IP6:Fe (A), IP6:Zn (B), IP6:Ca (C) and IP6*Ca:Zn (D)

In general, both processing methods (popping and fermentation) were effective in reducing the level of phytate to a greater extent. The effect of fermentation in reducing the content of phytic acid is far better than popping. However, due to the particularly high initial phytate

content of amaranth, almost all IP6-to-mineral molar ratios were above the recommended values except for fermented brown amaranth. It is therefore necessary to design further strategies to maximize phytate degradation and improve mineral bioavailability.

3.1.6. Conclusion

All the three types of *Amaranthus caudatus* grains cultivated in Ethiopia have been found to be rich source of protein and fat compared to most commonly consumed cereals. They are also a good source of iron, zinc and calcium making them a potential crop for complementing both cereals and legumes. However, all the three types considered under this study contained high amount of mineral absorption inhibitors mainly phytate that could actually significantly reduced by popping or fermentation. Despite the attempt done to decrease the level of mineral absorption inhibitors using popping and fermentation, the level is still high enough to bind minerals and consequently decrease their bioavailability. It is therefore necessary to investigate further optional processing methods to fully exploit the crop to its potential. Furthermore, investigating in vitro and/or in vivo bioavailability of key nutrients (iron, zinc and calcium) in the studied grain amaranth and their interaction with chelating factors such as phytate, polyphenols and oxalate is also strongly recommended.

3.2. Nutrient and Energy Adequacy of Amaranth based Complementary Foods

3.2.1. Introduction

It has been observed that popped amaranth is used as a raw material for complementary food formulation by the Me'enit people living in Bench Majji zone, SNNPR, Ethiopia. Based on the results obtained above (section 3.1), the nutrient adequacy of flour obtained from popped amaranth grain with respect to its protein, fat, energy, iron and zinc contents were evaluated and compared with the recommended nutrient content in complementary foods for breast-fed infants aged 6-11 and 12-23 months consuming the recommended daily ration size of 40 g/d and 60 g/d dry weight complementary food flour respectively (Lutter and Dewey, 2003). For the sake of comparison, selected seven commercial complementary foods processed in Ethiopia and sold in Addis Ababa supermarket were purchased and included in this study. Based on the results of this section recommendation was given on the suitability of amaranth based complementary foods.

3.2.2. Materials and Methods

3.2.2.1. Sample collection

Selected processed cereal-based complementary foods (CFs) were purchased from supermarkets in Addis Ababa, Ethiopia. The samples were packed in polyethylene bag and sent to Institut de recherche pour le développement (IRD), Montpellier, France for analysis of proximate composition and minerals (iron and zinc) using the protocol described in section 3.1.

3.2.2.2. Calculation of energy value

The energy values of the CFs were calculated using the Atwater coefficients 4 kcal/g for protein, 9 kcal/g for fat, 4 kcal/g for available carbohydrates (Atwater & Benedict, 1902) and 2 kcal/g for dietary fiber as recommended by FAO (2003).

3.2.2.3. Calculation of iron, zinc, protein, fat and energy requirement from complementary foods

Assuming average breast-fed children of 6-11 and 12-23 months old consume the recommended daily ration size of 40 g/d and 60 g/d dry weight cereal flour, respectively, calculation of the contribution of CFs to the recommended nutrient and energy requirement from CFs was made based on the nutrient and energy requirement of CFs as described in Lutter and Dewey (2003).

3.2.3. Results and Discussion

3.2.3.1. Description of Commercially available CFs

Among the seven complementary foods (CFs) collected, only five were fortified (Table 3.2.1). Two of the non fortified CFs (i.e CF6 and CF7) were found to be a mix of several cereals and pulses having closer nutrient contribution. This indicates that the formulation was done without the notion about nutrient contribution of each ingredient. Moreover, they also contained bulla, a product of *Ensete ventricosum*, having a high tendency to develop thicker consistency in the product thereby decreasing the energy and nutrient density of the products. On the other hand, CF3 contained alpha amylase responsible for hydrolyzing the starch which consequently contributed to an increase in energy and nutrient density.

Table 3.2.1 Product Description of commercially available complementary foods in Ethiopia

Sample	Major Ingredients	Mineral and vitamin premix added + or not -
CF1	Barely flour, oats, soybean and iodized salt	+
CF2	Wheat, soybean, chickpea, milk powder and sugar	+
CF3	Wheat, carrot, spinach, pumpkin, skimmed milk powder, full cream milk powder, palm oil, sugar, iodized salt and enzyme	+
CF4	Corn and soy blend	+
CF5	Corn, rice, soya, milk powder, sugar and fruits	+
CF6	Oats, barley, soybean, peanut, wheat, broad bean, lentil, pea, maize, sesame, bulla, red sorghum, fenugreek, linseed and teff.	-
CF7	Soybean, barley, red teff, lentil, pea, millet, peanut, wheat, fenugreek, linseed, broad bean, maize, sorghum, sesame, bulla, oats	-

3.2.3.2. Proximate composition of commercially available CFs

Table 3.2.2 presents the proximate composition and energy contents of commercial complementary foods. The protein, fat and ash contents varied markedly, ranging from 6.1 to 14.1 g/100g DM, 3.6 to 8.0 g/100g DM, 1.3 to 2.7g/100g DM, respectively. The contents were found to be lower than that present in popped amaranth (13.9-15.5 g/100g DM for protein, 7.5-7.7 g/100g DM for fat and 2.6-3.1 g/100g DM for ash). The carbohydrate and energy contents of the commercial CFs were found in the range of 76-81 g/100g DM and 416-438 kcal/100g DM, respectively. CF6 was found to contain highest amount of energy and this might be due to the presence of peanut and other oil seeds. In contrast, CF7 that was claimed to contain peanut, linseed, soybean, sesame seed and other oil seed was found to have low amount of fat.

Table 3.2.2 Proximate composition and energy contents of commercial complementary foods

Sample	Protein	Fat	Ash	CHO	Energy
			mg/100g DM		
CF1	13.20 ± 0.28	3.58 ± 0.12	2.74 ± 0.16	80.5 ± 0.24	415.6 ± 1.23
CF2	12.22 ± 1.64	5.44 ± 0.12	2.56 ± 0.03	79.8 ± 1.74	421.2 ± 0.68
CF3	11.21 ± 0.06	5.87 ± 0.03	2.21 ± 0.02	80.7 ± 0.01	422.9 ± 0.18
CF4	12.22 ± 0.01	7.97 ± 0.04	2.51 ± 0.06	77.3 ± 0.12	436.8 ± 0.00
CF5	6.13 ± 0.00	5.65 ± 0.06	1.28 ± 0.01	86.9 ± 0.05	426.5 ± 0.75
CF6	14.08 ± 0.00	7.75 ± 0.22	2.22 ± 0.01	75.9 ± 0.21	437.9 ± 1.40
CF7	13.77 ± 0.15	3.73 ± 0.03	1.96 ± 0.01	80.5 ± 0.19	418.5 ± 0.36

Table 3.2.3 shows mineral, mineral absorption inhibitors and estimated mineral bioavailability of commercially available complementary foods. As observed for macronutrients, the contents of micronutrients also varied markedly and were in the range of 6-22 mg/100g DM and 0.8-12.1 mg/100g DM for iron and zinc, respectively. ADF content was in the range of 1.2-4.3 g/100g DM. The range for IP6 content was 235-893 mg/100g DM with the highest IP6 content in complementary foods formulated with a mixture of many cereals, legumes and oilseeds showing that the ingredients were not properly processed to lower the level of mineral absorption inhibitors especially for oilseed which have high content of phytic acid (Abebe et al., 2007). The contents of IP6 in CF1, CF4, CF6 and CF7 were found to be higher than the sum of IP6 and IP5 contents of some complementary foods studied by Gibbs et al. (2011). IP6-to-mineral molar ratio that is used to estimate mineral bioavailability was also calculated and the result was in the range of 0.89-7.07 and 1.93-30.63 for [IP6]:[Fe] and [IP6]:[Zn], respectively. Only 1-out of 7 CFs and 4-out of the 7 CFs met the recommendation for [IP6]:[Fe] and [IP6]:[Zn], respectively (Table 3.2.3). CF3 was found to meet the recommendation for both [IP6]:[Fe] and [IP6]:[Zn]. However, the contents of phytate and ADF were found to be lower than that of a sole amaranth-based complementary foods. The lower level of phytate could significantly contribute to improved mineral absorption and lower level of fiber decreases the dietary bulk thereby increasing the food intake resulting in increased nutrient and energy intake for children.

Table 3.2.3 Mineral, mineral absorption inhibitors and estimated mineral bioavailability of commercially available complementary foods

Sample	Fe	Zn	ADF	IP6	IP6:Fe	IP6:Zn
	mg/100g DM		g/100g DM	mg/100g DM		
CF1	15.15 ± 0.10	7.64 ± 0.21	4.34 ± 0.02	677 ± 4	3.78	8.78
CF2	13.85 ± 0.40	4.74 ± 0.20	2.10 ± 0.03	326 ± 20	1.99	6.82
CF3	22.31 ± 0.23	12.07 ± 0.48	1.22 ± 0.07	235 ± 10	0.89	1.93
CF4	17.55 ± 0.34	10.50 ± 0.21	3.49 ± 0.02	723 ± 42	3.49	6.82
CF5	5.85 ± 0.24	0.80 ± 0.12	1.71 ± 0.20	247 ± 7	3.58	30.63
CF6	10.68 ± 0.11	2.97 ± 0.10	3.98 ± 0.13	892 ± 5	7.07	29.78
CF7	11.89 ± 0.27	2.57 ± 0.05	3.84 ± 0.22	754 ± 7	5.37	29.07

3.2.3.3. Contribution of commercial CFs and amaranth-based CFs to the estimated nutrient requirement from complementary food for 6-23 month old children

Assuming breast fed infants 6-11 months and children 12-23 months consume the recommended daily ration size of CF (i.e., 40 and 60 g of flour/day, respectively) (Lutter and Dewey, 2003), the percentage contribution of the commercial and amaranth-based complementary foods to the recommended nutrient requirement from CF for iron and zinc (at moderate bioavailability), protein and fat was 21-81% and 50-100%, 6-97% and 10-100%, 54-100% and 57-100%, and 31-73% and 26-64%, respectively. The contribution to the energy requirement from CF was averaged to 46%, 34% and 24% for 6-8, 9-11 and 12-23 month-old children, respectively (Figure. 3.2.1).

The result indicated that none of the CFs including amaranth-based ones meet the iron requirement for 6-11 month old children but 5 of the CFs contained adequate iron for 12-23 month-old children. The contribution of amaranth based complementary foods to the daily intake of iron was lower than that of fortified commercial complementary foods (CF1-CF4) but comparable to the non fortified CFs especially for the age group of 6-11 month old children (Figure 3.2.1). Regarding zinc, only 2 of the CFs could contribute to more than 80% of the requirement for 6-11 month old children and 3 of the CFs could contribute to more than 90% of the requirement for 12-23 month old children. Only the fortified CFs were found to better contribute to the daily requirement of zinc. Similar to that of the contribution for iron, the contribution of amaranth based CFs to the daily zinc requirement was comparable to the non fortified commercial complementary foods. CF5 that was claimed being fortified was found to contribute the lowest iron and zinc requirement for both age groups. The protein requirement of 6-23 month old children from CFs was met by both the commercial and amaranth-based complementary foods except CF5, which contributed to 54 and 57% of the requirement for 6-11 and 12-23 month-old children, respectively. None of the complementary foods (commercial or amaranth based ones) met the requirement for fat. However, amaranth-based CFs were found to contribute better than the commercial CFs for the daily fat requirement. Although CF7 was claimed to contain many oil seeds such as fenugreek, linseed, sesame and soybean, it was found to contribute the least to the daily fat content (30% and

27% for 6-11 and 12-23 month old children, respectively). None of the CFs met the energy requirement of all age groups of children. The low fat content is the primary reason why the CFs failed to meet the daily energy requirement. It is therefore necessary to add cooking oil during the preparation of porridge. In addition to the prediction to meet the daily nutrient requirement based on the total nutrient contents of these complementary foods, the issue of bioavailability should be considered. As seen from Figure 3.1.3 of section 3.1 and Table 3.2.3 of section 3.2, the predicted mineral bioavailability of some of the commercially complementary foods and all amaranth-based complementary foods was below the recommendation.

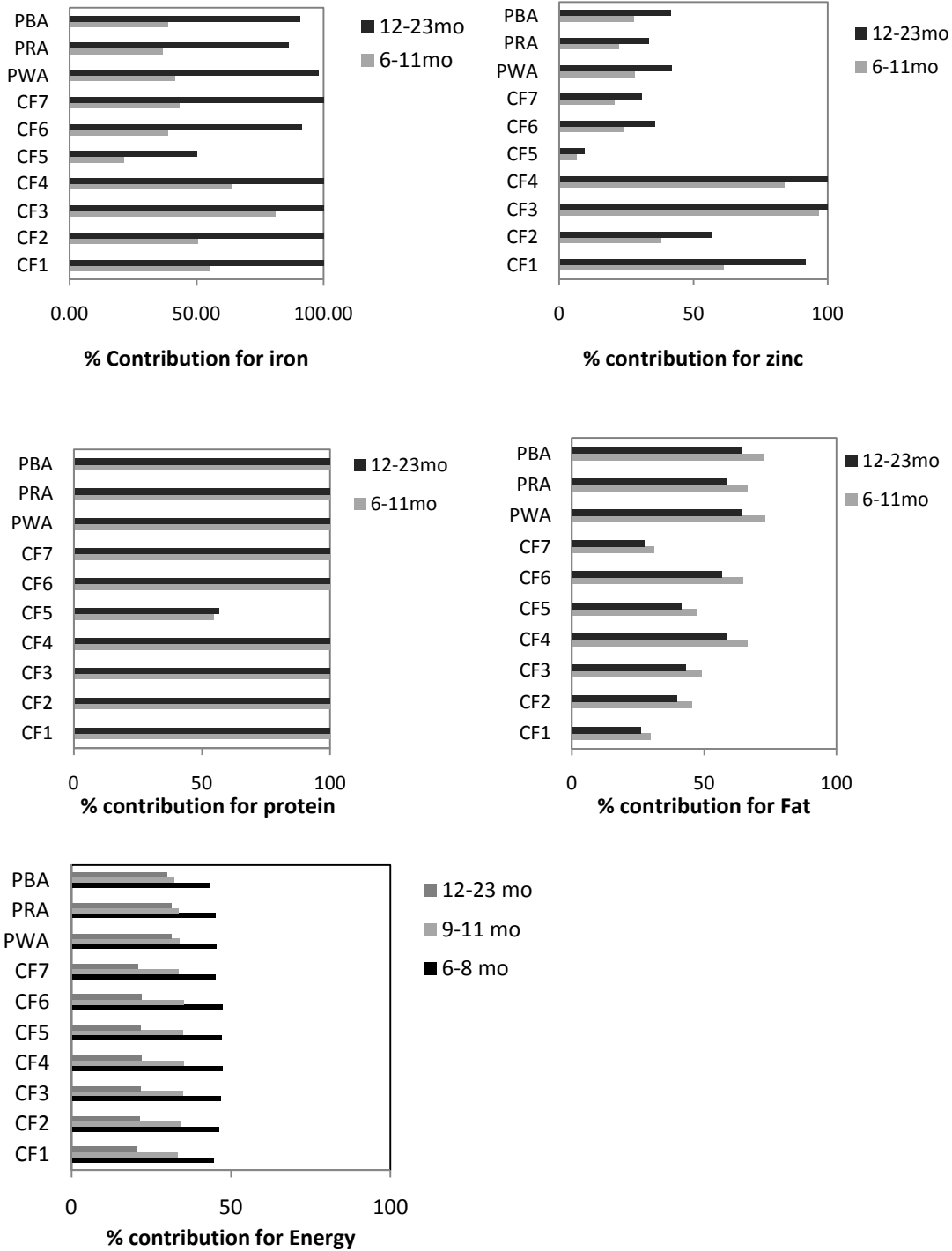


Figure 3.2.1 Percentage contribution of commercial CFs and amaranth-based CFs to the recommended nutrient requirement from CF for average breast fed children whose intake is 40 and 60 g flour/day for 6-11 and 12-23 month-old children, respectively. Estimated nutrient requirement values for iron (mg), zinc(mg), protein(g), and fat(g) were respectively, 11, 5, 4.5, and 4.8 for 6-11 month old children, and 7, 5, 6.5, and 8.2 for 12-23 month old children. Energy requirements were 356, 479 and 772 kcal for 6-8, 9-11, and 12-23 mo children, respectively. PWA-popped white amaranth, PRA-popped red amaranth and PBA-popped brown amaranth.

3.2.4. Conclusion

This section attempts to show the adequacy of amaranth-based complementary foods to the daily nutrient requirement from daily consumption of recommended amount of complementary food for 6-11 and 12-23 month old children and compared with some commercially available complementary foods processed in Ethiopia.

All amaranth-based and many of the commercial complementary foods provide sufficient amount of protein needed from complementary food for 6-23 month old children. But none of the complementary foods meet the requirement for fat. However, amaranth based CFs are better than the commercial complementary foods. Therefore, incorporation of oil seeds during formulation or use of cooking oil during porridge preparation for both amaranth-based and commercial complementary foods is recommended.

Many of the complementary foods considered have limitation to meet the recommendation of zinc for both age categories and iron for 6-11 month old children. However, the fortified commercial complementary foods are better than the non-fortified and amaranth-based complementary food. But one of the commercial complementary foods that was claimed fortified with micronutrient was found to contribute the least among all complementary foods. Moreover, the results of phytate-to-mineral molar ratio shows that many of complementary foods fail to meet the cut off point for both iron and zinc. Therefore, the use proper fortification levels in commercial complementary food, application of further dephytinization processes in both amaranth based and commercial complementary foods, enriching all CFs with animal source foods and consumption along with mineral absorption enhancers to improve the bioavailability of nutrients are strongly recommended to improve the nutritional status of children living either in the rural or urban areas.

3.3. Combined treatment of germination and soaking may improve both energy density and phytate degradation in amaranth porridge

3.3.1. Introduction

Amaranth has attracted a great deal of interest in recent decades due to its valuable nutritional and agricultural characteristics. However, the nutritional significance has been limited by the presence of high level of phytic acid (Egli et al., 2002; Amare et al., 2015 unpublished result). Phytic acid mainly chelates divalent metal ions such as iron, zinc, calcium, magnesium and manganese, making them unavailable for absorption in humans and monogastric animals (Hurrell et al., 2003; Egli et al., 2004; Koréissi-Dembélé et al., 2013). Moreover, amaranth grain contains about 60% starch (Capriles et al., 2008) and the presence of such high amount of starch will influence the energy density by forming a thick viscous porridge at low dry matter content. As a result, applying techniques that could partially hydrolyze the starch and/or degrade the phytic acid will significantly improve both the energy density and nutrient bioavailability.

Degradation of phytic acid (IP6) in to a lower inositol phosphates such as IP5 (*myo*-inositol penta phosphate), IP4 (*myo*-inositol tetra phosphate), IP3 (*myo*-inositol tri phosphate), IP2 (*myo*-inositol diphosphate) and IP1 (*myo*-inositol monophosphate) could be made possible by adding exogeneous phytases (Sandberg et al., 1996) or enhancing the activity of endogeneous phytase during food processing such as soaking, germination and fermentation (Egli et al., 2002; Reale et al., 2007; Liang et al., 2008) thereby improves mineral bioavailability. The lower inositol phosphates have lower mineral binding potency than *myo*-inositol hexaphosphate (Sandberg et al., 1989; Lönnerdal et al., 1989; Sandberg et al., 1999) and thus higher mineral bioavailability could be achieved after processing.

For an effective degradation of phytic acid using endogenous (plant origin) and exogeneous (plant or microbial origin) enzymes, it requires optimal conditions of pH and temperature (Beal and Mehta, 1985; Silva and Trugo, 1996; Bergman et al., 2000). Besides, endogenous phytase activity of cereals and legumes varies widely; rye, triticale, wheat and buckwheat

exhibited the highest phytase activity whereas legumes and other common cereals like maize, sorghum, millet and rice are known for their relatively low phytase activity (Egli et al., 2002). This wide variation in phytase activity indicates that selection of the best phytase producing grain is of paramount importance to achieve highest phytate degradation for the formulation of cereal and/or legume-based foods particularly for infants and young children.

It has been reported that amaranth, being categorized under pseudocereals, had high endogenous phytase activity compared to other cereals (Egli et al., 2002). Therefore, designing strategies to degrade the high phytate content in amaranth using endogenous enzyme is indispensable and preferred alternative. In order to optimize the conditions that activate phytases in cereals; the use of hydrothermal treatment that provides suitable condition; appears to be a promising method for maximum phytate degradation and this was observed in wheat (94.4-95.6%), rye (99.0-99.5%) and rice (99.8%) (Bergman et al., 2001).

Therefore, the aim of the present study was to improve the nutritional qualities of amaranth porridge by determining the optimal conditions of a treatment combining malt addition and soaking. First, an experimental design was conducted to determine optimal pH, temperature and incubation time of amaranth phytase. Secondly, the effects of hydrothermal treatment conducted previously on phytic acid degradation in amaranth flour and energy density at suitable consistency of gruel prepared from amaranth have been assessed.

3.3.2. Materials and methods

3.3.2.1. Sample collection

Grain *Amaranthus caudatus*, white in seed color, was purchased from six farmers living in Chat Kebelle, Bench Majji Zone, Southern Nations, Nationalities and Peoples region, Ethiopia in October 2011. The grains of the six origins were sorted to remove immature seeds, cleaned and washed to remove sand and soil. The washed seeds were sun dried and equal amount of the six samples were mixed to prepare a composite sample.

3.3.2.2. Sample preparation

i. Preparation of raw amaranth flour

The composite amaranth sample was milled, sieved using 425 μm sieve and stored at 4 °C until further analysis.

ii. Popping

Sun dried amaranth grains were popped as described in Amare et al. (2015). The popped grains were milled to pass a 0.425 mm sieve and stored in polyethylene bags at 4 °C until further analysis was done.

iii. Germination

For germinating amaranth grains the method of Colmenares de Ruiz and Bressani (1990) was used with modification. The seeds were washed and soaked by disinfecting solution of 70% ethanol for 3 min. Then it was thoroughly washed to remove residual solvent and soaked in distilled water (1:3, w/ v) for 5 h at room temperature (22 ± 2 °C). The soaked seed were kept between thick layers of cotton cloth and allowed to germinate for 48 h at 32 ± 2 °C. During germination, seeds were rinsed with distilled water three times a day. After germination; the seeds were dried in an electric oven at 50 °C for 20 h.

3.3.2.3. Extraction of phytases

Phytase from amaranth flour was extracted in triplicate by weighing 2 g of flour and adding 20 mL of 0.1 M sodium acetate buffer at 4 °C, pH 5.6, according to the method of Konietzny et al. (1995). The samples were magnetically stirred for 2 h at 4 °C and centrifuged at 10000g at 4 °C for 30 min. The supernatants were shaken with AG1-X8 anion resin (Bio-Rad Laboratories, Richmond, CA) at 4 °C for 10 min to remove free phosphorus and myo-inositol phosphates. Finally, the samples were centrifuged at 2600g at 4 °C for 10 min again, and the supernatants were used as enzyme extracts.

3.3.2.4.Determination of free phosphorus

The free phosphorus adsorbed on AG1-X8 anion resin described above was desorbed by adding 20 mL of 1 N HCl and stirred for 10 minutes at 4 °C. The solution was centrifuged at 2600 g for 10 min and the supernatant was transferred to another tube. To the residue 20 mL of 1 N HCl was added to repeat the desorption process. The two supernatants were mixed and homogenized. Aliquot (0.25 ml) was taken and mixed with 2 mL of a mixture of sulfuric acid, acetone and ammonium molybdate (1 volume of 5 N H₂SO₄, 2 volumes of pure acetone and 1 volume of 10 mM ammonium (hepta) molybdate) and allow it to stand for 10 minutes. Series of phosphorus standard solutions were also prepared and treated similarly as the sample extract. Absorbance reading was taken using spectrophotometer at 405 nm.

3.3.2.5.Measurement of phytase activity

Phytase activity was measured as explained by Lolas and Markakis (1977) with modification. Briefly, 3 mL of extracted enzyme, 2 mL of 2.5 mM sodium phytate (Sigma Aldrich), and 9 ml of acetate buffer (pH 5.6) were mixed and incubated for 60 minutes at 30 °C. The enzymatic activity was stopped after 60 min by adding 4 mL of 2 N HCl and the liberated inorganic phosphate was determined using the spectrophotometric method at 405 nm as described in Heinonen and Lahti (1981). Phytase activity is expressed in phytase units (PU) per g DM of flour. One PU is equivalent to the enzymatic activity that liberates 1 µmol of inorganic phosphate from sodium phytate per minute per gram (DM) of flour. Standard solutions of potassium phosphate (0-5 mM) were prepared to develop the calibration curve.

3.3.2.6.Experimental Design

Milled raw amaranth flour was mixed with germinated flour at a ratio of 9:1 (w/w) and 500 mg of the mixture was incubated at various temperature and pH using 5 mL of citric acid with concentration between 2 mM and 15 mM in order to achieve different pH values with regular shaking of the solution at 1 hr interval for different incubation times as described in Table

3.3.1. The enzymatic activity was terminated by the addition of 25 mL of 0.6 M hydrochloric acid.

To optimize the conditions for phytate degradation in amaranth flour, a central composite rotatable design with three factors and five levels (-1.68, -1, 0, 1, 1.68) was used. The experimental matrix is shown in table 3.3.1. The variables were coded according to the following equation:

$$X_i = (x_i - \frac{\bar{x}_i}{\Delta x_i}) \quad (1)$$

Where X_i is the coded value of an independent variable x_i , \bar{x}_i is the corresponding real value at the centre point, Δx_i is the step change. Therefore, the specific codes are:

$$X_1(\text{temperature}) = \frac{(x_1 - 50)}{8} \quad (2)$$

$$X_2(\text{pH}) = \frac{(x_2 - 5.0)}{0.5} \quad (3)$$

$$X_3(\text{Incubation time}) = \frac{(x_3 - 5.0)}{2.0} \quad (4)$$

For the mathematical description of the hydrothermal treatment results, a polynomial of second order was used having the form of:

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{11}X_1^2 + B_{22}X_2^2 + B_{33}X_3^2 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 + \varepsilon \quad (5)$$

Where Y = response variable (the content of phytic acid remaining after the application of the treatment); X_1 is the coded value of the temperature; X_2 is the coded value of the pH; and X_3 is the coded values of incubation time. The coefficient B_0 is a constant; b_1 , b_2 and b_3 are the main effects of x_1 , x_2 and x_3 , respectively; b_{12} , b_{13} and b_{23} shows the interaction between the variables; and the square coefficients b_{11} , b_{22} and b_{33} will tell if any of the variables has a maximum/minimum in the experimental domain. The difference between the experimental data (y_{obs}) and the model (y_{calc}) gives the residual (ε). The replicates at the center point make it possible to estimate the pure errors of the analyses, which are used to predict if the model give significant lack of fit.

Table 3.3.1 Specification matrix of the second order design: x1: temperature, x2: pH and x3: incubation time

Expt. No.	Coded variables			Actual values			IP6 (mg/100g DM)
	X ₁	X ₂	X ₃	X ₁	X ₂	X ₃	
	$X_1 = \frac{(x_1 - 50)}{8}$	$X_2 = \frac{(x_2 - 5.0)}{0.5}$	$X_3 = \frac{(x_3 - 5.0)}{2.0}$	(T, °C)	(pH)	(Time, h)	
1	0	0	-1.68	50	5	1.64	20
2	0	0	1.68	50	5	8.36	7
3	1	1	1	58	5.5	7	155
4	0	0	0	50	5	5	6
5	-1.68	0	0	36.6	5	5	199
6	-1	1	-1	42	5.5	3	354
7	-1	-1	-1	42	4.5	3	232
8	0	0	0	50	5	5	13
9	0	0	0	50	5	5	9
10	-1	1	1	42	5.5	7	2
11	1	-1	-1	58	4.5	3	937
12	1	1	-1	58	5.5	3	301
13	1	-1	1	58	4.5	7	827
14	0	1.68	0	50	5.84	5	118
15	-1	-1	1	42	4.5	7	56
16	1.68	0	0	63.5	5	5	806
17	0	0	0	50	5	5	9
18	0	-1.68	0	50	4.16	5	714
19	0	0	0	50	5	5	9

3.3.2.7. Determination of phytic acid

The final mixture after the addition of hydrochloric acid in section 3.3.2.6 was magnetically stirred for 6 min in a boiling water bath in order to extract the phytate. The solution was then centrifuged at 4500 g for 20 min at 4 °C and 6 mL of the supernatant was used for phytic acid determination using high performance anion exchange chromatography as described in Lestienne, Icard-Verniere, Mouquet, Picq, and Treche (2005), using an AS-11 pre-column and column kit (Dionex, Sunnyvale, USA) after drying on speed vac (JOUAN, Saint Herblain, France).

3.3.2.8. Kinetics of phytic acid degradation using optimum conditions

After optimizing the best pH, temperature and incubation time for maximum dephytinization, 11.2 g of a mixture of raw and germinated amaranth grain flour in a 9:1 ratio was mixed with 75 mL of distilled water in five other 100 mL beakers. Similarly, equal amount of 100% raw amaranth grain flour in the absence of germinated flour was also mixed with distilled water in five another different 100 mL beakers. The pH of these mixtures was adjusted to the optimum value (5.2) using lemon juice, chosen for maximizing the possible application in field, and incubated at optimum temperature (46 °C) for 0, 1, 2, 3½, and 5 h. Aliquots of these mixtures were taken and used for phytic acid analysis as explained previously.

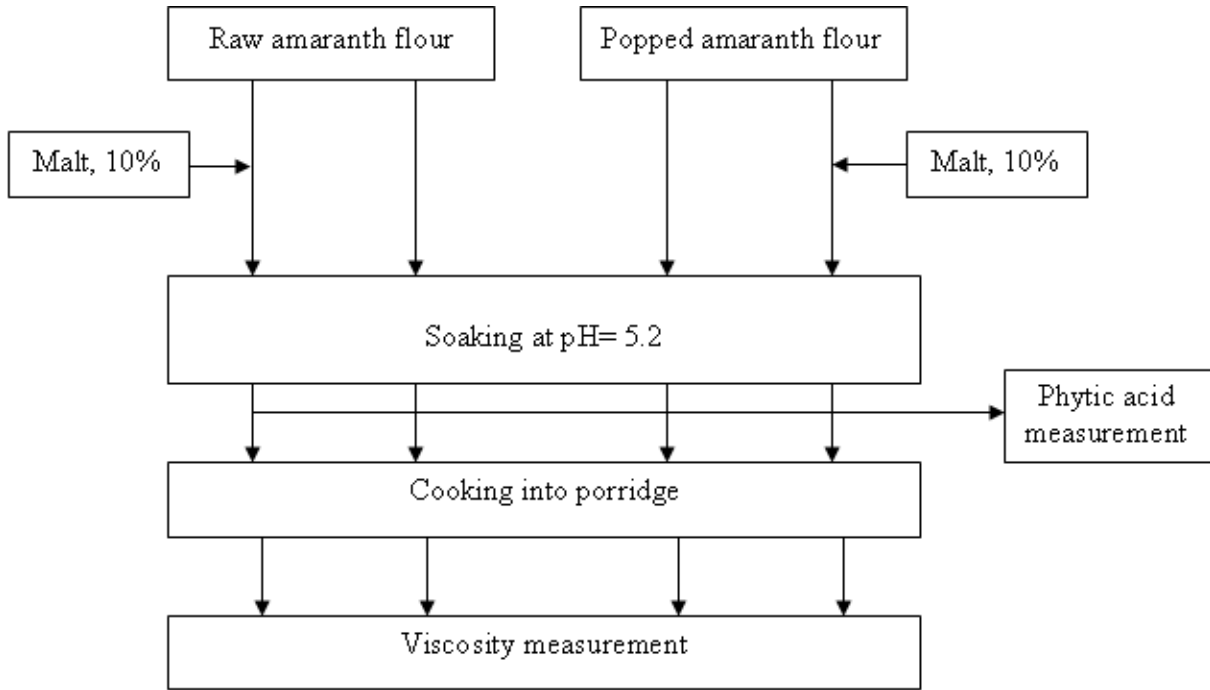


Figure 3.3.1 Flow chart for kinetics of phytic acid degradation under optimum conditions

3.3.2.9. Preparation of porridge and viscosity measurement

A portion of the slurry incubated using the above procedure (section 2.3.6) was poured into a pan and cooked for 5 min at boiling temperature. The dry matter content of the porridge was measured at 105 °C in an oven until constant weight was achieved. A viscometer Haake

VT550 with SV-DIN coaxial cylinder was used to measure the apparent viscosity of the porridge at a shear rate of 83 s^{-1} after 10 min as explained in Mouquet and Treche (2001). The measurements were done at $45 \text{ }^{\circ}\text{C}$.

3.3.3. Data Analysis

All measurements were done in triplicate and statistical analyses for the measurement of phytase activity were performed using SPSS version 19. Statgraphics Centurion XV (Statistical Graphics Corp., Englewood Cliffs, N.J., U.S.A., STSC. Inc. 1987n, USA) was used for statistical calculations and graphing for optimization of phytic acid degradation. The data were analysed by analysis of variance (ANOVA). Means were separated using Fischer's least significant difference tests with a probability $P < 0.05$.

3.3.4. Results and discussion

3.3.4.1. Estimation of Phytase activity

The results of endogenous phytase activity and inorganic phosphorous contents of raw, popped and germinated amaranth are presented in Table 3.3.2. The phytase activity of germinated amaranth was nearly three-fold higher compared to raw amaranth. Several studies also confirmed that germination increases phytase activity of cereals and legumes (Egli et al., 2002; Sung et al., 2005; Ramadan and Oraby, 2013).

The phytase activity of raw amaranth was much lower than reported elsewhere (Egli et al., 2002). This variation might be due to the difference in the pH of the buffer solution, incubation temperature and substrate concentration during extraction of endogenous phytase. Moreover, genotypic variation might also cause a difference in phytase activity as evidenced in wheat (Liu et al., 2006). However, after germination, for the same time, it was observed that the phytase activity obtained in this study was higher than that reported by Egli et al. (2002). These authors reported that the improvement in phytase activity after 48 h germination was 124% but it is 187% in the present study. Variation in germination

temperature might be the possible reason for the existing difference in phytase activity (Sung et al., 2005). The rheological property of popped amaranth doesn't allow the determination of phytase using the method used. But it is expected that heat treatment deactivates the enzymatic activity and could have lower phytase activity than raw amaranth.

The content of inorganic phosphorus in raw, popped and germinated amaranth was 25.9, 57.4 and 90.8 $\mu\text{mol/g DM}$, respectively. Higher phytase activity predicts higher phytic acid degradation that consequently increases the release of free phosphorus, and thereby improves phosphorus bioavailability. Inorganic phosphorus production during popping was higher than that of the raw amaranth (Table 3.3.2) which is associated with the decreased level of phytic acid (data not shown). The effect of heat treatment on phytic acid degradation with subsequent increase in free phosphorus has also been reported by Khan et al. (1991), where 18-47% and 12-53% phytate degradation was achieved for fresh and dried maize, respectively.

Table 3.3.2 Endogenous phytase activity of raw and processed white amaranth grain flour

Name of sample	Treatment	Phytase Activity (PU/g DM)	Inorganic phosphorus ($\mu\text{mol/g DM}$)
White Amaranth	Raw	0.61 \pm 0.06	25.91 \pm 0.56d
	Popped	ND	57.39 \pm 2.11c
	Germinated	1.75 \pm 0.03	90.79 \pm 4.09b

Values are mean \pm SD of triplicate measurement; means followed by different letters in a column are significantly different ($p > 0.05$), ND-Not determined

The endogenous phytase activity, as indicated in Table 3.3.2, was highest for germinated amaranth that explains the importance of using germinated flour as a power flour to blend with raw amaranth flour for maximum phytic acid degradation. Larsson and Sandberg (1992) also reported that addition of malted rye flour into oats reduced the phytic acid content to very low levels. Realizing the significant contribution of germination for phytic acid degradation that is caused by its high phytase activity, germinated amaranth flour (10%) was suggested for the optimization experiment. Moreover, addition of germinated flour promotes hydrolysis of

starch as the activity of amylases is also expected to increase during germination (Helland et al., 2002).

3.3.4.2. Determination of optimal condition for phytic acid degradation in amaranth

Optimal conditions for phytic acid degradation after incubating a mixture of raw and germinated amaranth flour in a 9:1 ratio for the three variables were determined by using the responses for each treatment combination. These results were analyzed using Statgraphics and the obtained mathematical model is shown in eq 6 below.

$$Y(\text{cal}) = 11881.1 - 54.5798*X_1 - 4060.21*X_2 - 23.3896*X_3 + 2.82724*X_1^2 - 43.0*X_1X_2 + 2.125*X_1X_3 + 601.433*X_2^2 - 26.5*X_2X_3 + 2.01356*X_3^2 \quad (6)$$

Based on the ANOVA results, factors that had a significant influence ($p < 0.05$) on the phytase activity were temperature (X_1 , $p = 0.0000$), pH (X_2 , $p = 0.0000$), incubation time (X_3 , $p = 0.0079$), the quadratic terms of temperature (X_{12} , $p = 0.0000$) and pH (X_{22} , $p = 0.0000$), and the interaction between temperature (X_1) and pH (X_2), $p = 0.0000$.

Using the model shown above (eq 6) the optimal conditions for phytate degradation were at a temperature of 46 °C, a pH of 5.2 and incubation time of 8.36 h (Table 3.3.3). The result indicated that under these optimal conditions, 100% degradation of phytic acid could be achieved. Fredlund et al. (1997) also reported that hydrothermal treatment of wheat, rye, hulled barley and dehulled barley at a temperature of 55 °C and pH 4.8 incubated for 24 h resulted in the degradation of 91, 92, 89 and 99% phytate, respectively.

3.3.4.3. Phytic acid degradation under optimum conditions of temperature and pH

Although three parameters: temperature, pH and incubation time were optimized, only the optimized values of temperature and pH were used. This is because the optimized incubation time was too long and the possibility for fermentation to commence was very high. In order to follow the phytic acid degradation at different times in the absence of fermentation, raw and popped amaranth flour with or without addition of 10% amaranth malt was incubated at

optimized temperature and pH for 1, 2, $3\frac{1}{2}$, and 5 h. The amount of phytic acid remaining and percent of degradation products after the treatment is shown in Table 3.3.4.

Table 3.3.3 Optimum conditions and predicted value of responses at optimum conditions.

Conditions	Coded levels	Actual levels
pH	0.4	5.2
Temperature (°C)	-0.5	46
Incubation time (h)	1.68	8.36
Phytic acid (g/100g DM)	-	0

3.3.4.4. Effect of flour type, malt addition and soaking on phytic acid degradation

The amount of phytic acid in raw amaranth was lower by 54% than that found in popped amaranth. This is because the endogenous phytase is highly active in raw amaranth to degrade the phytate after the treatments than that found in popped amaranth which could be deactivated during popping. Addition of germinated flour (10%) to raw amaranth flour had a significant positive effect on phytic acid content and the percentage of higher inositol phosphates (Table 3.3.4). Phytic acid was found to decrease by 14% and the proportion of IP5, IP4 and IP3 over the total phosphate was increased by 40%.

The phytase activity of germinated flour was more than 2 fold higher than that of raw amaranth (Table 3.3.2). Therefore, increasing the amount of malt used might further contribute to a better phytic acid degradation during incubation. On the other hand, addition of amylase rich flour has an advantage in improving mineral absorption, even if the effect on phytate degradation is low (Hurrell et al., 2002). This is due to the fact that amylase liquefies the porridge. *Myo*-inositol pentaphosphate (IP5), *myo*-inositol tetraphosphate (IP4) and *myo*-inositol triphosphate (IP3) are also contributors to inhibit mineral absorption and therefore degradation of these inositol phosphates will have paramount contribution to enhance mineral absorption (Sandberg et al., 1989; Sandberg et al., 1999).

Table 3.3.4 Effect of flour type, malt addition (10%), and incubation time on phytic acid degradation in amaranth grain[†]

Variables		IP6 (g/100g DM)	P-value	IP5,4,3 vs Total (%)	P-value
Type of flour*	Raw	0.69 ± 0.60	0.0000	12.41 ± 6.62	0.1128
	Popped	1.50 ± 0.19		14.20 ± 5.76	
Addition of malt*	With	1.02 ± 0.55	0.0362	9.98 ± 6.16	0.0000
	Without	1.18 ± 0.64		16.63 ± 4.25	
Incubation Time[¶]	0	1.59 ± 0.22a	0.0000	14.51 ± 6.22a	0.0412
	1	1.26 ± 0.38b		14.84 ± 4.35a	
	2	1.01 ± 0.56c		15.09 ± 7.50a	
	3.5	0.87 ± 0.66d		12.18 ± 6.23ab	
	5	0.76 ± 0.69e		10.27 ± 5.85b	

[†]Values are mean ± SD and means followed by different letters down the column are significantly different at $p < 0.05$.

Incubating amaranth flour, irrespective of the type of flour and use of malt, at optimized pH and temperature for 5 h decreased the phytic acid content only by 52% (Table 3.3.4). Similar result was also reported by Egli et al. (2003) in cereal and legume blends for the production of complementary foods. The authors showed that the use of high phytase sources such as wheat, rye and buckwheat allowed complete degradation of phytic acid in less than 3 h.

The percentage of IP5, IP4 and IP3 versus the total inositol phosphate content showed a statistically non significant increase until an incubation time of 3 h and decreased thereafter. This means that initially part of the IP6 was converted to those fractions but when the incubation time elongates those fractions might also be converted to the lower molecular weight phosphates such as *myo*-inositol diphosphate, *myo*-inositol monophosphate and inorganic phosphate. Generally, after 5 h incubation time, the proportion of IP5, IP4 and IP3 over the total phosphate showed a decrease by 29%. Further degradation of IP6 and lower inositol phosphate could be made possible by elongating the incubation time in order to maximize the nutrient bioavailability.

However, in this experiment, phytate degradation was only partial and remaining phytate content after 5h incubation at optimal phytase conditions was still high enough to complex minerals and reduces their bioavailability.

3.3.4.5. Effect of malt addition on apparent viscosity and energy density

Apparent viscosity of amaranth porridge, at 16% DM, decreased when malt, which is rich in amylase, was used for both raw and popped amaranth (Figure 1). An increase in incubation time did not have any clear effect on the apparent viscosity but adding malt brought a clearly visible reduction in apparent viscosity, 22-33% and 90-100% for raw and popped amaranth respectively, at the same dry matter content (16% DM). Therefore, this allows the porridge to be prepared at higher dry matter content which could consequently increase the energy and nutrient density. The power equation used to predict the dry matter content at the higher recommended viscosity (3 pa.s) (Mouquet and Treche, 2001) showed that the DM content increased by 2-4% and 11-31% in raw and popped amaranth, respectively, after malting for an incubation period of 0-5 h. This resulted in an increase in energy content of the porridge by a maximum of 21% in raw amaranth and 183% in popped amaranth. The very huge increment observed in popped amaranth could be attributed to dextrinization of the starch during popping making it sensitive to the action of amylase when malt was added. Therefore, porridge prepared after addition of malt in both raw and popped amaranth flour allowed the porridge to meet the minimum energy requirement (84 kcal/100g porridge) for an average breast fed 9-11 months old child receiving 2 meals per day (Dewey and Brown, 2003).

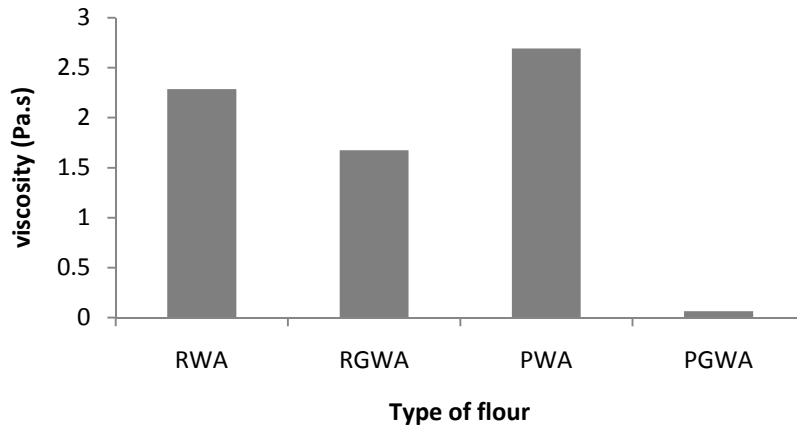


Figure 3.3.2 Effect of malt addition on apparent viscosity of amaranth porridge at 16% DM. RWA-raw white amaranth, RWGA-raw white amaranth with malt, PWA-popped white amaranth flour, PGWA-popped white amaranth with malt

3.3.5. Conclusion

The purpose of this research was to study the way in which combined treatment of malt addition and soaking influences the phytate content of amaranth flour and to establish the optimal conditions for this treatment in order to obtain amaranth flour with reduced phytate content together with partially hydrolysed starch. By applying a combined treatment of malting and soaking under the controlled conditions of temperature and pH, 100 % of the phytate present in amaranth was hydrolyzed at longer incubation time (8.36 h). This was possible because the conditions under which the treatment was performed were the optimal conditions under which the phytase acts upon the phytic acid. The experimental results indicated that optimal temperature and pH for phytic acid degradation in amaranth were found to be 46 °C and 5.2, respectively. The phytic acid content in raw amaranth was found to be lower than that found in popped amaranth due to the higher endogenous phytase activity in raw amaranth that could act on the phytic acid than popped amaranth whose endogenous phytase could be deactivated during popping. The addition of 10% of malted amaranth flour was found to enhance phytic acid degradation as germinated flour has a slightly greater phytase activity than raw flour. This was evidenced in the subsequent study after the hydrothermal treatment for optimization of the best conditions for phytate degradation with 14% further reduction in phytic acid during malt addition. Multivariate statistical analysis results also showed that incubation time (5h) degrades phytic acid by 52%. Moreover,

addition of malt favors the hydrolysis of starch and found to lower the apparent viscosity which consequently improved the energy and nutrient density of porridge and the effect was much more pronounced when used in popped flour than in raw flour.

3.4. Effect of processing on free and total amino acid profile, protein digestibility, protein extraction efficiency and fractionation of *Amaranthus caudatus* grain cultivated in Ethiopia

3.4.1. Introduction

The genus *Amaranthus* belongs to the family Amaranthaceae. It has a huge biodiversity and many of them are cultivated as leafy vegetables, grains, and ornamental plants while others are weeds (Rastogi and Shukla, 2013). Among all the species, *Amaranthus caudatus*, *Amaranthus hypochondriacus* and *Amaranthus cruentus* are mainly cultivated for their seeds (Bressani, 2003; Kaur et al., 2010).

Amaranth was neglected from the food table for many decades after the arrival of the Spanish conquistadors in Latin America as it was used in ceremonial dishes associated with human sacrifice by the Aztecs (Rastogi and Shukla, 2013). It is known for its high tolerance to arid conditions and poor soils, its resistance to drought, heat, and pests, and its ability to adapt to environments where conventional cereal crops do not grow well. Amaranth can thus contribute to food security, especially in resource poor setting (Barba de la Rosa et al., 1992; Rastogi and Shukla, 2013). Despite the very wide distribution of amaranth in Ethiopia, it is cultivated as intercropped with sorghum and maize mainly by Me'etit people who live in the Southern Nations, Nationalities and Peoples region (SNNPR). The most frequent mode of consumption of amaranth grains by these people is after popping and milling, and by mixing the flour with other cereal flours such as teff, sorghum, barley and wheat to prepare bread, injera and porridge.

Concerning the nutritional quality, protein content of amaranth grains is higher than that of most common cereals (Pedersen, 1987; Gamel et al., 2004). In addition, amaranth grains contain useful amino acid profile (Gamel et al., 2004; Pisarikova et al., 2005a, 2005b). Despite the high protein content and interesting amino acid profile of amaranth grains, the overall protein quality is dependent on the digestibility. In this regard, popping, the most commonly used processing method for amaranth, and fermentation, which is widely used in

Africa to prepare cereal based foods like injera, could influence protein digestibility (Hassan and El Tinay, 1995; El-Adawy et al., 2002; El Hag et al., 2002 and Pranoto et al., 2013). This is due to the fact that these techniques are associated with the decrease in the content of exogenous factors such as tannin, phytate and trypsin inhibitors which could lower the protein digestibility. Moreover, processing could also change the proportion of protein fractions (Yousif and El Tinay 2000; Fageer and El-Tinay 2004) which consequently influence the nutritional, structural and functional properties of seed storage proteins.

Other processing techniques such as boiling, microwave cooking, and autoclaving also affect protein digestibility. According to El-Adawy et al. (2002), boiling, microwave cooking and autoclaving improved the digestibility of chickpea protein. On the other hand, Nunes et al. (2004) reported that cooking decreased the digestibility of sorghum and maize protein. Similar controversies surround the effect of popping on the digestibility of amaranth protein. According to a study conducted by Gamel et al. (2004), popping improves the IVPD whereas Pedersen et al. (1987) and Písaříková et al. (2005b) reported a decrease in IVPD during popping of amaranth. On the other hand, fermentation has been found to improve in vitro protein digestibility of cereals due to the degradation of protein binding molecules (Hassan and El Tinay, 1995; El Hag et al., 2002 and Pranoto et al., 2013).

In developing countries like Ethiopia, complementary foods for young children are mainly prepared from mixes of cereals and legumes. It is known that among essential amino acids, legumes are rich sources of lysine but lack methionine and cysteine, while cereals are rich in these two amino acids but are poor sources of lysine. Mixing legumes and cereals during complementary food formulation is a common strategy to meet essential amino acid requirements. However, a too high proportion of legumes sometimes compromises the acceptability of the product and, in addition, legumes contain high levels of antinutritional factors like trypsin inhibitors and hemagglutinins. It is thus advisable to reduce the proportion of legumes in complementary food formulation.

The aim of the present study was, therefore, to evaluate the protein quality of *Amaranthus caudatus* grain cultivated in Ethiopia in the raw state and after popping or fermentation by

analyzing the contents of free and total amino acids and in vitro protein digestibility of amaranth grain porridge to assess its potential application as an ingredient for complementary food formulation. Moreover, the effect of processing on the extraction of protein and protein fractions was also evaluated.

3.4.2. Materials and methods

3.4.2.1. Materials

Three different types of *Amaranthus caudatus* grains, white, red and brown in color, were purchased from six farmers living in Chat Kebelle, Bench Majji Zone, Southern Nations, Nationalities and Peoples region, Ethiopia in October 2011. The grains of the six origins were sorted to remove immature seeds, cleaned and washed to remove sand and soil. The washed seeds were sun dried and equal amount of the six samples were mixed to prepare a composite sample for each amaranth types.

3.4.2.2. Solvents, chemicals and reagents

Citrate buffer pH 2.2 and ninhydrin reagent were purchased from Biochrom (France). Sodium hydroxide (BioXtra, $\geq 98\%$ acidimetric), pellets (anhydrous), L-Norleucine (N8513 suitable for amino acid analysis), aminobutyric acid (A2129; $\geq 99\%$), methanesulfonic acid (M4141; 4 M with 0.2% (w/v) tryptamine) were purchased from Sigma-Aldrich (Saint-Louis, Missouri, USA). Amino acid standards (AA-S18; analytical standard), hydrochloric acid 0.1N were purchased from Fluka (Fluka Chemicals, Buchs, Switzerland). All reagents and chemicals used were of analytical grade.

3.4.2.3. Sample preparation

i. Preparation of raw amaranth flour

The composite samples for each type of amaranth grain were milled, sieved using 0.425 mm sieve and stored at 4 °C until further analysis.

ii. Popping

Cleaned and sun dried amaranth grains were popped as described in Amare et al. (2015). The popped grains were milled to pass a 0.425 mm sieve and stored in polyethylene bags at 4 °C until further analysis was done.

iii. Fermentation

Natural fermentation was carried out according to the method described by Ibrahim et al. (2005) with modifications. Briefly, 250 g of flour prepared from raw amaranth grains was mixed with 500 ml distilled water and then left to ferment spontaneously for 48 h at room temperature (22±2 °C). The sample was mixed, transferred into aluminum cups and dried in a hot oven (Heraeus UT 5042, Germany) at 50 °C for 20 h. The dried sample was then ground to pass through a 0.425 mm sieve and stored in polyethylene bags at 4 °C.

3.4.2.4. Preparation of porridge

Amaranth porridge was prepared using the method described in Mouquet and Treche (2001).

3.4.2.5. Protein and dry matter measurement

The nitrogen content of all samples was determined using Kjeldhal method and a conversion factor of 5.85 was used. The dry matter content was determined by oven drying at 105 °C to a constant weight.

3.4.2.6. Free and total amino acid analysis

a) Extraction procedure

Free amino acids

Free amino acids were analyzed following the method used by Moore et al. (1958) with modifications. Briefly, 150 mg of sample was weighed and placed in a sealable test tube. To this, 50 μ l of internal standard Norleucine (25 μ M) and 4.95 ml of citrate buffer (pH 2.2) were added. The solution was mixed for 1 h on a rotational shaker. All extractions were performed in triplicate.

Total amino acids

Samples (10-20 mg) of dried flour were weighed in a Schlenk tube and 50 μ l of 25 μ M Norleucine and 450 μ L of 4 M methanesulfonic acid were added. The tube was flushed with nitrogen, closed and heated at 150 °C for 2 h. After cooling, 450 μ L of 4 M NaOH was added to the hydrolysate, which was diluted to 5 ml with a loading buffer (citrate buffer at pH 2.2). All extractions were performed in triplicate.

b) *Amino acid analysis*

Sample extracts of free and total amino acid analysis were filtered using a 0.45 μ m membrane filter and injected into the amino acid analyzer (Biochrom30⁺, Biochrom, France), using a lithium cation exchange resin column and ninhydrin as detection compound. All total and free amino acid contents were determined except total tryptophan, which is the most fragile amino acid and is destroyed by the extraction procedure. Amino acid standards were also run in a similar way as the samples.

3.4.2.7. Protein extraction

Protein extraction was done based on the method described in Ju et al. (2001) with modification. Amaranth grain flour was defatted with diethyl ether with flour to solvent proportion (1:10 w/v). The defatted amaranth flour was dried overnight at ambient temperature. Two gram flour was then extracted by shaking with 10 mL distilled water at room temperature (22 ± 2 °C) for 4 h (albumin extract) and centrifuged at 3000g for 30 min. After water extraction, the flour was extracted with 10 mL of 5% NaCl at room temperature (22 ± 2 °C) for 4 h (globulin extract) and centrifuged at 3000 g for 30 min. The flour was then extracted for glutelin fraction with 10 mL of 0.02 M NaOH, at room temperature (22 ± 2 °C) for 30 min followed by prolamin extraction with 7.5 mL of 70% ethanol at room temperature (22 ± 2 °C) for 4 h. Each extraction was repeated two times in order to remove all the protein of each fraction. The nitrogen content in each fraction was determined by Kjeldhal method and the result was multiplied by 5.85 to convert into protein (Berghofer & Schoenlechner 2002).

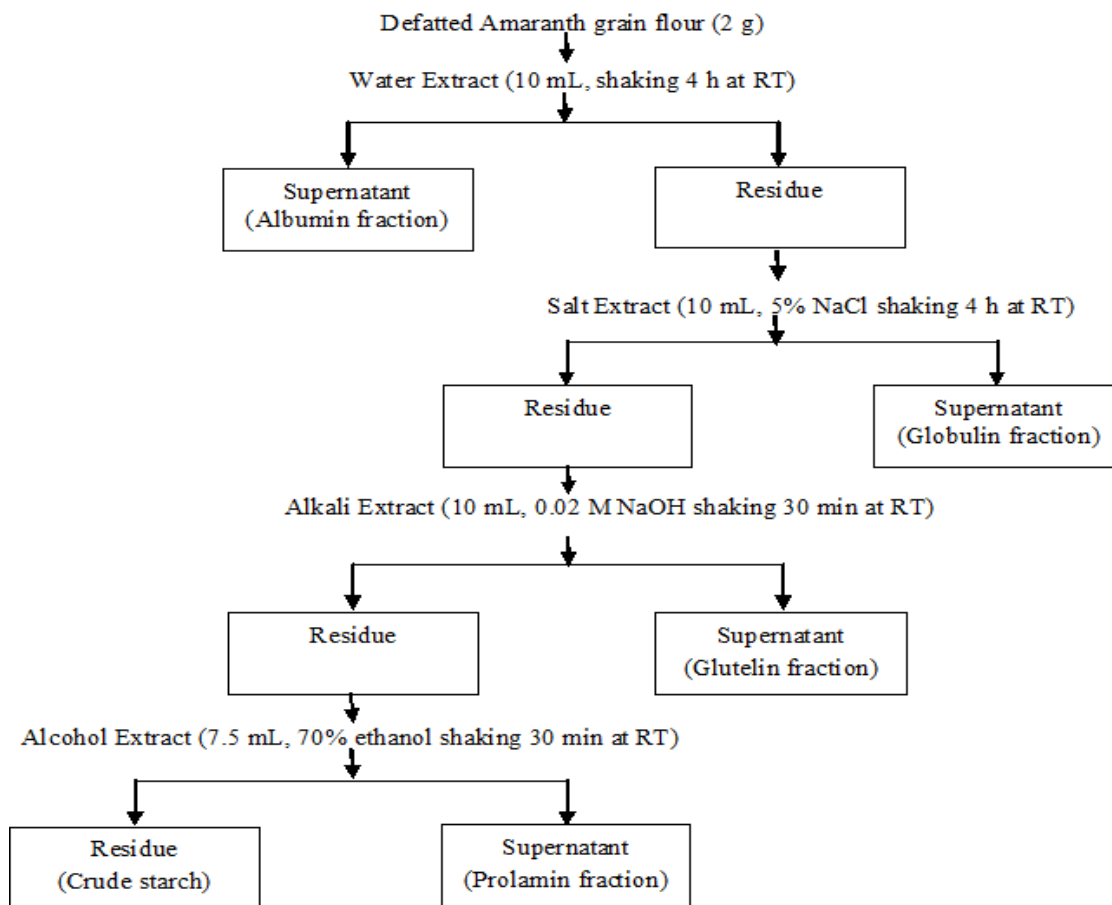


Figure 3.4.1 Schematic diagram for sequential extraction of amaranth protein fractions

3.4.2.8. Determination of *in vitro* protein digestibility

In vitro protein digestibility was determined according to the method of Akeson and Stahmanna (1964) with modifications. To 10 g of amaranth porridge (with approximately 10% assumed DM content), 15 ml of 0.16 M HCl containing 1.5 mg pepsin (Sigma, P-7000, 14,900 u/mL) was added and incubated at 37 °C for 2 h in a shaking water bath. The resulting suspension was neutralized with 7.5 ml of 0.32 M NaOH and treated with 4 mg of pancreatin (Sigma, P-7545, 8*USP specifications) in 7.5 ml of 0.32 M phosphate buffer (pH 8.0). The mixture was incubated for an additional 2 h at 37 °C. Enzyme blank was prepared by incubation under the same conditions except that the sample was omitted. After incubation, the sample was treated with 10 ml of 10% trichloroacetic acid (Sigma) to remove undigested

protein and larger peptides and centrifuged at 5000 g for 20 min at room temperature (22±2 °C). The solution was filtered using Whatman No. 1 filter paper, and the protein in the supernatant was then determined using Kjeldahl method.

3.4.2.9. Amino acid score and protein digestibility corrected amino acid score

The amino acid score (AAS) is the ratio of the amino acid content in the protein of a food/diet to the content of the same amino acid in the requirement pattern. The score determines the effectiveness with which absorbed dietary nitrogen can meet the indispensable amino acid requirements at the safe level of protein intake. This is thus a measure of the actual amounts of individual amino acids in a food with respect to the need for this amino acid. And the quality of the protein will finally depend on the indispensable amino acid for which the AAS is the lowest. However, AAS does not account for the digestibility of the protein. Therefore, another scale called the protein digestibility corrected amino acid score (PDCAAS) was adopted by WHO/FAO (2007) to better explain digestibility in relation to the needs of humans and the scoring of foods (Eq. (1)):

$$\text{PDCAAS} = \text{Amino acid score} \times \text{digestibility} \quad (1)$$

3.4.2.10. Calculation of the percentage contribution of amaranth to estimated amino acid requirements

From the amino acid data, intakes of indispensable amino acids were calculated for comparison with the WHO estimated needs (WHO, 2007), assuming breast-fed infants aged 6-23 months consume the recommended daily ration size of 50 g/d dry weight complementary food (Lutter and Dewey, 2003). The amount of cereal used in complementary food formulation was assumed to be 64% (Nguyen, 2013) and, assuming that this amount of cereal is replaced by amaranth, its percent contribution to the daily indispensable amino acid requirements was calculated.

3.4.3. Data Analysis

All measurements were done in triplicate and statistical analyses were performed using the software Statgraphics plus 5.1 (Statpoint, Warrenton, USA). Two-way ANOVA was applied to the experimental data and means were separated using Fischer's least significant difference tests with a probability $P < 0.05$.

3.4.4. Results and Discussion

3.4.4.1. Protein, total and free amino acid content in amaranth grains

Table 3.4.1 indicates total and free amino acid contents of the three raw *Amaranthus caudatus* grains. The amount of almost all essential amino acids in the raw samples was above the new FAO/WHO standard pattern for all age groups (WHO, 2007) implying that there is no limiting amino acid in *Amaranthus caudatus* grain. Tryptophan, one of the indispensable amino acids, was not analyzed in this study but Gamel et al. (2004) reported that their amaranth samples also contained appreciable amounts of tryptophan.

The lysine content of raw amaranth ranged from 65 to 74 mg.g^{-1} , protein which is close to the lysine contents in legumes (70-75 mg.g^{-1} protein) including chickpeas, cowpeas and lentils but twice higher than that found in many other cereals, such as barley, wheat, rice, rye and maize: 22-37 mg.g^{-1} protein. Methionine and cysteine contents ranged from 24 to 29 and 43 to 51 mg.g^{-1} protein, respectively, which were higher than that found in the legumes and cereals mentioned above (Iqbal et al., 2006; Shewry, 2007).

Table 3.4.1 Total and free amino acid profile (mg.g⁻¹ protein) of three types of raw *Amaranthus caudatus* grain[†]

Protein (g/100g DM) Amino acid	White amaranth		Red amaranth		Brown amaranth		Standard pattern[¶]
	13.88 ± 0.16		15.15 ± 0.08		15.53 ± 0.24		
	Total	Free	Total	Free	Total	Free	
Cysteine*	50.7 ± 8.3	0.9 ± 0.0	42.7 ± 3.0	1.3 ± 0.2	45.5 ± 4.3	0.7 ± 0.1	
Histidine*	37.2 ± 3.1	1.4 ± 0.1	33.9 ± 2.5	1.5 ± 0.0	34.8 ± 2.0	1.2 ± 0.1	18.0
Isoleucine*	39.4 ± 3.0	0.4 ± 0.1	37.2 ± 2.6	0.4 ± 0.1	34.9 ± 2.4	0.3 ± 0.1	31.0
Leucine*	67.1 ± 2.9	0.4 ± 0.0	60.8 ± 2.6	0.4 ± 0.0	59.5 ± 0.2	0.2 ± 0.0	63.0
Lysine*	73.9 ± 2.3	1.2 ± 0.0	66.7 ± 7.2	1.1 ± 0.0	65.3 ± 1.6	0.9 ± 0.0	52.0
Methionine*	28.8 ± 0.5	0.4 ± 0.1	23.8 ± 3.4	0.5 ± 0.2	25.8 ± 0.7	0.3 ± 0.1	
Phenylalanine*	46.5 ± 3.6	0.2 ± 0.0	42.6 ± 1.4	0.2 ± 0.0	40.4 ± 3.5	0.1 ± 0.0	
Threonine*	40.3 ± 1.9	0.0 ± 0.0	35.0 ± 3.4	0.0 ± 0.0	36.7 ± 1.1	0.0 ± 0.0	27.0
Tryptophan*	ND	1.0 ± 0.0	ND	1.4 ± 0.1	ND	0.7 ± 0.0	7.4
Valine*	48.3 ± 1.2	0.7 ± 0.1	42.0 ± 4.6	0.7 ± 0.1	41.3 ± 2.7	0.5 ± 0.1	42.0
Alanine	44.0 ± 2.0	1.1 ± 0.0	38.2 ± 5.8	0.5 ± 0.1	39.0 ± 1.5	1.0 ± 0.0	
Arginine	109.8 ± 8.0	1.6 ± 0.0	102.2 ± 10.0	3.6 ± 0.3	100.6 ± 4.0	1.0 ± 0.0	
Aspartic acid	94.4 ± 5.2	1.1 ± 0.0	86.0 ± 9.3	2.2 ± 0.1	89.4 ± 10.0	1.2 ± 0.0	
Glutamic acid	203.5 ± 14.0	2.9 ± 0.0	183.5 ± 15.0	4.6 ± 0.3	181.8 ± 3.0	1.8 ± 0.0	
Glycine	100.1 ± 4.0	1.3 ± 0.0	89.1 ± 6.4	0.4 ± 0.1	100.0 ± 4.0	1.1 ± 0.0	
Proline	135.7 ± 25.6	2.4 ± 0.0	125.0 ± 30.0	3.7 ± 0.2	118.4 ± 17.4	1.5 ± 0.0	
Serine	65.5 ± 4.0	1.3 ± 0.2	58.5 ± 4.8	2.1 ± 0.1	68.9 ± 1.6	0.6 ± 0.0	
Tyrosine	52.9 ± 6.0	0.5 ± 0.0	45.8 ± 2.7	0.7 ± 0.0	49.7 ± 3.4	0.4 ± 0.0	
AAA [§]	99.4 ± 6.3		88.4 ± 2.6		90.1 ± 3.4		46.0
SAA [¥]	79.5 ± 7.9		66.5 ± 5.9		71.3 ± 4.9		26.0
NH ₃	78.3 ± 6.1	3.2 ± 0.0	70.0 ± 14.3	2.0 ± 0.1	76.6 ± 12.2	1.4 ± 0.1	

[†]Values are means of results obtained in triplicate ± SE expressed in mg.g⁻¹ protein and means followed by different letters in the same row are significantly different at p < 0.05

[§]AAA: aromatic amino acids (phenylalanine and tyrosine). [¥] SAA: sulfur amino acids (cysteine and methionine), ND: not determined

*Indispensable amino acids (WHO 2007)

[¶] Standard FAO/WHO reference pattern for indispensable amino acids for children aged 1-2 years (WHO, 2007).

The concentrations of essential amino acid in *Amaranthus caudatus* grain cultivated in Ethiopia (Table 3.4.1) were in good agreement with those reported by Chavez-Jauregui et al. (2000) and slightly higher than those reported by Gamel et al. (2004) in same species of amaranth cultivated in other parts of the world. The percentage of indispensable amino acids, excluding tryptophan, was highest in raw white amaranth (49%) followed by 43% in both raw red and brown amaranth. These values are higher than the reference protein pattern for 1-2 years old children (31%). The amaranth amino acid profile thus provides a good balance of total indispensable amino acids, and some of the limiting amino acids, especially lysine in cereals and methionine and cysteine in legumes, could be complemented by amaranth.

The free amino acid content of the three types of raw amaranth is listed in Table 3.4.1. The main essential free amino acid was histidine followed by lysine and tryptophan with 1.2-1.5, 0.9-1.2 and 0.7-1.4 mg.g⁻¹ protein, respectively. A study by Nimbalkar et al. (2012) on *Amaranthus hypocondriacus* grain reported that the top three essential free amino acids were threonine, phenylalanine, and methionine and that the amount of threonine was twice that of the other free amino acids. However, in the present study, no free threonine was detected in *Amaranthus caudatus* grains, and glutamic acid and proline were the main non-essential free amino acids with values in the range of 1.8 to 4.6 and 1.5 to 3.7 mg.g⁻¹ protein, respectively in all three types of amarantha.

3.4.4.2. Effect of popping and fermentation on total amino acid content

Popping decreased the amount of six amino acids including three indispensable amino acids: lysine, methionine, and cysteine. The decrease was highest for lysine and cysteine and reached 36 and 37% reduction, respectively (Table 3.4.2). In a study conducted on two different species of amaranth (*Amaranthus caudatus* and *Amaranthus cruentus*) by Gamel et al. (2004), the highest loss of amino acid was recorded for tyrosine followed by phenylalanine and methionine with 35, 24 and 18% loss for *Amaranthus caudatus* and 32, 20 and 19% for *Amaranthus cruentus*, respectively. Another study by Tovar et al. (1989) in different species of amaranth reported highest loss for lysine (56%) followed by arginine (29%). While Pisarikova et al. (2005b) reported that the decrease was highest for histidine followed by

lysine and leucine during heat treatment. Despite the improvement in sensory attribute during popping, the associated loss of amino acids decreased the overall quality of amaranth proteins.

Table 3.4.2 Effect of popping and fermentation on total amino acid content of *Amaranthus caudatus* grain (mg.g-1 protein)[†]

Amino acid	p-value	Processing				
		Raw	Popped		Fermented	
		Mean ± SE	Mean ± SE	% variation	Mean ± SE	% variation
Cysteine	0.0023*	46.3 ± 3.0a	29.4 ± 3.0c	-37	38.9 ± 3.0b	-16
Histidine	0.8097	35.3 ± 0.8	35.0 ± 0.8	--	34.6 ± 0.8	--
Isoleucine	0.1014	37.2 ± 1.1	33.7 ± 1.1	--	35.4 ± 1.1	--
Leucine	0.4034	62.5 ± 1.2	60.3 ± 1.2	--	60.8 ± 1.2	--
Lysine	0.0000*	68.6 ± 2.4a	44.0 ± 2.4c	-36	55.0 ± 2.4b	-20
Methionine	0.0062*	26.1 ± 1.1a	23.0 ± 1.1b	-12	20.8 ± 1.1b	-20
Phenylalanine	0.3940	43.2 ± 1.0	41.4 ± 1.0	--	41.4 ± 1.0	--
Threonine	0.0836	37.3 ± 0.7	35.1 ± 0.7	--	35.4 ± 0.7	--
Tyrosine	0.1473	49.5 ± 1.4	48.5 ± 1.4	--	45.7 ± 1.4	--
Valine	0.0832	43.9 ± 1.1	40.2 ± 1.1	--	42.2 ± 1.1	--
Alanine	0.3488	40.4 ± 0.8	38.7 ± 0.8	--	39.8 ± 1.6	--
Arginine	0.0003*	104.2 ± 3.2a	88.6 ± 3.2b	-15	83.2 ± 3.2b	-20
Aspartic acid	0.0191*	89.9 ± 1.6a	84.8 ± 1.6b	-6	83.1 ± 1.6b	-8
Glutamic acid	0.0711	189.6 ± 6.9	183.6 ± 6.9	--	176.3 ± 6.9	--
Glycine	0.6262	96.4 ± 1.9	93.8 ± 1.9	--	95.4 ± 1.9a	--
Proline	0.0584	126.4 ± 5.6	112.9 ± 5.6	--	106.5 ± 5.6	--
Serine	0.0049*	64.3 ± 1.4a	59.1 ± 1.4b	-8	57.2 ± 1.4b	-11
Ammonia	0.1330	75.0 ± 5.7	71.9 ± 5.7	--	80.7 ± 5.7	--

[†]Values are means of results obtained in triplicate ± SE for the three amaranth types; *The effect of processing is significant (p < 0.05) and means followed by different letters in the same row are significantly different at p < 0.05

In the present study, fermentation also led to a significant decrease in the contents of the same six amino acids as that of popping (Table 3.4.2). However, the decrease in essential amino acids, lysine and cysteine, due to fermentation was significantly lower than the decrease due to popping ($p < 0.05$). But methionine was subject to a higher (20%) percent reduction during fermentation although not statistically significant ($p < 0.05$) compared to the result obtained after popping. The reduction could be ascribed to metabolism of amino acids by microorganisms into ammonia and volatile compounds that are responsible for the flavor of the fermented product (Pranoto et al., 2013).

3.4.4.3. Effect of popping and fermentation on free amino acid content

Popping and fermentation led to highly significant changes in free amino acid contents ($p < 0.01$) (Table 3.4.3). Popping led to a significant decrease in almost all free amino acid contents (Table 3.4.3). Free aromatic amino acids were strongly affected, phenylalanine and tyrosine completely vanished and 90% of tryptophan vanished during popping. Non-enzymatic browning reaction is the most probable explanation for the decrease in the level of free amino acids during heat treatment (Ibanoglu et al., 1997).

Conversely, fermentation increased the amount of almost all free amino acids, except tyrosine and glutamic acid, which remained unchanged, and arginine, which strongly decreased (Table 3.4.3) due to its conversion in to non protein amino acid called γ -Hydroxy-arginine (Kuo et al., 2004) and Ornithin in urea cycle (Kuensch et al., 1974). Lysine and phenylalanine increased to a greater extent than the other free amino acids. Increased microbial enzyme activity coupled with protein hydrolysis is the likely explanation for the increase in the amount of free amino acids during fermentation (Pranoto et al., 2013; Sripriya et al., 1997). Hamad and Fields (1979) also showed that there was a significant increase in free lysine content during fermentation in other type of cereals.

Table 3.4.3 Effect of popping and fermentation on free amino acid content of *Amaranthus caudatus* grain (mg.g-1 protein)†

Amino acid	p-value	Processing				
		Raw	Popped		Fermented	
		Mean ± SE	Mean ± SE	% variation	Mean ± SE	% variation
Arginine	P < 0.01	2.0 ± 0.2a	0.8 ± 0.2b	-60	0.3 ± 0.2c	-85
Cysteine		1.0 ± 0.1b	0.5 ± 0.1c	-50	3.7 ± 0.1a	270
Histidine		1.4 ± 0.4b	0.4 ± 0.4c	-71	4.6 ± 0.4a	229
Isoleucine		0.4 ± 0.1b	0.1 ± 0.1c	-75	2.8 ± 0.1a	600
Leucine		0.3 ± 0.2b	0.1 ± 0.2c	-67	6.5 ± 0.2a	2067
Lysine		1.1 ± 0.1b	0.7 ± 0.1c	-36	2.0 ± 0.1a	82
Methionine		0.4 ± 0.1b	0.1 ± 0.1c	-75	2.6 ± 0.1a	550
Phenylalanine		0.2 ± 0.1b	0.0 ± 0.1c	-100	3.5 ± 0.1a	1650
Threonine		0.0 ± 0.0b	0.0 ± 0.0b	--	0.2 ± 0.0a	100
Tryptophan		1.0 ± 0.1b	0.1 ± 0.1c	-90	2.1 ± 0.1a	110
Tyrosine		0.5 ± 0.1a	0.0 ± 0.1b	-100	0.5 ± 0.1a	--
Valine		0.6 ± 0.1b	0.3 ± 0.1c	-50	4.0 ± 0.1a	344
Alanine		0.9 ± 0.2b	0.5 ± 0.2c	-44	5.2 ± 0.2a	478
Aspartic acid		1.5 ± 0.1b	0.5 ± 0.1c	-67	2.3 ± 0.1a	53
Glutamic acid		3.1 ± 0.4a	0.6 ± 0.4b	-81	3.2 ± 0.4a	--
Glycine		0.9 ± 0.2b	0.8 ± 0.2c	-11	3.6 ± 0.2a	300
Ornithine		0.2 ± 0.2b	0.1 ± 0.2c	-50	2.1 ± 0.2a	950
Proline		2.5 ± 0.3a	0.4 ± 0.3c	-84	2.4 ± 0.3b	4
Serine		1.3 ± 0.1b	0.3 ± 0.1c	-77	1.7 ± 0.1a	31
Ammonia	2.2 ± 0.4b	3.2 ± 0.4c	+46	14.1 ± 0.4a	541	

†Values are means of results obtained in triplicate ± SE expressed for the three amaranth types. Means followed by different letters in the same row are significantly different at p < 0.01

3.4.4.4. Effect of popping and fermentation on protein extraction efficiency

The protein extraction efficiencies for raw white, red and brown amaranth were 70, 71 and 67% (table 3.4.4) and the values were close to each other. Variation in extraction efficiency could arise from variation in granular size of the flour, the type of solvent used and temperature during extraction (Gornistein et al., 2002). The use of multiple solvents to extract each amaranth protein fractions was reported to improve the extraction efficiency (Segura-Nieto et al., 1992).

Popping significantly decreased the extraction efficiency in all the three types of amaranth ($p < 0.05$). The percentage decrease in white, red and brown amaranth was 28, 34 and 36%, respectively. The decrease in extraction efficiency could be attributed to the strong starch-protein complexation that was intensified during popping due to denaturation of protein and starch gelatinization allowing better chances for interaction between protein and starch. The effect of heat treatment in lowering the protein extraction efficiency in peanut was also reported by Pomes et al. (2004). On the other hand, fermentation improved the extraction efficiency significantly ($p < 0.05$) by about 13-21%, where larger improvement was observed in the white and red amaranth. The improvement observed during fermentation might be due to the hydrolysis of protein binding molecules and loosening of starch-protein interaction due to the action of enzymes.

Table 3.4.4 Effect of processing on protein extraction efficiency of amaranth grain*

Sample	Extraction Efficiency (%)		
	Raw	Popped	Fermented
White Amaranth	70.0 ± 0.9 ^b	50.2 ± 2.2 ^c	84.7 ± 0.4 ^a
Red Amaranth	70.7 ± 0.4 ^b	46.6 ± 0.2 ^c	83.7 ± 0.1 ^a
Brown Amaranth	66.8 ± 0.6 ^b	42.6 ± 0.3 ^c	75.2 ± 0.5 ^a
Mean	69.14 ± 0.2 ^b	46.47 ± 0.3 ^c	81.16 ± 0.2 ^a

*values are mean of triplicate measurements ± SD and values with different letters across the row are significantly different at $p < 0.05$

3.4.4.5. Effect of popping and fermentation on amaranth protein fractions

Seed proteins have four major fractions according to the Osborne classification (Shewry, 2002). These are water soluble, albumin; salt soluble, globulin; alkali soluble, glutelin and alcohol soluble, prolamin. Each fraction has its own peculiar functional property depending on the type and amount of amino acid present (Ma and Harwalkar, 1984). For example, albumins extracted from two Mexican *Amaranthus caudatus* varieties showed excellent foaming capacity and foaming stability at pH 5, suggesting that amaranth albumin could be used as whipping agents (Silva-Sanchez et al., 2004). The authors also showed that maximum water and oil absorption capacities were reached at acidic pH, suggesting that amaranth albumins could be appropriate in preparation of acidic foods. Moreover, supplementation of 1% amaranth albumin into wheat flour was reported to improve the dough properties due to higher mixing stability and the bread had better crumb characteristics.

Figure 3.4.2 shows variations in the protein fractions of raw, popped and fermented amaranth. In the raw white colored amaranth the percent distribution followed the decreasing order from albumin, glutelin, globulin and prolamin with corresponding values of 40.0, 29.8, 21.7 and 8.5%, respectively. The red amaranth also followed the same trend in its protein distribution albumin (39.3%), glutelin (30.6%), globulin (21.4%) and prolamin (8.8%). While the brown amaranth had a different sequence: Albumin (36.8%), globulin (34.1%), glutelin (24.8%) and prolamin (4.3%).

Nevertheless, in all the three types of *Amaranthus caudatus* grain, albumin + globulin was the major and prolamin was the minor fraction with values of 62-71% and 4-9%, respectively. This is in contrast with that of other cereals such as teff and sorghum having high content of prolamin (~40%) and low content albumin + globulin, 11 % in teff and 6% in sorghum (Adebowale et al., 2011). Similar results were also reported elsewhere (Segura-Nieto et al., 1992, Barba de la Rosa et al., 1992) in *Amaranthus hypochondriacus*. On contrary, in the study by Bressani and Garcia-Vela (1990) on three different species of amaranth: *Amaranthus caudatus*, *Amaranthus hypochondriacus* and *Amaranthus cruentus*, glutelin was found to be the major fraction. Such variation in results of protein fractions was also reported by

Adebowale et al. (2011) in teff grains prolamin content. In their report they showed that prolamin fraction in teff exhibits 25-37% increment than previous report by Endeshaw (1995). The possible explanation given was the difference in the method of extraction.

Popping significantly decreased the albumin and glutelin fractions and increased the globulin and prolamin fractions in both white and red amaranth at $p < 0.05$ (Figure 3.4.2 A and B). In the brown amaranth, the effect seemed to slightly differ in that albumin and prolamin increased and, globulin and glutelin fractions decreased significantly ($p < 0.05$) (Figure.3.4.2 C). Similar result was also reported by Gamel et al. (2005). Because of the fact that each fraction has a different amino acid composition (Bressani and Garcia-Vela, 1990; Segura-Nieto et al., 1992, Gorinstein, 2002), the fraction that contains highest proportion of heat labile amino acids decreased significantly and contributed to a proportional increment in the fraction that contains thermally less sensitive amino acids. Moreover, the fraction that contains amino acid involved in complexation reaction with gelatinized starch during popping caused a decrease in that fraction.

Fermentation increased the amount of albumin at the expense of decreasing the amount of glutelin and globulin (Figure 3.4.2 A, B and C). The prolamin fraction significantly decreased ($p < 0.05$) in white amaranth and a statistically non significant increase was observed in red and brown amaranth due to fermentation. Hydrolysis of tannin during fermentation might contribute to an increase in water soluble protein fractions. Studies conducted in sorghum also showed that fermentation generally increased the amount of albumin, globulin and glutelin and decreased the content of prolamin (El Khalifa and El Tinay, 1994) indicating that fermentation plays a significant role in modifying the protein fractions which consequently affect the functional property of the protein.

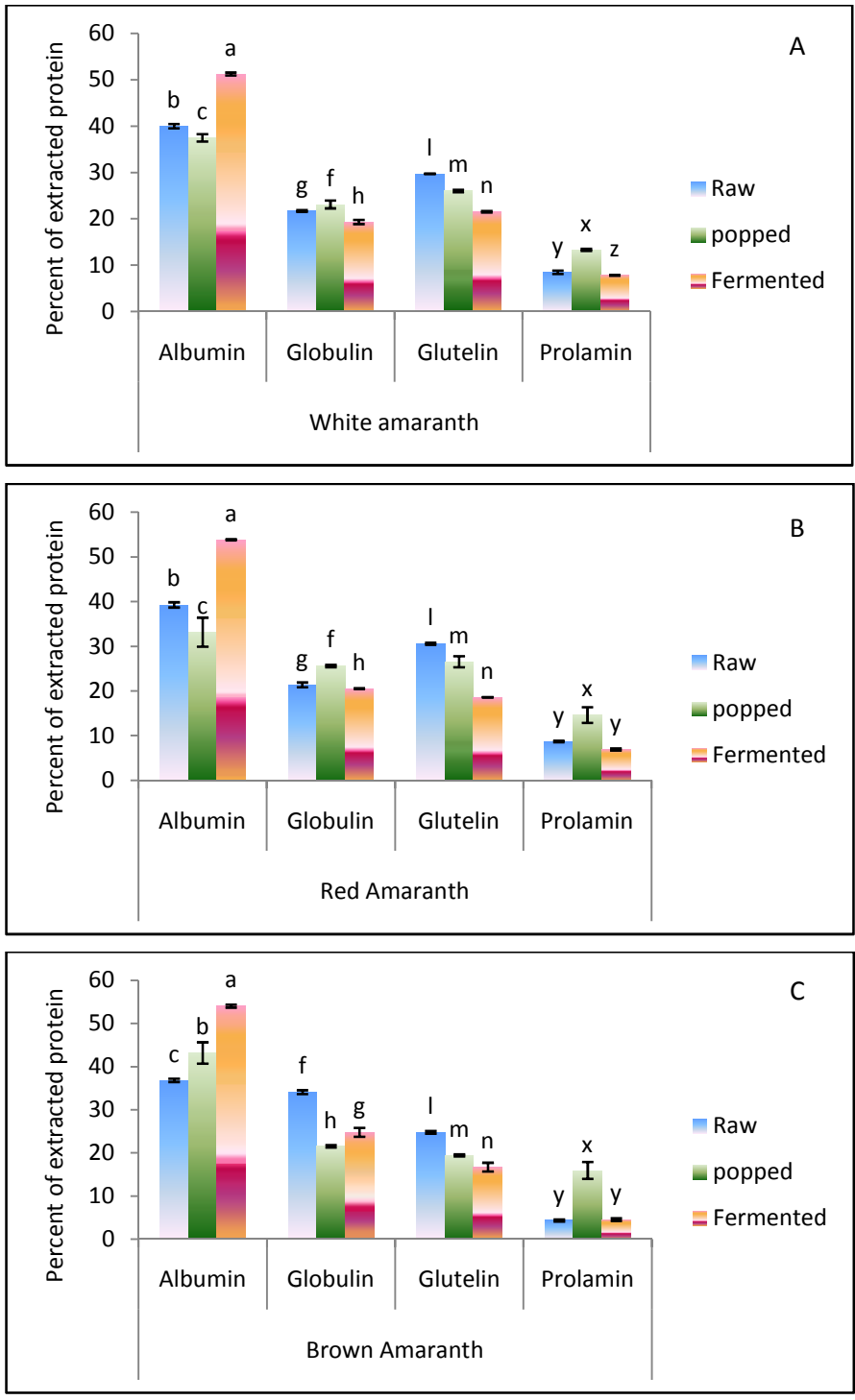


Figure 3.4.2 Effect of processing on protein fractions expressed as percentages of total extracted protein. Values are mean \pm SD of triplicate measurement and values with different letters are significantly different at $p < 0.05$.

3.4.4.6. Effect of popping and fermentation on in vitro protein digestibility (IVPD) and protein digestibility corrected amino acid score (PDCAAS)

In vitro protein digestibility (IVPD) is shown in Figure 3.4.3. When the digestibility of porridge made from raw amaranth samples was compared, it was highest for white (82.4%) followed by red (77.6%) and brown (71.2%) amaranth. The lower IVPD in the colored amaranth might be related to the presence of higher amount of dietary fiber and polyphenols (Chapter 3.1 and 3.6), which could bind the protein and reduce its susceptibility to enzymatic attack (Pedersen, 1987).

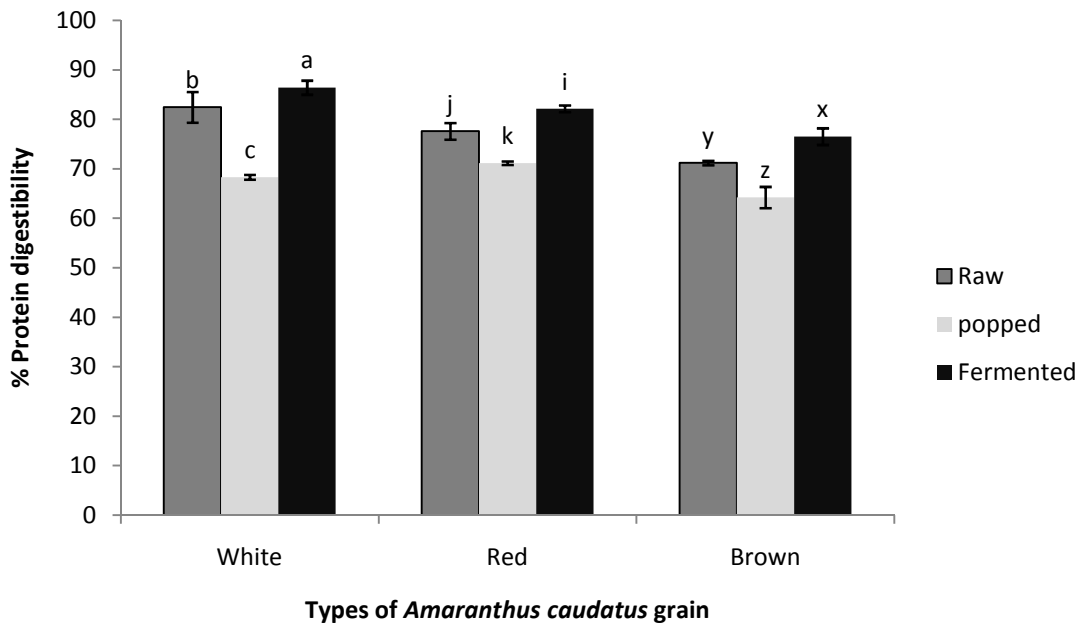


Figure 3.4.3 Effect of processing on in vitro protein digestibility (%) in amaranth. Means with different letters are significantly different at $p < 0.05$.

Both popping and fermentation significantly affected, ($P < 0.05$), the IVPD (Figure 3.4.3). Popping decreased protein digestibility by 17.14, 8.3 and 9.8% for white, red and brown colored amaranth, respectively. This is because amino acids undergo several chemical reactions during severe heat treatment, including Maillard reaction between amino acids and reducing sugars, which reduces the availability of amino acids (Ibanoglu et al., 1997). Therefore, during dry heat processing (popping) where the temperature often exceeds 150 °C, this reaction is likely to contribute to the decrease in protein digestibility. Unlike popping,

fermentation improved IVPD by 4.8, 5.9 and 7.5% for white, red and brown colored amaranth, respectively. This could be due to degradation of phytic acid (data not shown), which is a potent inhibitor of proteolytic enzymes. Besides, hydrolysis of tannins, which potentially complex proteins, may also increase the accessibility of protein molecules for enzymatic attack and hence increase IVPD (Duodu et al., 2003). In addition, hydrolysis of proteins by microorganisms during fermentation, evidenced by the increase in free amino acids, could increase IVPD. Studies by El Hag et al. (2002), Pranoto et al. (2013) and Hassan and El Tinay (1995) on finger millet and sorghum, indeed showed that tannin hydrolysis occurs under the action of microorganisms during fermentation.

Table 3.4.5 Percent protein digestibility corrected amino acid score (PDCAAS) for three types of raw and processed amaranth grains*

Amino acid	White Amaranth			Red Amaranth			Brown Amaranth		
	Raw	Popped	Fermented	Raw	Popped	Fermented	Raw	Popped	Fermented
AAA [§]	178	135	174	149	138	159	139	125	133
Histidine	170	128	170	146	143	162	138	126	139
Isoleucine	105	81	107	93	70	94	80	70	79
Leucine	88	68	89	75	66	79	67	61	69
Lysine	117	68	110	100	66	104	89	40	49
SAA [¥]	252	157	248	198	134	224	195	119	99
Threonine	123	94	122	101	88	110	97	83	90
Valine	95	74	95	78	60	82	70	60	71

*The scoring pattern for indispensable amino acids was considered for children aged 1-2 years (WHO, 2007).

[¥] SAA: sulfur amino acids (cysteine and methionine)

[§] AAA: aromatic amino acids (phenylalanine and tyrosine)

For all the indispensable amino acids, the PDCAAS, which takes both the amino acid score and protein digestibility into account, was highest for the white followed by the red and brown amaranth (Table 3.4.5). In all the three types of amaranth, the popped samples had the lowest PDCAAS values due to the loss of heat labile amino acids during popping. Although there was a significant improvement ($P < 0.05$) in protein digestibility during fermentation (Figure 3.4.3), the PDCAAS of almost all essential amino acids showed only a slight increase or remained unchanged after fermentation except lysine in the brown amaranth, which decreased considerably. In white and red amaranth, leucine in raw samples, leucine and lysine in popped samples and leucine in fermented samples were decreased, while in the brown

amaranth, leucine in raw samples and lysine in popped and fermented samples were decreased.

3.4.4.7. Percentage contribution of amaranth as an ingredient in complementary foods to the daily amino acid requirement of 6-23 month old children

Table 3.4.6 shows the percent contribution of amaranth to the daily indispensable amino acid requirements of 6-23 month old children. All the three types of amaranth contributed more than 50% of the daily indispensable amino acid requirements based on the consumption of 50 g of complementary foods per day of which amaranth accounts for 32 g. Compared to the cereals commonly used in complementary food formulation such as wheat and maize, the contribution of amaranth is appreciably higher for all indispensable amino acids except leucine (Table 3.4.6). Moreover, the daily needs of sulfur-containing amino acids were fully met by consuming the suggested amount of amaranth. However, if only the digestible fraction of protein is taken into consideration, the contribution to amino acid requirements would decrease by about 18-29%.

Table 3.4.6 Percentage contribution of raw amaranth to the daily indispensable amino acid requirements for children aged 6-23 months, compared to that of more commonly used cereals (maize or wheat)*

Amino acid	Amount of amino acid (mg/100 g DM)					Requirement [§]	Percentage contribution (%) [¥]				
	Wheat [†]	Maize [†]	Amaranth				Wheat	Maize	Amaranth		
			White	Red	Brown				White	Red	Brown
AAA	764	795	1379	1340	1400	458-476	51-53	53-56	92-96	90-93	94-98
His	274	244	516	514	540	171-178	49-51	44-46	92-96	92-97	98-102
Ileu	441	325	547	563	542	279-321	44-51	32-37	54-62	56-64	54-62
Leu	811	1129	932	921	925	566-642	40-46	56-64	46-53	46-52	46-53
Lys	334	244	1026	1011	1014	497-535	20-22	15-16	61-66	61-65	60-65
SAA	418	316	1103	1008	1108	241-262	51-56	39-42	136-148	124-135	135-147
Thr	346	334	559	531	570	264-273	40-42	39-41	65-67	62-64	67-70
Val	523	433	671	636	641	380-428	39-44	32-36	50-56	48-54	48-54

*Assuming daily consumption of 50 g of complementary food on a dry weight basis formulated with 64% of cereals or amaranth

[†]Shewry et al. (2007)

[§]The lower and upper values refer to the requirements for 6 and 23 month-old children, respectively (WHO, 2007)

[¥]The lower and upper values refer to the contribution of unprocessed cereals to the daily amino acid needs for 23 and 6 month old children, respectively

3.4.5. Conclusion

All three types of *Amaranthus caudatus* grains investigated in this study can be considered as a potential source of protein. Due to its high essential amino acid contents, amaranth could potentially substitute other more common cereals used in complementary food formulation for young children and thus reduce the proportion of legumes of high antinutritional content. Although popping improved the sensorial attributes of amaranth porridge, it reduced protein quality through the loss of heat labile amino acids and this simultaneously affect the proportion of protein fraction causing a decrease in the fraction that contains high percentage of heat labile amino acids. On the other hand, fermentation was better than popping in maintaining the amino acid profile and improving the percentage of aqueous soluble fraction with its added advantage of improving the protein digestibility although it resulted in a marked decrease in lysine content.

3.5. In vitro starch digestibility and functional property of *Amaranthus caudatus* grain as affected by popping and fermentation.

3.5.1. Introduction

Starch is the commonest storage carbohydrate in plants and also the largest source of carbohydrates in human food. It consists of two types of molecules: amylose (linear polymer of α -D-glucose units linked by α -1,4 glycosidic linkages) and amylopectin (branched polymer of α -D-glucose units linked by α -1,4 and α -1,6 glycosidic linkages) (Singh et al., 2010). Starch is also the main component in amaranth grain and plays an important role in its food applications, such as for food thickeners, for soups, fat replacers, and in breakfast cereals, muffins, cookies, snacks, pastas, health foods and etc (Kong et al., 2009).

The degree of starch hydrolysis determines the postprandial blood glucose level and highly processed, low fiber and energy-dense carbohydrate food products can lead to over consumption and obesity related diseases (Englyst & Englyst, 2005). According to Englyst and Cummings (1985) and Englyst et al. (1992), starch can be classified into rapidly digestible (RDS), slowly digestible (SDS) and resistant starch (RS). The method of classification is based on the in vitro digestion of starch by simulating stomach and intestinal conditions and measuring glucose release at different time interval. Accordingly, RDS and SDS are digested in the small intestine, and RS is the part of the starch fraction that escapes the digestion and enters the large intestine in humans. Besides, according to the physiological classification by Cummings & Stephen (2007) and Englyst et al. (2007), RDS and SDS fractions are classified as available or glycemic carbohydrates, whereas RS is regarded as a 'non-glycemic' carbohydrate and higher RDS content of a food shows that the food has a high glycemic index (Englyst et al., 1999; Englyst et al., 2003).

The duration and extent of starch digestion in any type of food depend on several intrinsic and extrinsic factors (Singh et al., 2010). Starch granule morphology, amylose to amylopectin ratio, molecular structure, degree of branching in terms of steric hindrance, interaction of starch with fiber, antinutrients (eg, phytate) and protein in the food, the physical form of the

starch or the effect of processing (eg, raw or cooked, ground or whole) could significantly influence the starch digestibility (Thorne et al., 1983). X-ray diffraction pattern indicated that amaranth has an “A” pattern of granular structure (Kong et al., 2010) and according to Biliaderis (1989), cereals having this type of pattern are highly digestible. Moreover, amaranth had low amylose content (from 4.7% to 12.5%) (Kong et al., 2009) rendering the starch being more digestible than cereals that contain higher amylose (Aarathi et al., 2003; Sun et al., 2006; Sagum and Arcot, 2000).

Amaranth has many advantages compared to most commonly utilized cereals in terms of its contents of micro and macronutrients. The high quality amino acid profile is also an interesting attribute to complement with other cereals and legumes during complementary food formulation for young children in developing countries (Amare et al., 2015). One additional important attribute required from ingredients used for complementary food formulation is, high rate of starch digestion. This is because children do have a limited gastric capacity and thus they are not able to accommodate bulky foods of low rate of digestion that could stay longer in the stomach. They consume a small amount of food at a higher frequency to meet their daily nutrient requirement. As a result food types that could provide the necessary nutrient in a very short period of time by having fast rate of digestion after ingestion of the food are strongly recommended.

Although amaranth contains high amount of starch, there are few studies about its digestibility. Therefore, this study attempts to evaluate starch digestibility of three types of *Amaranthus caudatus* grain cultivated in Ethiopia which are most often used to formulate complementary food for young children and compared against starch digestibility of corn flakes. The effect of two potential processing methods utilized prior to the consumption of amaranth, popping and fermentation, on starch digestibility, consistency, and energy density of amaranth porridge was evaluated.

3.5.2. Materials and Methods

3.5.2.1. Sample collection

Three different types of *Amaranthus caudatus* grains, white, red and brown in color, were purchased from six farmers living in Chat Kebelle, Bench Majji Zone, Southern Nations, Nationalities and Peoples region, Ethiopia in October 2011. The grains of the six origins were sorted to remove immature seeds, cleaned and washed to remove sand and soil. The washed seeds were sun dried and equal amount of the six samples were mixed to prepare a composite sample for each amaranth types.

3.5.2.2. Solvents, Chemicals and reagents

The enzymes used were pepsin from Sigma (catalog no. P-7000; St Louis), amyloglucosidase from Megazyme (EC 3.2.1.3., 3300 U/ml), pancreatin from Sigma (P-7545, activity 8USP/g), and invertase from Megazyme (EC 3.2.1.26, 300 U/mg). All chemicals and reagents were of analytical grade.

3.5.2.3. Sample preparation

i. Preparation of raw amaranth flour

The composite samples for each type of amaranth grain were milled, sieved using 0.425 mm sieve and stored at 4°C until further analysis.

ii. Popping

Cleaned and sun dried amaranth grains were popped as described in Amare et al. (2015). The popped grains were milled to pass a 0.425 mm sieve and stored in polyethylene bags at 4 °C for further analysis.

iii. Fermentation

Natural fermentation was carried out according to the method described by Ibrahim et al. (2005) with modification. Briefly, 250 g of amaranth flour was mixed with 500 ml distilled water in a 600 ml beaker and then left to ferment for 48 hrs at room temperature ($25 \pm 2^\circ \text{C}$). Thereafter, the sample was mixed with a glass rod and transferred to three aluminum dishes (30 cm diameter each) and dried in a hot oven (Heraeus UT 5042, Germany) at 50°C for 20hrs. The dried sample was then ground to pass a 0.425 mm screen and stored in polyethylene bags at 4°C for further analysis.

3.5.2.4. Preparation of porridge by cooking and with instant procedure

Amaranth porridge was prepared at different concentrations as suggested by Mouquet and Treche (2001). Briefly, the flour was mixed with cold demineralized water into slurry and cooked with continuous stirring over a hot plate for 5 min once the mixture starts to boil. The porridge was allowed to cool to 45°C before viscosity measurements and the dry matter content was determined by oven drying at 105°C to constant weight. The instant character of popped amaranth was also checked according to a method described in Mouquet, Salvignol, Van Hoan, Monvois, & Trèche (2003). Briefly, popped amaranth flour was mixed with hot water at 70°C and the viscosity was measured at the same dry matter content as that of porridge prepared using the cooking procedure described above. If the apparent viscosity of the porridge prepared with the “instant procedure” is equal or slightly higher than the viscosity of the porridge prepared with the “cooking procedure”, then the flour can be considered as “instant”. If it is lower, it implies that part of the flour starch is not totally precooked/pregelatinized during popping and will continue to swell during cooking of the porridge, thus leads to an increase in viscosity.

3.5.2.5. Viscosity Measurement

A viscometer Haake VT550 with SV-DIN coaxial cylinder was used to measure the apparent viscosity of the porridge at a shear rate of 83s^{-1} for 10min as described in Mouquet and Treche

(2001). The measurements were done at 45⁰C, a temperature which porridge is served for infants and children.

3.5.2.6. Calculation of energy density

The energy values were calculated using the Atwater coefficients 16.7 kJ/g (4 kcal/g) for protein, 37.4 kJ/g (9 kcal/g) for fat, 16.7 kJ/g (4 kcal/g) for carbohydrates (Atwater & Benedict, 1902) and for fiber 8.35 kJ/g (2 kcal/g), as recommended by FAO (2003).

3.5.2.7. Total starch

A total starch assay kit (Megazyme International, Ireland) was used to determine the starch content. Samples (100 mg) were wetted with 0.2 mL of aqueous ethanol (80% v/v), and starch was predissolved in 2 mL of 2M KOH at 4 °C. The pH was then adjusted with 8 mL of 1.2 M sodium acetate buffer, pH 3.8, and starch was hydrolysed with, 0.1 mL each, thermostable α -amylase and amyloglucosidase in a water bath at 50 °C. Liberated glucose was quantified using the glucose oxidase-peroxidase assay kit (K-GLUC, Megazyme), and TS was calculated as glucose*0.9.

3.5.2.8. Free sugars

The free sugar content of amaranth porridge was determined in a separate sample after gelatinization of starch and hydrolysis of sucrose with invertase as described in (Englyst, Englyst, Hudson, Cole, & Cummings, 1999) with modification. Briefly, about 10 g of pre-prepared porridge was weighed and mixed with 25 ml of distilled water. The mixture was vigorously vortex mixed and incubated in a boiling water bath for 30 minutes, followed by vigorous mixing and cooling to 37 °C. Two hundred μ l of invertase was added to the mixture and incubated for 30 minutes at 37 °C in a horizontal shaker. Then, 200 μ l of the extract was taken and mixed with 4 ml of 80% ethanol to deactivate the enzyme and glucose determination was followed using the glucose oxidase-peroxidase assay kit (K-GLUC, Megazyme).

3.5.2.9. Preparation of enzyme mixture

Pancreatin (4.5 g) was weighed into a centrifuge tube and a magnetic stirring bar and 30 mL water was added to each. The pancreatin was suspended by vortex mixing and then mixed for 10 min on a magnetic stirrer. The tubes were centrifuged at 1500 g for 10 min; the cloudy supernatant was transferred into a flask and 1 mL amyloglucosidase and 0.8 mL invertase were added and mixed well.

3.5.2.10. In vitro Starch digestibility

In vitro starch digestibility was determined following the method of Englyst et al. (1999). Briefly, about 10 g of pre-prepared porridge (containing < 0.6 g carbohydrate) was weighed into 50-mL polypropylene centrifuge tubes. Five milliliters of distilled water and 10 mL freshly prepared pepsin–guar gum solution (5 g pepsin/L and 5 g guar gum/L in 0.05 mol HCl/L) was added to the sample. The tubes were capped and the contents were vortex mixed and placed into a water bath at 37 °C for 30 min to allow hydrolysis of proteins by pepsin. The guar gum standardizes viscosity, keeps the sample in suspension, and prevents its sedimentation. Five milliliters of 0.5 N sodium acetate buffer (pH=5.2) (equilibrated to 37 °C) was added to each tube followed by addition of 5 ml of enzyme mixture (pancreatine, invertase and amyloglucosidase) prepared as described above. The tubes were immediately capped and the contents were mixed gently by inversion before it was secured horizontally in the 37 °C shaking water bath. The shaking action of the water bath was started at this time, which was taken as time zero for the incubation and was not interrupted until all the G120 portions were collected. The enzyme mixture was added to the rest of the sample tubes at 1-min intervals, to aid timing of incubations, and they were placed into the shaking water bath. Each tube was removed from the bath exactly 20 min after the enzyme mixture was added and 0.2 mL of the contents was added to 4 mL 80% ethanol and vortex mixed to stop the hydrolysis; this was the G20 portion (glucose released from rapidly digestible starch, RDS). The tube was returned to the shaking water bath immediately after the sample was taken. After another 100 min, another 0.2 mL aliquate was taken and added to 4 mL of 80% ethanol

and vortex mixed; this was the G120 portion (glucose released from slowly digestible starch, SDS).

All values were corrected for free glucose, which includes the glucose part of sucrose and represented by equations 1-4 below. The amounts of glucose released from the hydrolysis of starch were measured by the glucose oxidase–peroxidase assay kit (K-GLUC, Megazyme). Each sample was analyzed in triplicate and values were expressed as g starch/100 g DM.

$$TS = (TG - FG) * 0.9 \dots \dots \dots (1)$$

$$RDS = (G_{20} - FG) * 0.9 \dots \dots \dots (2)$$

$$SDS = (G_{120} - G_{20}) * 0.9 \dots \dots \dots (3)$$

$$RS = (TG - G_{120}) * 0.9 \dots \dots \dots (4)$$

The starch digestible rate index (SDRI = RDS expressed as percentage of TS) and rapidly available glucose (RAG = free glucose + glucose from sucrose + glucose released from starch with in 20 minute incubation) were also calculated. In order to express the nutritional value of starch more effectively, potentially bioavailable starch (BAS) was also calculated from the difference between total starch and resistant starch (Periago, Englyst and Hudson, 1996; Bravo, 1998).

The in vitro starch digestibility was evaluated on the basis of total starch (TS) and resistant starch (RS) determined after in vitro enzymatic digestion (Świeca, Baraniak and Gawlik-Dziki, 2013).

$$SD[\%] = 100 - \left(\frac{RS}{TS} \times 100 \right)$$

Where SD is the in vitro starch digestibility, RS and TS are the resistant and total starch contents, respectively.

3.5.3. Statistical Analysis

Data were submitted to Statgraphics plus 5.1 (Statpoint, Warrenton, USA) software and analyzed using analysis of variance (ANOVA) to determine significant differences among the

processing methods. Duncan multiple range test was used to compare the means. Pearson correlation coefficient (R^2) and p-value were used to show correlations and their significance. Differences were considered significant when $p < 0.05$.

3.5.4. Results and Discussion

3.5.4.1. Free sugars, total starch and starch fractions of raw *Amaranthus Caudatus* grain porridge

Table 3.5.1 shows total starch and its fractions in three types of *Amaranthus caudatus* and the effect of processing. Total starch content was in the range of 48-56 g/100g DM. The amount of starch obtained in brown amaranth is lower than that obtained in the two other amaranth types. The total starch content of amaranth was found to be higher than that of barley (Rosin et al., 2002) and lower than that of rice, sorghum and teff (Bultosa and Taylor, 2004; Saravanabavan et al, 2013; Zhu et al., 2011). The result is also comparable to that found in *Amaranthus cruentus*, *Amaranthus hybridus* and *Amaranthus hypochondriacus* (Cai et al., 2004; Capriles et al., 2008).

The free sugar content in raw samples was found to be in the range of 0.6-0.7 g/100g DM. Rapidly digestible starch content ranges between 27.4-33.2 g/100g DM, the highest and lowest values being for the white and brown amaranth, respectively. Slowly digestible starch was found in the range of 10.9-13.9 g/100g DM. Alike RDS, highest SDS was obtained for white amaranth and the lowest was obtained for brown amaranth. The percentage of RDS was twofold higher than SDS and foods having high percentage of RDS are known to have high glycemic response (Englyst et al., 1999). In connection to this, Roopa and Premavalli (2008) reported that finger millet exhibited different characteristics where the content of SDS was found to be three times higher than that of RDS showing a better therapeutic effect by modulating blood glucose level through a slow release of the available carbohydrate.

The resistant starch content was also calculated from the difference between total starch, RDS and SDS and the highest value was obtained for brown (9.43 g/100g DM) followed by white

(8.95 g/100g DM) and red (8.48 g/100g DM) amaranth. This value is far more than that reported in literatures so far in *Amaranthus cruentus* (Capriles et al., 2008). Such large difference could possibly arise from the difference in species, cooking effect and difference in the methodology used. In many literatures the RS content was determined after digestion for more than 16 h while the amount of starch left undigested after 2 h digestion was considered as resistant starch in the present study.

3.5.4.2. Effect of processing on free sugars, total starch and starch fractions in amaranth grain porridge

Popping exhibited no significant effect ($p < 0.05$) on the contents of free sugars and this might be due to the lower efficiency of the applied heat to convert free sugars (reducing sugars) into maillard reaction products. But it significantly increased ($p < 0.05$) the amount of RDS, SDS and TS contents by a percentage of 8.6, 8.2 and 5.2 %, and a statistically non significant decrease ($p < 0.05$) in RS content was observed. The increase in RDS and SDS during popping could be attributed to the decrease in the contents of antinutrients (such as phytic acid and tannins) (Amare et al., 2015, unpublished result) that binds the starch molecule and denaturation of protein that could contribute to loosening the protein-starch interaction (Alonso et al., 2000a; 2000b). A decrease in resistant starch that leads to an increase in starch digestibility after popping has been reported elsewhere on sorghum (Saravanabavan et al., 2013).

Fermentation significantly increased ($p < 0.05$) free sugars (95%), RDS (11%) and SDS (18%) fractions but it significantly decreased total starch (1.6%) and RS (75%) contents. Similar results were reported in finger millet (Antony & Chandra, 1998) and sorghum (Pranoto et al., 2013). The decrease in total starch content could be ascribed to the hydrolysis of starch into lower molecular weight compounds such as maltodextrins, disaccharides, and monosaccharides by amylase producing microorganisms or by endogenous alpha amylases which are activated during fermentation (Sripriya et al., 1997; Elkhalfa et al., 2006). Whereas, the decrease in RS with subsequent increase in the amount of both RDS and SDS

during fermentation could be due to proteolysis of the protein matrix that surrounds the starch granules resulting in increased access for enzymatic attack (De Mesa-Stonestreet et al., 2010).

Table 3.5.1 Total starch and its fractions in raw and processed *Amaranthus caudatus* grain (g/100g DM)*

Treatment	White	Red	Brown	Mean ± SD
<i>Free sugars</i>				
Raw	0.67	0.60	0.64	0.64 ± 0.01c
Popped	0.63	0.59	0.53	0.60 ± 0.03c
Fermented	1.47	1.13	1.15	1.25 ± 0.05b
Corn flakes				3.30 ± 0.04a
<i>Rapidly digestible starch</i>				
Raw	33.18	32.61	27.41	31.06 ± 0.24d
Popped	35.63	35.11	30.48	33.74 ± 0.11c
Fermented	36.58	36.00	31.13	34.56 ± 0.18b
Corn flakes				50.89 ± 0.34a
<i>lowly digestible starch</i>				
Raw	13.89	13.03	10.89	12.61 ± 0.28d
Popped	14.65	14.21	12.07	13.64 ± 0.31c
Fermented	16.33	15.33	13.12	14.93 ± 0.16b
Corn flakes				24.51 ± 0.50a
<i>Total starch</i>				
Raw	56.02	54.12	47.73	52.62 ± 0.22c
Popped	58.15	57.14	50.81	55.37 ± 0.15b
Fermented	54.95	53.36	46.97	51.76 ± 0.27d
Corn flakes				76.82 ± 0.39a
<i>Resistant starch</i>				
Raw	8.95	8.48	9.43	8.96 ± 0.37a
Popped	7.87	7.82	8.26	7.98 ± 0.37a
Fermented	2.04	2.03	2.72	2.28 ± 0.37b
Corn flakes				1.42 ± 0.53b

*Mean values with different letters in column are significantly different at $p < 0.05$

3.5.4.3. In vitro starch digestibility in raw amaranth

Table 3.5.2 shows the contents of BAS, starch digestibility (SD), SDRI and RAG. The amount of BAS ranges from 38-47 g/100g DM, the highest and lowest content was found in

white and brown amaranth, respectively. The difference might occur as a result of the existing difference in their total starch content (Table 3.5.1). The percent SD is almost the same for all the three types of amaranth and falls in the range 80-84%. Capriles et al. (2008) reported that 99% of the starch in *Amaranthus cruentus* is digestible, although a different method was used to quantify the resistant starch content. The high SD of amaranth could be attributed to several factors such as small starch granule size, high levels of amylopectin, and low gelatinization temperature (Caprilles et al., 2008).

Starch digestibility rate index, which indicates the fraction of starch hydrolyzed 20 minutes after the ingestion of the food expressed as percentage of TS was found to be in the range of 57-60%. Despite the larger difference in the starch content of brown amaranth with that of white and red amaranth, no huge difference was observed in SDRI. The high SD is a desirable attribute to use amaranth as an ingredient for complementary food formulation. This is because children have limited gastric capacity and the rapid release of glucose after ingestion of those foods promotes the rapid return of hunger which in turn encourages eating frequently to meet the daily nutrient requirement (Icard-Verniere et al., 2010). Rapidly available glucose also indicates the amount of glucose available to the cells after 20 minutes of ingestion of food to raise the postprandial blood glucose level. The result showed that RAG values in the three types of *Amaranthus caudatus* grain porridge is in the range of 28 to 34 g/100g DM.

Table 3.5.2 Bioavailable and rapidly available glucose, starch digestibility and starch digestibility rate index of raw and processed *Amaranthus caudatus* grain porridge*

Treatment	White	Red	Brown	Mean ± SE
<i>BAS (g/100g DM)</i>[¶]				
Raw	47.07	45.64	38.30	43.67 ± 0.31d
Popped	50.28	49.31	42.55	47.38 ± 0.23c
Fermented	52.90	51.30	44.24	49.48 ± 0.18b
Corn flakes				75.40 ± 1.16a
<i>SD [%]</i>[§]				
Raw	84.03	84.34	80.24	82.87 ± 0.55d
Popped	86.47	86.32	83.76	85.52 ± 0.32c
Fermented	96.27	96.15	94.21	95.54 ± 0.29a
Corn flakes				98.16 ± 0.51a
<i>SDRI (%)</i>[‡]				
Raw	59.22	60.28	57.43	58.98 ± 0.68b
Popped	61.27	61.45	60.00	60.91 ± 0.51b
Fermented	66.57	67.43	66.28	66.76 ± 0.76a
Corn flakes				68.00 ± 1.18a
<i>RAG (g/100g DM)</i>[‡]				
Raw	33.85	33.21	28.05	31.70 ± 0.23d
Popped	36.26	35.70	31.01	34.34 ± 0.28c
Fermented	38.05	37.13	32.28	35.81 ± 0.17b
Corn flakes				54.19 ± 0.39a

*Mean values with different letters in row and column are significantly different at $p < 0.05$. BAS-Bioavailable starch, SD-Starch digestibility, SDRI-Starch digestibility rate index, RAG-Rapidly available glucose.

3.5.4.4. Effect of processing on BAS, RAG, SD and SDRI in amaranth

Popping significantly increased ($p < 0.05$) the BAS and SD by 8.5% and 3.2%, respectively. In addition, the SDRI and RAG were also increased significantly ($p < 0.05$) by 3.2% and 8.3%, respectively. The increase could be attributed to the better exposure of seed's starch matrix and pregelatinization due to popping making it more susceptible to enzymatic attack (Capriles et al., 2008). Fermentation also significantly increased ($P < 0.05$) the amount of BAS and SD by 13% and 15%, respectively. Similarly, both the percent SDRI and RAG increased by 13%. The improvement in SD after popping and fermentation could be attributed

to the huge degradation of antinutrients especially phytates (Amare et al., 2015, unpublished result) that consequently improves the bioavailability of starch (Singh et al., 2010). Moreover, during fermentation the protein starch interaction will be lost due to the action of proteases that promote the accessibility of starch molecules to amylase (De Mesa-Stonestreet et al., 2010).

The BAS and RAG values of corn flakes were significantly higher than that of all raw and processed amaranth samples. However, SD and SDRI values of corn flakes were significantly higher than that of popped amaranth but no significant difference was observed when compared with fermented samples. This indicates that most of the starch fractions of amaranth are digestible.

3.5.4.5. Correlation

Linear regression correlation analysis was carried out to evaluate the relationship among starch fractions, SD, SDRI, protein and fat (Table 3.5.3). The values of the correlation coefficients are given in Table 3.5.3. There were positive and significant correlations observed between free sugar content and RDS ($r = 0.88^{**}$), SDS ($r = 0.90^{**}$), TS ($r = 0.79^{**}$), SDRI ($r = 0.62^{**}$), BAS ($r = 0.90^{**}$), SD ($r = 0.72^{**}$) and RAG ($r = 0.91^{**}$) but it is negatively correlated with RS ($r = -0.71^{**}$), protein ($r = -0.85^{**}$) and fat ($r = -0.81^{**}$) content. RDS, SDS and TS had significant and positive correlation with each other and with other parameters such as SDRI, BAS, SD and RAG. But they negatively correlated with RS, protein and fat content. On the other hand, RS is positively correlated with both protein and fat but the correlation is only significant with protein. BAS is positively correlated with SD and RAG but negatively correlated with protein and fat. SDRI, SD and RAG are significantly negatively correlated with protein showing the presence of high amount of protein reduces the SD due to the strong starch-protein interaction as confirmed in many studies (Rooney and Pflugfelder 1986; Kim et al., 2008).

Table 3.5.3 Selected relationships among nutritional components of *Amaranthus caudatus* grain

	FG	RDS	SDS	TS	RS	SDRI	BAS	SD	RAG	Protein
RDS	0.88**									
SDS	0.90**	0.95**								
TS	0.79**	0.95**	0.91**							
RS	-0.71**	-0.60**	-0.65**	-0.33*						
SDRI	0.62**	0.59**	0.58**	0.34*	-0.90**					
BAS	0.90**	0.99**	0.98**	0.95**	-0.62**	0.59**				
SD	0.72**	0.67**	0.71**	0.42**	-0.99**	0.91**	0.69**			
RAG	0.91**	0.99**	0.96**	0.94**	-0.62**	0.60**	0.99**	0.68**		
Protein	-0.85**	-0.92**	-0.89**	-0.97**	0.34*	-0.33*	-0.92**	-0.40*	-0.92**	
Fat	-0.81**	-0.79**	-0.79**	-0.86**	0.25	-0.22	-0.80**	-0.28	-0.81**	0.91**

FG-free sugars, RDS-Rapidly digestible starch, SDS-slowly digestible starch, TS-Total starch, RS-Resistant starch, SDRI-Slowly digestible rate index, BAS-BAS, SD-Starch Digestibility, RAG-rapidly available glucose

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

3.5.4.6. Effect of processing on the consistency of amaranth grain porridge

In order to evaluate the extent to which both popping and fermentation bring a change on the rheology of amaranth porridge, the viscosity of raw, popped and fermented amaranth for all the three amaranth types were measured with changing the dry matter content (Figure 3.5.1). The viscosity of raw amaranth sample was higher than that of both popped and fermented amaranth at the same dry matter content. The lower viscosity observed after popping compared to the raw amaranth might be due to pregelatinization of starch during heat treatment. Similar results were reported by Muyonga et al. (2014). While the lower viscosity observed during fermentation could be due to the partial hydrolysis of starch as evidenced by lower content of starch in fermented samples compared to raw samples (Table 3.5.1). Among the three amaranth types the white and the red had comparable starch content, higher than that of the brown amaranth resulting in lower gelatinization and lower viscosity for all raw, popped and fermented samples compared to the corresponding processes in white and red amaranth.

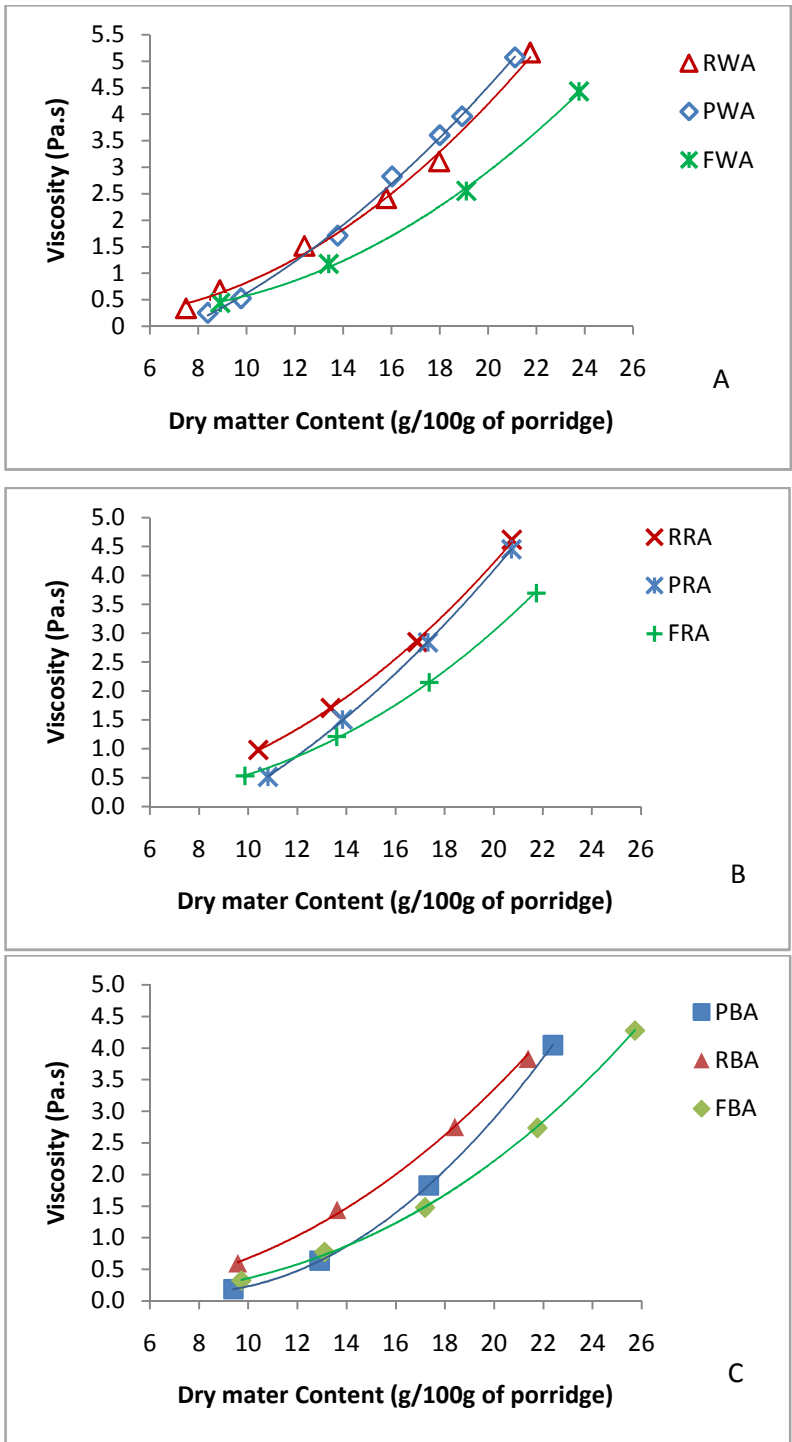


Figure 3.5.1 Viscosity of porridge prepared from three types of *Amaranthus caudatus* grain A) White B) Red and C) Brown measured at a shear rate of 83s⁻¹ after 10 minutes shear at a porridge temperature of 45 °C. RWA, RRA, RBA, PWA, PRA, PBA, FWA, FRA and FBA are raw white, raw red, raw brown popped white, popped red, popped brown, fermented white, fermented red and fermented brown amaranth, respectively.

3.5.4.7.Evaluation of the instant character of popped amaranth flour

In order to assess the instant character of popped amaranth flour, the apparent viscosity of porridge prepared at different dry matter content according to the two different procedures: “instant” and “cooking” were compared (Figure 3.5.2). The result indicated that for all the three types of amaranth the viscosity curve for the cooked porridge and the instant porridge are superimposed. This shows that the entire starch molecule in amaranth was gelatinized during popping and thus it could be concluded that popped amaranth could be used as an instant powder to prepare porridge and could be mixed with roasted barley powder locally called “besso”, the most commonly used instant powder in Ethiopia to prepare an instant “besso juice”.

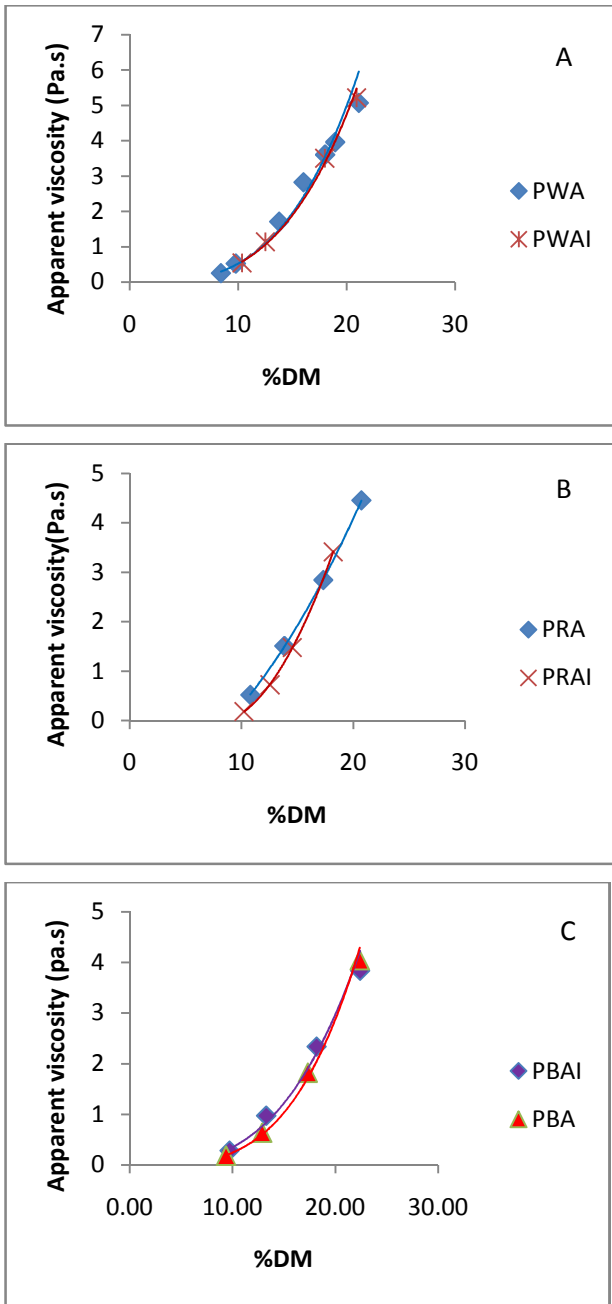


Figure 3.5.2 Viscosity of three types of popped amaranth A) white B) red and C) Brown measured with cooking and instant procedure at a shear rate of $83s^{-1}$ for 10 minutes at a porridge temperature of $45\text{ }^{\circ}C$.

3.5.4.8. Energy Density of amaranth porridge and the effect of processing

Mouquet and Treche (2001) reported that a porridge having a consistency close to that of yoghurt is spoonable and has a viscosity in the range of 1-3 Pa.s measured using Haake VT500 viscometer with SV-DIN coaxial cylinder at a shear rate and porridge temperature of 83s^{-1} and $45\text{ }^{\circ}\text{C}$, respectively. Muyonga et al. (2014) also reported that porridge with 3 Pa.s has a drinking consistency although the measurement conditions were not declared. Therefore, porridge with such consistency could be recommended for 6-23 month-old children. Taking into account the aforementioned facts, the energy density of the porridge prepared from all the three types of amaranth in raw state and after popping and fermentation was calculated (Table 3.5.4). The minimum meal frequency required to meet the energy requirement from amaranth based complementary foods was also calculated at the two viscosity extremes for different age groups.

The dry matter content showed better increment after fermentation than after popping especially at the higher viscosity (3 Pa.s) in all the three amaranth compared to porridge prepared from raw amaranth. This is because part of the starch was hydrolyzed during fermentation which will in turn contribute to an increase in the energy density of the porridge. The minimum number of meals needed to meet daily energy requirement from CF increases as the age of the child increases. The required meals for 6-8, 9-11, 12-23 months old children are 2.4-3.3, 2.8-3.9 and 3.8-5, respectively at a viscosity of 1 Pa.s and 1.6-2.1, 1.9-2.4 and 2.5-3.2, respectively at a viscosity of 3 Pa.s. Porridge prepared from fermented amaranth have high energy density than porridge prepared from raw and popped amaranth. Therefore, the daily energy requirement of children could be met with less number of meals if the porridge is prepared from fermented amaranth than it is from popped and raw.

Table 3.5.4 Energy density and minimum daily number of meals required to attain the level of energy needed from amaranth based complementary foods of different viscosity for children with average breast milk energy intake according to age group†

Sample	Treatment	DM content		Energy Density (kcal/g porridge)		No. of meals					
						6-8		9-11		12-23	
		1 Pa.s	3 Pa.s	1 Pa.s	3 Pa.s	1 Pa.s	3 Pa.s	1 Pa.s	3 Pa.s	1 Pa.s	3 Pa.s
White	Raw	11.0	16.8	0.45	0.69	3.2	2.1	3.8	2.4	5.0	3.2
	Popped	11.4	17.2	0.45	0.70	3.2	2.1	3.7	2.4	4.9	3.2
	Fermented	12.9	20.2	0.54	0.85	2.6	1.7	3.1	2.0	4.1	2.6
Red	Raw	10.5	17.3	0.43	0.71	3.3	2.0	3.9	2.4	5.2	3.1
	Popped	12.4	17.8	0.50	0.72	2.9	2.0	3.4	2.3	4.5	3.1
	Fermented	12.8	20.0	0.54	0.84	2.7	1.7	3.1	2.0	4.2	2.7
Brown	Raw	12.0	19.0	0.48	0.76	3.0	1.9	3.5	2.2	4.7	2.9
	Popped	14.5	20.3	0.56	0.78	2.6	1.8	3.0	2.2	4.0	2.9
	Fermented	14.8	22.5	0.59	0.91	2.4	1.6	2.8	1.9	3.8	2.5

†Viscosity of porridge (1-3 Pa.s) was considered based on the recommendation by Mouquet and Treche (2001) and estimated total energy requirement is based on new US longitudinal data averages plus 25% (2SD). Assumed functional gastric capacity (30 g/kg reference body weight) is 249 g/meal at 6-8 months, 285 g/meal at 9-11 months, and 345 g/meal at 12-23 months.

3.5.5. Conclusion

The in vitro starch digestion assay showed that amaranth had more rapidly digestible starch than slowly digestible starch making it a high glycemic food. Moreover, both popping and fermentation significantly contributed to an increase in starch digestibility of amaranth which resulted in lowering its potential to use it as a therapeutic food for diabetes. However, the increased digestibility and improvement in energy and nutrient density during popping and fermentation was found to be a good attribute to use amaranth as an ingredient in complementary food formulation. Besides popping was found to gelatinize all the available starch in amaranth, and as a result, popped amaranth starch could be used as instant flour for consumption using boiled water. Popping and fermentation increased the dry matter content of porridge prepared at the same viscosity recommended for children making the porridge energy dense than the one prepared from raw amaranth. Therefore, the use of the aforementioned processing techniques prior to the preparation of porridge allows decreasing the feeding frequency and thus saves care givers time and energy.

3.6. Bioactive components and in vitro antioxidant activity of *Amaranthus caudatus* grain as affected by processing

3.6.1. Introduction

Amaranthus is a fast growing crop that spread throughout the world, growing under a wide range of climatic conditions. The plant is resistant to drought, hot climate, and pests and thus it needs little requirements on cultivation inputs and thus attracted the attention to the world's food supply (National Academy of Sciences, 1985; Omami et al., 2006). It is able to produce edible grains and leafy vegetables. There are three species of the genus *Amaranthus* that produce relatively large inflorescences with often more than 50 000 edible seeds per plant. These are *A. hypochondriacus*, *A. cruentus* and *A. caudatus*. Vegetable amaranths are represented by various amaranth species, such as *A. tricolor*, *A. dubius*, *A. cruentus*, *A. edulis*, *A. retroflexus*, *A. viridis*, and *A. hybridus* (Bressani 2003).

With regard to its nutritional value, amaranth has higher protein, fat and dietary fiber contents than conventional cereals (Nascimento et al., 2014). Moreover, it has better protein quality with high amount of lysine and sulfur containing amino acids (Amare et al., 2015).

Several studies showed that the leaves of amaranth has a wide spectrum of potential health benefits such as antidiabetic, anticholestrolemic, antioxidant activities etc. (Girija et al., 2011; Kumara et al., 2012; Lopez Mejia et al., 2014). Being a gluten free cereal, the grain of amaranth was also utilized in the formulation of different functional foods (Alvarez Jubete et al., 2010a). The small size of amaranth grain allows the whole grain to be used for consumption without removing the outer cover, which may contain high amount of bioactive compounds and dietary fiber. As a result high antioxidant activity is expected and this was also proven in studies conducted in different amaranth species (Nsimba et al., 2008; Gorinstein et al., 2008; Alvarez-Jubete et al., 2010b; Lopez-Mejia et al., 2014).

However, many polyphenols, especially phenolic acids, which are mainly responsible for the antioxidant capacity, are directly involved in plant response to abiotic and biotic stresses. As a

result, different amaranth species grown under different agroecological conditions are expected to demonstrate different levels of bioactive components responsible to react against the stress. Moreover, the application of different processing methods may have significant effect on the levels of bioactive components which are responsible to impart antioxidant property (Chandrasekara et al., 2012, Alvarez Jubete et al., 2010b) and other health promoting actions like lowering of blood pressure and prevention of diabetes demonstrated by γ -Aminobutyric acid (GABA) (Thitinunsomboon et al., 2013; Coda et al., 2010). γ -Aminobutyric acid (GABA) is a four-carbon non-protein amino acid produced from L-glutamate by the catalytic action of an enzyme glutamate decarboxylase (Ueno, 2000). The amount in raw cereals and legumes is too small but improved during processing depending on the amount of its precursor, L-glutamate, and the activity of the enzyme, glutamate decarboxylase involved in conversion process.

The aim of the present study was, therefore, to determine the levels of bioactive components (total polyphenols, total flavonoids and γ -aminobutyric acid (GABA)) and antioxidant capacity of three different types of amaranth grain cultivated in Ethiopia. The effect of popping, germination and fermentation on the contents of those bioactive compounds and antioxidant capacity was also evaluated.

3.6.2. Materials and Methods

3.6.2.1. Sample collection

Three different types of *Amaranthus caudatus* grains, white, red and brown in color, were purchased from six farmers living in Chat Kebelle, Bench Majji Zone, Southern Nations, Nationalities and Peoples region, Ethiopia in October 2011. The grains of the six origins were sorted to remove immature seeds, cleaned and washed to remove sand and soil. The washed seeds were sun dried and equal amount of the six samples were mixed to prepare a composite sample for each amaranth types.

3.6.2.2.Solvents, Chemicals and reagents

The chemicals 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS), potassium persulfate, (+)-catechin, γ -Aminobutyric acid, norleucine were purchased from Sigma–Aldrich Chemie GmbH (Taufkirchen, Germany), Folin-Ciocalteu reagent and methane sulfonic acid were purchased from Merck & Co., Inc. (New York, USA), and all other chemicals and reagents were of analytical grades.

3.6.2.3.Sample preparation

i. Preparation of raw amaranth flour

The composite samples for each type of amaranth grain were milled, sieved using 0.425 mm sieve and stored at 4°C until further analysis.

ii. Popping

Cleaned and sun dried amaranth grains were popped as described in Amare et al. (2015). The popped grains were milled with to pass a 0.425 mm sieve and stored in polyethylene bags at 4 °C for further analysis.

iii. Germination

For germinating the Amaranth grains the method of Colmenares de Ruiz and Bressani (1990) was used with modification. The seeds were washed and soaked by disinfecting solution of 70% ethanol for 3 min. Then it was thoroughly washed to remove residual solvent and soaked in distilled water (1:3, w/ v) for 5 h at room temperature (22 ± 2 °C). The soaked seed were kept between thick layers of cotton cloth and allowed to germinate for 48 h at $32 \pm 2^{\circ}\text{C}$. During germination, seeds were rinsed with distilled water three times a day. After germination, the seeds were dried in an electric oven at 50 °C for 20 h.

iv. Fermentation

Natural fermentation was carried according to the method described by Ibrahim et al., (2005) with modification. Briefly; 250 g of raw amaranth grain flour was mixed with 500 ml distilled water in a 600 mL beaker and then left to ferment for 48 h at room temperature (22 ± 2 °C). Thereafter, the sample was mixed with a glass rod and transferred to three aluminum dishes (30 cm diameter each) and dried in a hot oven (Heraeus UT 5042, Germany) at 50 °C for 20 h. The dried sample was then ground to pass a 0.425 mm screen and stored in polyethylene bags at 4 °C for further analysis.

3.6.2.4. Sample extraction

Sample extraction was done according to the method described by Saura-Calixto et al. (2007). Briefly, 0.3 g of dried powdered sample was mixed with 12 mL of acidified methanol water solution (50:50v/v, pH 2) and extracted for 3 h. The mixture was centrifuged at 2500g for 10 min and the supernatant was transferred to another test tube. To the residue, 12 mL of acetone water (70:30v/v) was added and extracted for another 3 h, centrifugation takes place and the supernatant was mixed with the first extract and stored at 4 °C until analysis.

3.6.2.5. Determination total phenolic content

Total phenolics were determined following the method of Singleton and Rossi (1965). Briefly, 50 mg of sample was mixed with 1.5 mL acetone/water 70/30 (v/v) for phenolic compound extraction. The mixture was shaken for 1 h and centrifuged at 5000g for 10 minutes. The supernatant was transferred to another tube and the extraction was repeated one more time. To a 600 µL of the supernatant, 4.7 mL distilled water, 300 µL Folin-Ciocalteu's reagent (F9252; Sigma-Aldrich, Saint-Quentin-Fallavier, France) and 400 µL of 20% sodium carbonate solution was mixed and incubated for 30 min at room temperature under dark. Color formed during the reaction was measured spectrophotometrically (760 nm). Gallic acid (G7384; Sigma-Aldrich) was used as standard and the results are expressed in mg of gallic acid equivalent (GAE) per 100 g DM.

3.6.2.6.Determination of Total flavonoid

Total flavonoid content was determined using a colorimetric method described previously Xu and Chang (2007). Briefly, 0.25 mL of extract prepared as described in section 3.6.2.4 above or (+)-catechin standard solution was mixed with 1.25 mL of distilled water in a test tube, followed by adding 75 μ L of a 5% NaNO₂ solution. After 6 min, 150 μ L of a 10% AlCl₃.6H₂O solution was added and allowed to stand for another 5 min before adding 0.5 mL of 1 M NaOH. The mixture was brought to 2.5 mL with distilled water and mixed well. The absorbance was measured immediately at 510 nm using a UV-Visible Spectrophotometer (UV 160, Shimadzu, Japan). The results were calculated and expressed as milligrams of (+)-catechin equivalents (mg of CE/g DM) using the standard curve of (+)-catechin. Linearity range of the standard curve was 0 to 500 μ g/mL ($r = 0.9925$). The analysis was conducted in triplicate.

3.6.2.7.Determination of γ -aminobutyric acid (GABA)

Ten to twenty milligram of dried sample flour was weighed in a Schlenk tube and 50 μ L of 25 μ M Norleucine and 450 μ L of 4 M methanesulfonic acid were added. The tube was flushed with nitrogen, closed and heated at 150 °C for 2 h. After cooling the hydrolysate, 450 μ L of 4 M NaOH was added and diluted to 5 ml with a loading buffer (citrate buffer pH 2.2). All extractions were done in triplicate. The extract was then filtered using a 0.45 μ m membrane filter and injected into a Biochrom 30⁺ amino acid analyser (Biochrom Ltd, France) using a lithium cation exchange resin column. GABA was used as a standard and the measurement was done at 570 nm.

3.6.2.8. Determination of Antioxidant capacity (AC)

i. DPPH Assay

DPPH• radical (2,2-diphenyl-1-picrylhydrazil) inhibition capacity by antioxidant compound in the extract was done by the method described in Lopez-Mejia et al. (2014) with modification. Briefly, 0.1 ml of extract or standard solution was mixed with 3.9 ml of DPPH• radical solution (3.94mg in 100ml of methanol). The mixture was shaken vigorously with vortex and left to stand at room temperature in the dark for 30 min. Thereafter, the absorbance was measured spectrophotometrically using the Perkin Elmer Lamda 950 UV/Vis/NIR spectrophotometer at 517 nm against methanol as a blank. The results were calculated and expressed as mg of Trolox equivalents (TE) per 100 gram dry matter of the sample using the calibration curve of Trolox (concentration 0-200µg/ml).

ii. ABTS Assay

Antioxidant capacity of the extract was evaluated by the method, as described by Re et al. (1999) with slight modification. Briefly, ABTS⁺ radical cation was generated by a reaction of 7 mM ABTS with 2.45 mM potassium persulfate. The reaction mixture was allowed to stand in the dark for 16 h at room temperature and used within two days. The ABTS⁺ radical solution was diluted with ethanol, to give an absorbance of 0.700 ± 0.050 at 734 nm. 100 µl of diluted sample was mixed with 1.9 ml of diluted ABTS⁺ radical solution. The mixture was allowed to stand for 6 min at room temperature and the absorbance was immediately recorded at 734 nm. Trolox solution (concentration 0–200µg/ml) was used as a reference standard. The results were expressed as µg Trolox/g dry weight of sample.

3.6.3. Data Analysis

Data were analyzed by two way analysis of variance (ANOVA) using Statgraphics Centurion XV (Statpoint,Warrenton,USA) to determine effect of processing methods and type of grain. Duncan multiple range test was used to compare the means. Pearson correlation coefficient

(r) and p-value were used to show correlations and their significance, respectively. Differences of $p < 0.05$ were considered significant. All tests were done in triplicate.

3.6.4. Results and Discussion

3.6.4.1. Total phenolics and total flavonoid contents of raw amaranth grains

Table 3.6.1 shows the contents of bioactive components namely total polyphenols and flavonoids in three types of raw amaranth grain. The amount of total polyphenols and total flavonoids in the brown colored amaranth was 257 mg GAE/100g DM and 0.68 mg CE/ g DM, respectively. These values are higher than that obtained in white amaranth: 146 mg GAE/100g DM and 0.52 mg CE/ g DM, respectively and red amaranth: 158 mg GAE/100g DM and 0.60 mg CE/ g DM, respectively as colored substances contains high level of bioactive compounds than pale seeded once. The amount of total polyphenols obtained in all the three types is less than that reported by Chlopicka et al., (2012) but higher than that obtained by Nsimba et al. (2008) and Alvarez-Jubete et al. (2010b). The content of total polyphenols in all the studied type of amaranth is less than that of other pseudocereals such as quinoa and buckwheat (Alvarez-Jubete et al., 2010b) but higher than wheat, rice and oats (Adom and Liu 2002). The total flavonoid content of amaranth was also found to be higher than commonly utilized cereals like corn, wheat, rice and oats (Adom and Liu 2002).

Table 3.6.1 Total polyphenol and total flavonoid contents in three types of raw *Amaranthus caudatus* grain*

Type of Sample (Raw grain)	Total (mg DM)	Polyphenol GAE/100g	Total (mg CE/ g DM)	Flavonoid
White Amaranth	146 ± 3		0.52 ± 0.06	
Red Amaranth	158 ± 3		0.60 ± 0.01	
Brown Amaranth	257 ± 16		0.68 ± 0.13	

*values are mean ± SD of triplicate measurements

3.6.4.2. Effect of processing on total polyphenol and total flavonoid of *Amaranthus caudatus* grains

Popping decreased the total polyphenol content significantly ($p < 0.05$) by 15 %. Alvarez Jubete et al. (2010) have also shown that heat treatment decreases the total polyphenol content in bread prepared from amaranth, quinoa, buckwheat and wheat. The automatic loss of pericarp during popping could also have contributed to the decrease in polyphenol content as phenolic compounds are concentrated in the pericarp of cereal grains (Taylor and Dewar, 2001; Dykes and Rooney, 2007). On contrary, a significant increment was observed during germination (95%) and fermentation (66%) (Table 3.6.2). The increase during germination could be attributed to the release of more bound phenolics as a result of structural breakdown of cell walls which could be resulted from increased activity of cell wall degrading enzymes such as xylanases and cellulases and/or synthesis of bioactive compounds (Katina et al., 2007). Binqiang et al. (2010) also showed that germination of oat resulted in a significant increase in total phenolic content. Similarly, during fermentation, the action of microbial enzymes could allow better extractability of bound phenolics that could explain fermentation induced increase in total phenolic compounds (Đorđević et al., 2010).

The content of flavonoids significantly increased during germination ($p < 0.05$) but no significant effect ($p < 0.05$) was observed after popping and fermentation. If germination is preceded with long hours of soaking, total content of phenolic compounds decreases due to leaching out of the extractable phenolics (Towo et al., 2003). But in the present study the soaking time was too short and the grain was also too hard for the soaking media to impart this effect.

Table 3.6.2 Effect of processing on the content of total polyphenols and total flavonoids in *Amaranthus caudatus* grain.

Bioactive compounds	p-value	Processing			
		Raw	Popped	Germinated	Fermented
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Total polyphenol (mg GAE/100g DM)	0.0000	187 ± 53b	159 ± 55a	365 ± 73d	310 ± 32c
Total Flavonoid (mg CE/ g DM)	0.0087	0.60 ± 0.09a	0.58 ± 0.09a	0.72 ± 0.14b	0.55 ± 0.09a

Means followed by different letters across the row are significantly different at $p < 0.05$.

3.6.4.3. Antioxidant activity of three types of raw *Amaranthus caudatus* grains

Table 3.6.3 shows the results of antioxidant activity of the three types of amaranth using DPPH and ABTS Assays. The results of the study shows that brown amaranth had highest antioxidant activity with values of 43.29 mg TE/100g DM and 93.08 mg TE/100g DM on DPPH and ABTS assays, respectively followed by red then white amaranth with corresponding values: 35.52 mg TE/100g DM and 85.66 mg TE/100g DM and 16.65 mg TE/100g DM and 79.61 mg TE/100g DM, with DPPH and ABTS assays, respectively. The higher antioxidant activity of brown amaranth could be explained by the presence of high amount of bioactive compounds responsible to impart antioxidant activity (Table 3.6.1).

Table 3.6.3 Antioxidant activity of three types of raw *Amaranthus caudatus* grain*

Type of Sample (Raw grain)	DPPH	ABTS
	(mg TE/100g DM)	
White Amaranth	16.65 ± 1.54	79.61 ± 1.43
Red Amaranth	35.52 ± 1.88	85.66 ± 3.59
Brown Amaranth	43.29 ± 3.22	93.08 ± 0.72

*values are mean of triplicate ± SD

3.6.4.4. Effect of processing on antioxidant capacity of *Amaranthus caudatus* grain

Figure 3.6.1 shows the effect of popping, germination and fermentation on antioxidant capacity of amaranth grain. The antioxidant activity during popping, germination and fermentation improved by 131, 213 and 21% with DPPH assay, respectively and 21, 80 and 13% with ABTS assay, respectively. Although the total phenolic content of amaranth, that could strongly influence the antioxidant capacity, decreased during popping, the antioxidant activity increased. This might be due to production of Maillard reaction products (Manzocco et al., 2000). The increase in antioxidant activity during germination could be due to the cumulative effect of increased bioactive compounds (total polyphenols and flavonoids) (Table 3.6.2) and the synthesis of compounds like tocopherols which are also responsible to impart antioxidant activity (Dicko et al., 2005). During fermentation, the action of enzymes such as amylases, xylanases, cellulases and proteases derived from the grain (endogeneous enzymes) and microorganism in the fermentation media (exogeneous enzymes) contribute to the modification of grain composition and could also enhance the release of bound phenolics due to hydrolysis of cell wall prior to extraction (Đorđević et al., 2010; Hur et al., 2014). As a result, the antioxidant capacity of fermented amaranth was found to increase in both DPPH and ABTS assays.

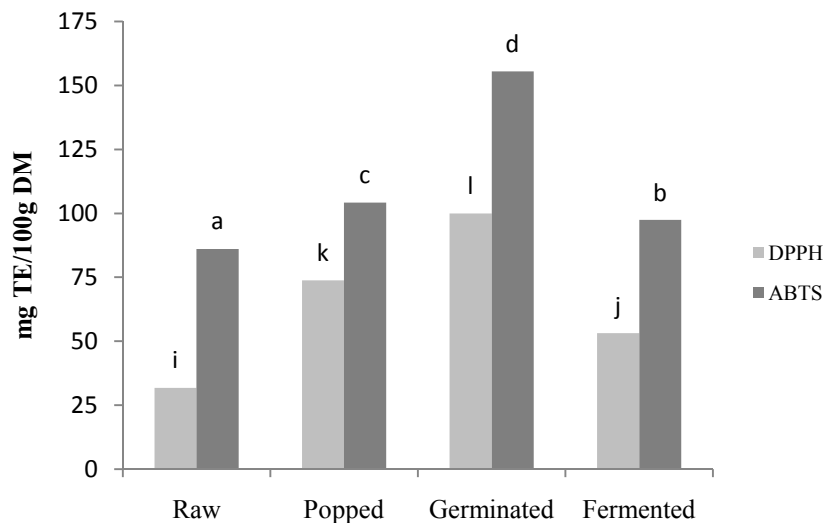


Figure 3.6.1 Effect of processing on antioxidant capacity of *Amaranthus caudatus* grain. Result bars designated with different letters on each assay are significantly different at $p < 0.05$.

3.6.4.5. Correlation between total polyphenol and antioxidant activity in amaranth

The correlation between the radical scavenging activity and total polyphenol content of the raw and processed amaranth was studied using a linear regression analysis. As indicated in Fig. 3.6.2A and 3.6.2B, the correlation coefficient between total phenolics and DPPH values ($r = 0.613, y = 0.2244x + 7.4111$) and ABTS scavenging activity ($r = 0.755, y = 0.235x + 50.818$). Polyphenols were anticipated to show a very strong correlation with the antioxidant activity but the comparatively low correlation values between total phenols and the antioxidative activity observed in the present study suggest that the major antioxidant compounds in studied seeds might be non-phenolics. Non phenolic compounds such as phytic acid, tocopherols, sterols, carotenoids, amino acids and peptides might also contribute to the antioxidant activity of raw and processed amaranth. The correlation between DPPH and ABTS assays was found to be ($r = 0.885, y = 1.0397x - 50.513$), signifying the fact that both assays have closely related reaction chemistry.

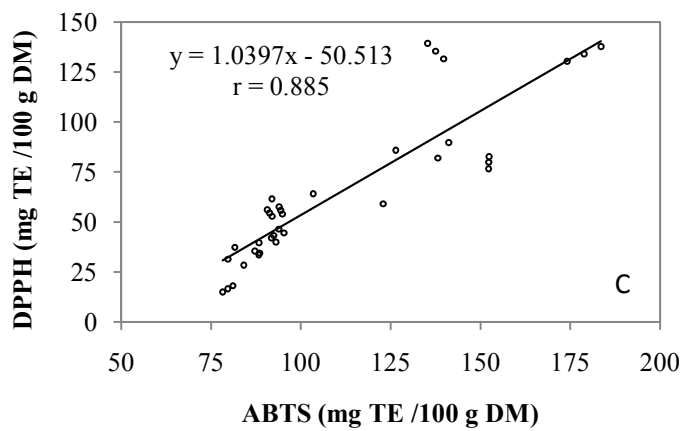
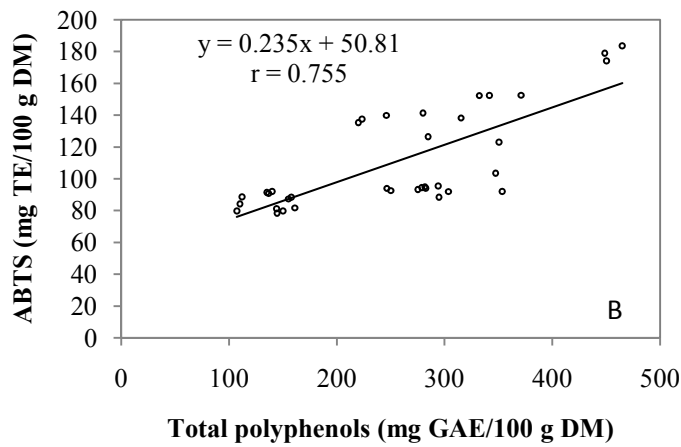
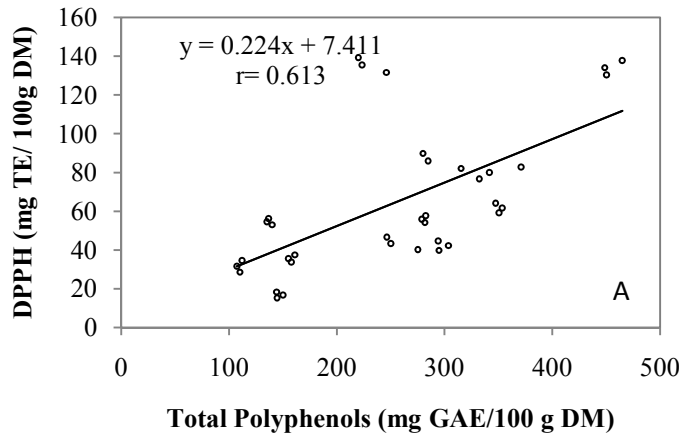


Figure 3.6.2 Correlation between total polyphenol content and DPPH assay (A), total polyphenol content and ABTS assay (B) and DPPH and ABTS assay (C).

3.6.4.6. Effect of popping, germination and fermentation on the content of GABA in three types of *Amaranthus caudatus* grain

Table 3.6.4 shows the effect of processing on GABA content of three types of amaranth grains. The amount of GABA in raw amaranth is in close agreement with the report by Coda et al (2010). But its content was found to be less than other known pseudocereals such as quinoa and buckwheat (Coda et al., 2010) and rice (Thitinunsomboon et al., 2013). Thitinunsomboon et al (2013) and Kuo et al (2004) also reported that too small amount of GABA was obtained in raw rice and legumes, respectively.

Table 3.6.4 Effect of processing on γ -Aminobutyric acid content of *Amaranthus caudatus* grain[†]

Sample	GABA (mg/g protein)
Raw	0.10 ± 0.2c
Popped	0.57 ± 1.1c
Germinated	2.77 ± 1.1b
Fermented	6.03 ± 1.3a

[†] values are mean ± SD of nine measurements and means followed by different letters down the column are significantly different at $p < 0.05$.

Popping showed a statistically non significant increase in the GABA content (Table 3.6.4) while germination and fermentation significantly increased the content of GABA ($p < 0.05$) from 0.1 to 2.77 and 6.03 mg/g protein, respectively (Table 3.6.4). This is tremendous improvement in the functional characteristics of amaranth only referring to its content of GABA. Some studies also showed that enriching the soaking media with L-glutamate could further improve GABA content during malting (Zhang et al., 2014) and inoculating with lactic acid bacteria responsible for GABA production during fermentation (Yang et al., 2008).

Therefore, the availability of L-glutamate and the enzyme responsible to convert L-glutamate, i.e. glutamate decarboxylase, determines the applicability of both malted and fermented amaranth as a functional food (Yang et al., 2008, Seo et al., 2012; Coda et al., 2010; Zhang et al., 2014; Khwanchai et al., 2014).

Okada et al. (2000) reported that a daily intake of 26.4 mg helps to improve the symptoms of mental disorder and a report by Inoue et al. (2003) showed that a daily intake of 10–12 mg reduces mild hypertension symptoms. Thus, in order to obtain the health improvement benefits of amaranth, too high amount of raw and popped amaranth based food is needed due to the low content of GABA, which is impractical. But the amount of germinated or fermented amaranth based food seemed to be feasible to demonstrate the required health improvement (Table 3.6.5).

Table 3.6.5 Amount of amaranth based food required to meet recommendation for physiological function

Type of sample	Recommended Amount of GABA in the diet (mg)		Amount of amaranth based food required to meet the recommendation (g)	
	For improving mental disorder	For reducing hypertension	For improving mental disorder	For reducing hypertension
Raw			1772	805
Popped	26.4	12	324	147
Germinated			56	25
Fermented			29	13

3.6.5. Conclusion

The study shows that amaranth was found to have high levels of bioactive compounds of health promoting activity and all processing techniques applied was found to affect the content of bioactive compounds. Germination brought significant improvement in all the bioactive components determined in this study and thus found to better enhance the nutraceutical value than popping and fermentation. Raw amaranth had too small amount of GABA like other cereals and legumes but processing especially germination and fermentation brought significant increment showing that malted and fermented amaranth could be considered in the DASH (Dietary Approach to Stop Hypertension) diet. Among the studied amaranth types, brown amaranth exhibited higher contents of total polyphenols and flavonoids and thus demonstrated higher antioxidant capacity than the two other amaranth types. Amaranth, being gluten free, in addition to the high levels of the aforementioned

bioactive compounds, is an alternative crop for celiac patients. Although the crop was declared as one of the 36 crops to feed the alarmingly growing global population due to its nutritional, functional and agricultural characteristics, the promotion is still lagging behind. We, therefore, strongly recommend the cultivation and utilization of this crop, which could easily grow even in marginalized areas, to fully exploit its nutritional and functional applications.

3.7. Physicochemical property, nutritional quality and sensory acceptability of teff-amaranth blended injera

3.7.1. Introduction

Injera is the staple diet Ethiopians'. It is a pancake like soft, spongy, sour, circular flatbread made from cereals such as teff, barley, sorghum, maize and wheat (Umeta et al., 2005). Flour prepared from these cereals is mixed with water and "ersho", the left over of a previous fermentation that is used as a starter culture to trigger the fermentation process, kneaded thoroughly and allowed to rest for 2-3 days to ferment. About one-third of the fermented batter is cooked for about five minutes and allowed to cool to about 60-70 °C. This cooked batter is mixed with the other portion and left to ferment for about 2 h followed by baking on a clay pan. The baked injera is consumed with "wot", a stew made with spices, meats and pulses, such as lentils, beans and split peas. Among all the aforementioned cereals teff was found to be the preferred cereal for a good quality injera (Yetneberk et al., 2004). However, the price of teff is increasing drastically and it is mainly consumed in urban areas where household incomes are relatively higher (Berhane et al., 2011). Due to the high demand for teff and the rapid increase in its price, substitution and/or blending of teff flour with other cereal flours is becoming a common practice in Ethiopia to prepare injera.

So far injera made solely from teff, sorghum, barley and maize or a blend of two or three of these cereals have been reported (Gebrekidan and Gebrehiwot 1982, Yetneberk et al., 2004, 2005, Abraha et al., 2013, Baye et al., 2013). Preparing injera from different cereal blends promotes food diversification for improved nutrition. Moreover, if low cost cereals are blended with teff during injera making it will be of benefit to the economically disadvantaged population.

Amaranth grain is an underutilized cereal in many parts of Ethiopia but in Bench Majji Zone, Southern Nations, Nationalities and Peoples Region a particular ethnic group called Me'entit cultivates the crop intercropping with maize and sorghum. The common mode of consumption of amaranth in Ethiopia is in the form of porridge prepared from flour of popped

grain. Utilizing amaranth to prepare injera is rarely practiced. Considering the drastic increase in the price of teff and good nutritional quality and agricultural advantages of amaranth like high yield, adaptability to harsh environment and high species diversity, the present study aims to develop teff-amaranth blended injera and evaluate its physicochemical properties, nutritional quality and sensory acceptability. Moreover,

3.7.2. Materials and Methods

3.7.2.1. Sample collection

White *Amaranthus caudatus* grain collected from Chat Kebelle, Bench Majji Zone, Southern Nations, Nationalities and Peoples region, Ethiopia in October 2011 and teff grain purchased from Addis Ababa Market were used.

3.7.2.2. Preparation of amaranth and teff flour

Both amaranth and teff grains were sorted to remove immature seeds, sand and soil then followed by washing with cold tap water and thoroughly rinsed with distilled water. The washed seeds were oven dried at 50 °C, milled and sieved in a 0.425 mm sieve.

3.7.2.3. Composite flours

Teff flour was composited with white *Amaranthus caudatus* grain flour at six levels with the following blending proportion: 100% teff (Control), 90% teff + 10% amaranth, 80% teff + 20% amaranth, 70% teff + 30% amaranth, 60% teff + 40% amaranth, 100% amaranth and mixed properly.

3.7.2.4. Preparation of Injera

Injera preparation followed the procedure described in Yetneberk et al. (2004) and Baye et al. (2014) with modification and the flow diagram of the procedure is presented in figure 1. Five hundred gram of the flour blend was taken in duplicate and 750 mL distilled water and 85 mL

back slop starter culture was added. The mixture was kneaded for about 3-5 minutes and left to ferment for 40 h. Sixty gram of the dough from each mixture was taken and 250 mL water was added to it. This mixture was boiled with continuous stirring with the aim to offer cohesiveness of the dough and to provide easily fermentable carbohydrate to leaven the injera. The gelatinized batter was cooled to approximately 45 °C under room temperature and added back to the fermenting dough. The final mixture was left to ferment for 2 h and about 500 g of the fermented batter was poured in a circular manner on a hot clay griddle, covered, and baked for 2-3 min. Aliquots from each mixture was taken at different time interval to follow the pH drops throughout the fermentation process.

Preparation of Injera

Grains
Dirt removing
Sorting
Washing
Drying
Milling
Blending

Addition of
• Water
• Backslope starter
Mixing
Kneading
Dough fermentation (1⁰)

Mixing
Dough fermentation (2⁰)
Baking

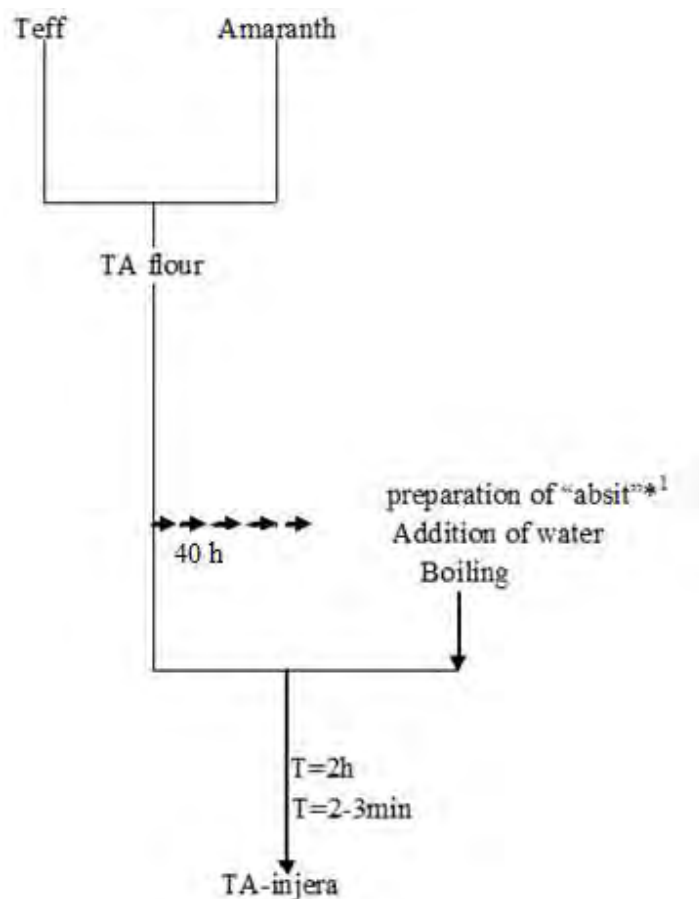


Figure 3.7.1 Flow diagram for injera preparation (Adapted from Yentneberk et al., 2004; Baye et al., 2014). TA:teff-amaranth

*Absit: a portion of batter cooked for about 5 minutes to be mixed with the entire batter to induce faster fermentation.

3.7.2.5. Physicochemical properties

i. Bulk density

The bulk density of amaranth/teff/amaranth-teff blend flour was determined as described in Flade (2014). Briefly flour samples were gently transferred into 10 mL graduated cylinders that were previously weighed. The bottom of the cylinder was gently tapped on a laboratory bench several times until no further diminution of the sample level was observed after it was filled up to the 10 mL mark. Bulk density is defined as the weight of the sample per unit volume of the sample (g/mL). Measurements were made in triplicate.

ii. Water absorption capacity

Water absorption of flours was measured by the centrifugation method as described in Kaur and Singh (2005) with modification. For water absorption, samples (0.5 g) were dissolved in 5 mL of distilled water and placed in 15 mL pre-weighed centrifuge tubes. The mixtures were stirred at 5 min intervals and held for 30 min, followed by centrifugation for 30 min at 3000 g. The supernatant was decanted, the excess moisture was removed by draining for 25 min at 50 °C, and the sample was reweighed. Triplicate determinations were carried out and water absorption capacity was expressed as gram of water bound per gram of the sample on a dry basis.

iii. Oil absorption capacity

Oil absorption capacity of flours was measured by the method described in Kaur and Singh (2005) with minor modification. Samples (0.5 g) were mixed with 5 mL soybean oil in pre-weighed centrifuge tubes and stirred for 1 min. After a holding period of 30 min, the tubes were centrifuged at 3000 g for 30 min. The oil was then removed with a pipette when it formed a separate layer; the tubes were inverted for 25 min to drain the oil prior to reweighing. Triplicate determinations were carried out and oil absorption capacity was expressed as gram of oil bound per gram of the sample on a dry basis.

iv. Measurement of pH

During fermentation, the pH of the slurry was recorded using a WTW 340i pH meter. The rate of change in pH ($-dpH/dt$) was also calculated for each combination.

3.7.2.6. Determination of proximate composition

The proximate composition was analyzed using AOAC procedures (AOAC 2000) and results were expressed as g/100 g dry matter

i. Dry matter (DM)

The dry matter content of each sample was determined by oven drying at 105 °C to a constant weight (AOAC 925.09).

ii. Crude Protein

The Nitrogen content of all samples was determined using Kjeldhal method (AOAC 979.09) and a conversion factor of 5.85 was used to calculate crude protein content (Berghofer & Schoenlechner 2002).

iii. Crude Fat

Crude fat determination was performed by extraction using Soxhlet apparatus (Soxtec™ 2050) for 4 h with diethyl ether as extraction solvent (AOAC 920.39)

iv. Ash

Total ash content was determined gravimetrically by weighing 2.5 g of milled sample in pre-dried crucibles and incinerating at 550 °C until the colour of the ash became white/gray (AOAC 923.03).

3.7.2.7. Mineral analysis

The analysis of minerals such as iron and zinc was made by flame atomic absorption spectrophotometry (AA800, Perkin Elmer, Les Ulis, France) after digesting 0.4 g of flour with a mixture of 7 mL of concentrated nitric acid and 3 mL hydrogen peroxide for 30 minutes.

3.7.2.8. Phytic Acid Determination in Injera

i. Sample Extraction

Phytic acid was determined based on the Megazyme phytic acid/total phosphorus assay procedure (Megazyme International, Ireland). Briefly, 1 g of sample was weighed into 75 mL glass beaker. To this 20 mL of 0.66 N HCl was added and stirred for 3 h at room temperature (22 ± 2 °C) for extraction of phytic acid and free phosphorus. After extraction, aliquot (about 1.5 mL) was transferred into a test tube and centrifuged for 20 minutes at 3000 rpm. Exactly 0.5 mL of the resulting extract was transferred into another test tube and neutralized with 0.5 mL of sodium hydroxide (0.75 M). The neutralized sample extract was used for the enzymatic dephosphorylation reaction.

ii. Enzymatic Dephosphorylation Reaction

a) *Determination of free phosphorus*

To a 50 μ L sample extract (obtained from section 3.7.2.6. i), 620 μ L water was added and the pH was adjusted using 200 μ L buffer solution (pH 5.5). The mixture was vortex mixed and incubated at 40 °C for 10 min. After incubation, 20 μ L distilled water and 200 μ L buffer (pH 10.4) was added and this mixture was vortex mixed and incubated at 40 °C for 15 minutes followed by addition of trichloroacetic acid (50% w/v). The mixture was centrifuged at 3000 rpm for 20 minutes and the supernatant was used for colorimetric determination.

b) *Determination of Total phosphorus*

To a 50 μ L sample extract (obtained from section 3.7.2.6. i), 600 μ L water was added and the pH was adjusted using 200 μ L buffer solution (pH 5.5) followed by addition of 20 μ L phytase suspension. The mixture was vortex mixed and incubated at 40 °C for 10 minutes. After incubation, 200 μ L buffer (pH 10.4) was added immediately followed by addition of 20 μ L alkaline phosphatase. This mixture was vortex mixed and incubated at 40 °C for 15 minutes.

The reaction was stopped by adding 300 μ L trichloroacetic acid (50% w/v) and centrifuged for 20 minutes at 3000 rpm. The total phosphorus was determined using colorimetric method.

c) Colorimetric Determination of phosphorus

One mL of the supernatant from section 3.7.2.6.ii a and b was carefully transferred into 15 mL plastic test tubes and 0.5 mL of color reagent (mixture of ascorbic acid (10%) and ammonium molybdate (5% w/v)) was added. The mixture was vortex mixed and incubated for 1 h at 40 °C. Absorbance was measured spectrophotometrically at 655 nm after incubation using Perkin Elmer Lambda 950 UV/Vis/NIR spectrophotometer. Phosphorus standard solutions of concentration ranging from 0 to 10 μ g/mL was used to draw calibration curve. The phytic acid content was determined by taking the difference between total phosphorus and free phosphorus concentration.

3.7.2.9. Sensory Evaluation of Injera Samples

Sensory evaluation for the prepared injera samples was conducted by 50 semi-trained consumer panelists, 25 males and 25 females, using acceptability test for nine attributes such as color, surface gas holes, suppleness, appearance, texture, mouthfeel, taste, flavor and overall acceptability. Panelists who were composed of staff and students of the Center for Food Science and Nutrition, Addis Ababa University were instructed to evaluate all the five injera samples prepared from 100% teff (control), 90% teff and 10% amaranth (90/10TA), 80% teff and 20% amaranth (80/20TA), 70% teff and 30% amaranth (70/30TA), 60% teff and 40% amaranth (60/40TA) in a randomized order which are labeled with three digit code for the aforementioned sensory attributes using a seven point hedonic scale (dislike extremely = 1; dislike very much = 2; dislike moderately = 3; neither like nor dislike = 4; like moderately = 5; like very much = 6; like extremely = 7). Panelists were instructed to cleanse their mouth before testing the next sample with odour and flavor free water. Test evaluation was conducted at the Center for Food Science and Nutrition, College of Natural Science, Addis Ababa University.

3.7.3. Statistical analysis

Data were submitted to the software SPSS version 19 and analyzed using one way analysis of variance (ANOVA). Duncan multiple range test was used to compare the means and p-value were used to show their significance. Differences of $p < 0.05$ were considered significant.

3.7.4. Results and discussion

3.7.4.1. Physicochemical properties of teff, amaranth and a blend of teff and amaranth flour

i. Bulk density

Bulk density is a useful parameter in determining packaging requirement and material handling in the food industry. The bulk density of teff flour (6.26 g/mL) was significantly higher than that of amaranth flour and a blend of teff and amaranth flour with values ranging between 6.13-6.21 g/mL (Table 3.7.1). Within the same volume of packaging material higher amount of teff flour could be packaged than amaranth flour or a blend of teff and amaranth flour indicating a relatively low cost requirement to package teff flour than amaranth flour. On the contrary, low bulk density would be an advantage in the formulation of complementary foods (Akpata and Akubor, 1999) and thus amaranth is the most suitable for the production of complementary foods.

ii. Water and oil absorption capacity

Water absorption capacity (WAC) is strongly related to the nature of the starch granule and those having low level of disintegration will have low potential absorption capacities. As there was no processing method applied that could damage the starch, except the milling process, a low level of water absorption capacity for pure teff flour, a blend of teff and amaranth flour and pure amaranth flour was obtained in the range of 0.99-1.11 g/g. However, pure amaranth flour was found to have significantly higher WAC than teff flour. This might be due to the presence of higher polar or charged protein molecules in amaranth than in teff

(Lawal and Adebawale, 2004). The high water absorption capacity of amaranth flours suggests that they would be useful functional ingredients in bakery products.

The oil absorption capacity (OAC) indicates the emulsifying capacity and necessary to improve the flavor and mouthfeel characteristics (Escamilia-Silva et al., 2003). The mechanism of oil absorption may be explained as a physical entrapment (hydrophobic interaction) between non-polar amino acid side chains and hydrocarbon chains of lipid (Jitngarmkusol et al., 2008). Therefore, both the type and amount of protein contribute to the oil-retaining properties of food materials (Ravi and Sushelamma 2005). The oil absorption capacity for pure teff flour, a blend of teff and amaranth flour and pure amaranth flour was obtained in the range of 0.79-0.86 g/g. Amaranth, being known to have higher protein content than teff (table 3.7.3), showed a higher OAC though this is statistically insignificant. The result indicates that both amaranth and teff flour may have closely related amino acid composition to demonstrates comparable OAC (Lawal and Adebawale, 2004). High OAC is a highly desirable quality in the formulation of food systems like sausages, cake, batters, mayonnaise and salad dressings.

Table 3.7.1 Functional properties of teff, amaranth and a blend of teff-amaranth flour[†]

Sample	Bulk density	Water absorption capacity	Oil absorption capacity
	(g/ml)		(g/g)
Control	6.26 ± 0.33a	1.03 ± 0.02b	0.83 ± 0.05ab
90/10TA	6.21 ± 0.01b	0.99 ± 0.03c	0.82 ± 0.02ab
80/20TA	6.20 ± 0.00b	1.01 ± 0.02ab	0.79 ± 0.02b
70/30TA	6.20 ± 0.02b	1.01 ± 0.00ab	0.81 ± 0.02ab
60/40TA	6.19 ± 0.01b	0.99 ± 0.00c	0.81 ± 0.07ab
100A	6.13 ± 0.01c	1.11 ± 0.02a	0.86 ± 0.02a

[†]Values are mean ± SD of triplicate measurements. Means followed by different letters in the same column are significantly different at p < 0.05. control-100% teff, 90/10TA-90% teff and 10% amaranth, 80/20TA-80% teff and 20% amaranth, 70/30TA-70% teff and 30% amaranth, 60/40TA-60% teff and 40% amaranth, 100A-100% amaranth flour.

iii. pH kinetics of teff and teff-amaranth blend sourdough

The initial and final pH of sourdough made from teff and amaranth flour blends with increasing proportion of amaranth from 0 to 40% showed very little or no difference (Table 3.7.2). Moreover, the pH kinetics also showed no visible difference as seen in the superimposed curves in Figure 3.7.2. The maximum rate of pH decrease $(-dpH/dt)_{max}$ was the same for both a sole teff and teff-amaranth blended dough than the control and this is close to the reports for teff-sorghum and barley-wheat blended dough (Baye et al., 2013).

Table 3.7.2 Kinetic parameters of pH change during injera sourdough fermentation

Sample*	Initial pH	Final pH	$(-dpH/dt)_{max}$	Time to reach $(-dpH/dt)_{max}$ (h)
Control	5.92 ± 0.01	4.15 ± 0.00	0.13	6
90/10TA	5.91 ± 0.08	4.18 ± 0.04	0.14	6
80/20TA	5.99 ± 0.03	4.18 ± 0.00	0.14	6
70/30TA	5.99 ± 0.01	4.12 ± 0.01	0.15	6
60/40TA	5.97 ± 0.01	4.02 ± 0.01	0.15	6

*Control-100% teff, 90/10TA-90% teff + 10% amaranth, 80/20TA-80% teff + 20% amaranth, 70/30TA-70% teff + 30% amaranth, 60/40TA-60% teff + 40% amaranth.

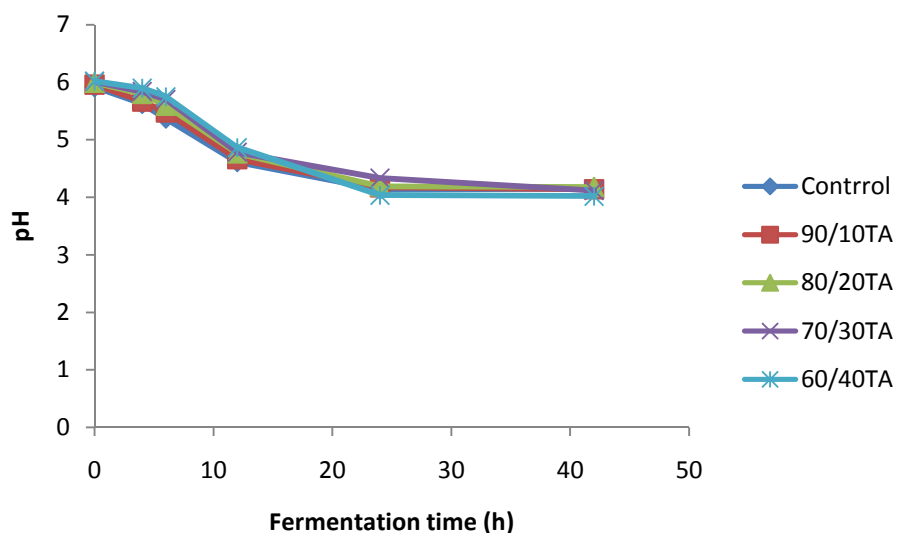


Figure 3.7.2 pH kinetics for teff and teff-amaranth flour blend doughs during fermentation. Control-100% teff, 90/10TA-90% teff and 10% amaranth, 80/20TA-80% teff and 20% amaranth, 70/30TA-70% teff and 30% amaranth, 60/40TA-60% teff and 40% amaranth.

3.7.4.2. Proximate composition of teff and teff-amaranth blended Injera

Table 3.7.3 shows the proximate composition of amaranth-teff blended injera. The protein, fat and ash contents of amaranth-teff blend injera significantly increased ($p < 0.05$) as the percentage of amaranth used increased. Forty percent addition of amaranth to teff improved the protein, fat and ash contents of injera by 20, 86 and 18%, respectively, showing the better nutrient content of amaranth than teff. The total carbohydrate contents determined by difference showed decreasing trend as the percentage of amaranth increased.

Table 3.7.3 Proximate composition of teff and teff-amaranth blended injera†

Sample*	DM	Protein	Fat	Carbohydrate	Ash
	(g/100g)	(mg/100g DM)			
Control	93.50	12.14 ± 0.10d	2.34 ± 0.07e	83.01 ± 0.15	2.51 ± 0.01e
90/10TA	93.40	13.01 ± 0.11c	2.68 ± 0.14d	81.69 ± 0.19	2.62 ± 0.04d
80/20TA	94.60	13.78 ± 0.28b	3.20 ± 0.09c	80.22 ± 0.20	2.79 ± 0.04c
70/30TA	95.09	13.98 ± 0.09b	3.82 ± 0.02b	79.33 ± 0.09	2.87 ± 0.02b
60/40TA	94.12	14.59 ± 0.32a	4.35 ± 0.05a	78.10 ± 0.34	2.96 ± 0.01a

†Values are mean of triplicates ± SD. Means followed by different letters in the same column are significantly different at $p < 0.05$.

*Control-100% teff, 90/10TA-90% teff and 10% amaranth, 80/20TA-80% teff and 20% amaranth, 70/30TA-70% teff and 30% amaranth, 60/40TA-60% teff and 40% amaranth.

3.7.4.3. Mineral content of teff and teff-amaranth blended Injera

The iron content of the control injera was significantly lower ($p < 0.05$) than teff-amaranth blended injera and showed a general increasing trend as the percentage of amaranth blended increased. Thirty percent amaranth blended injera had significantly higher ($p < 0.05$) iron content than 40% amaranth blended one. This could possibly be due to contamination from the baking clay pan or other source of contamination. Previous reports on iron content of teff injera showed that there exist high variation due to the contamination from soil iron (Umeta et al., 2005, Abebe et al., 2007) but washing with water or acid and threshing the stalk in the lab reduced the susceptibility to contamination with extrinsic iron (Hallberg and Bjorn-Rasmussen, 1981, Ambaw, 2013). In this study, teff grain was properly cleaned to remove sand and dust and washed several times with distilled water as a result the content of iron in injera prepared from this sample was found to be very low but slightly higher than that of

presumptive non-contaminated teff flour, taken from the US National Nutrient Database (U.S. Department of Agriculture and N.D.L.H.P., 2012), 7.6 mg/100 g.

Addition of amaranth to teff also showed a significant increase ($p < 0.05$) in the content of zinc compared to the control injera and the maximum increment was by about 10% upon blending 40% amaranth (Table 3.7.4). The content of both iron and zinc in the control injera (white teff injera) was higher than injera prepared from white teff collected from Sidama, Ethiopia (Abebe et al., 2007). Variation in the type of soil that the crop grows could affect the content of minerals.

Table 3.7.4 Iron and Zinc and phytic acid content of teff and teff-amaranth blended injera[†]

Sample*	Fe	Zn (mg/100g DM)	Phytic acid	Phytate:	
				Fe	Zn
Control	9.12 ± 0.20d	3.15 ± 0.01d	459.44 ± 6.00d	4.26	14.45
90/10TA	9.79 ± 0.21bc	3.23 ± 0.00c	596.49 ± 3.22a	5.16	18.30
80/20TA	9.68 ± 0.11c	3.38 ± 0.04b	540.79 ± 2.03b	4.73	15.86
70/30TA	12.03 ± 0.49a	3.41 ± 0.04b	527.56 ± 6.04c	3.71	15.33
60/40TA	10.29 ± 0.26b	3.47 ± 0.03a	447.25 ± 1.95e	3.68	12.77

[†]Values are mean of triplicates ± SD. Means followed by different letters in the same column are significantly different at $p < 0.05$. *Control-100% teff, 90/10TA-90% teff + 10% amaranth, 80/20TA-80% teff + 20% amaranth, 70/30TA-70% teff + 30% amaranth, 60/40TA-60% teff + 40% amaranth.

3.7.4.4. Phytic acid content of teff and teff-amaranth blended Injera

The fermentation process used “ersho”, left over of a previous fermentation, to trigger the fermentation process and the phytic acid content obtained in the final baked injera from teff was 460 mg/100g DM which is lower than that reported by Fisher et al. (2014) utilizing the same starter culture to commence the fermentation but much higher than that found in teff injera from Sidama, Ethiopia (Abebe et al., 2007). However, in the same report, inoculating high phytase producing lactic acid bacteria (*L. buchneri*) as a starter culture resulted in lower content of phytate. When 10% amaranth was added the content increased to 596 mg/100g DM but further increase in the percent of amaranth blended resulted in a decrease in the level of phytic acid although the content of phytic acid in amaranth was higher, 2.2 g/100g DM (Amare et al., 2015, unpublished result) than that found in teff (Fisher et al. 2014). This might

be attributed to the higher phytase activity of amaranth (Egli et al., 2002) than teff, predicted from the results for teff and sorghum blend (Baye et al., 2013, Egli et al., 2002) promoting high phytic acid degradation when the percentage of amaranth increased. Injera prepared by blending 40% amaranth to teff resulted in a significantly lower phytic acid content than the control. Further degradation in phytic acid could also be possible in the product if the fermentation was conducted by isolating high phytase producing strains.

3.7.4.5. Estimated mineral bioavailability of teff and teff-amaranth blended Injera

The molar ratios of phytate-to-mineral were calculated to predict the bioavailability of iron and zinc. The result shows that none of the products meet the recommendation for phytate:Fe suggested by Hurrell (2004). But the suggested recommendation for phytate:Zn (Gibson 2006) was fulfilled in all the products except injera prepared by blending 10% amaranth. It is therefore necessary to enhance the bioavailability of these problem nutrients either by degrading the phytic acid further with inoculating high phytase producing microorganisms or fortifying with highly bioavailable fortificants or consuming the product with foods containing mineral absorption enhancers.

3.7.4.6. Sensory acceptability of teff and amaranth-teff blended Injera

Table 3.7.5 shows the results of sensory evaluation. The color of the control injera and teff-amaranth blended injera exhibited no significant difference. The type of teff used for the current study was white and thus may not be different in color with that of the color of white amaranth used in the study. Increasing the percentage of amaranth used for preparing injera resulted in a statistically non significant decrease in the surface gas holes. Blending amaranth until 20% did not bring any significant effect on the suppleness of injera but significantly decreased ($p < 0.05$) thereafter. The sensory score results for the front and back side appearance of injera decreased as the percentage of amaranth blended increased. There was no significant difference ($p < 0.05$) observed in the texture of control and amaranth-teff blended injera but a slight variation was observed among teff-amaranth blended injera. The score for mouthfeel and taste of the products decreased as the percentage of amaranth blended

increased but significant for 40% addition in mouth feel and after 30% addition in taste of the products. The flavor of the product significantly decreased ($p < 0.05$) as the percentage of amaranth increased. The overall acceptability result shows that up to 20% amaranth addition to teff will not bring significant effect ($p < 0.05$) but beyond that the acceptability significantly decreased ($p < 0.05$) compared to the control product. In terms of cost, blending 20% amaranth could reduce the total cost of injera by about 14% (informal communication).

Table 3.7.5 Sensory acceptability test for teff and teff-amaranth blended injera†

Sample*	Injera quality attributes									
	Color	Surface gas holes	Suppleness	Appearance		Texture	Mouth feel	Taste	Flavor	Overall acceptability
				Back	Fore					
Control	5.6 ± 0.7a	5.2 ± 1.2a	5.7 ± 0.7a	5.4 ± 1.1ab	5.3 ± 1.4a	5.0 ± 1.4abc	5.2 ± 1.2a	5.5 ± 1.2a	5.6 ± 0.9a	5.4 ± 0.9a
90/10TA	5.5 ± 1.1a	5.0 ± 1.3a	5.7 ± 0.9a	5.6 ± 0.9a	5.2 ± 1.1a	5.4 ± 1.0a	5.2 ± 1.3a	5.4 ± 1.5a	5.5 ± 1.3ab	5.3 ± 1.3a
80/20TA	5.6 ± 0.9a	5.1 ± 1.1a	5.7 ± 1.1a	5.3 ± 1.1ab	5.2 ± 1.2a	5.3 ± 1.1ab	4.9 ± 1.3a	5.0 ± 1.2a	4.9 ± 1.2b	5.1 ± 1.2a
70/30TA	5.1 ± 1.3a	4.9 ± 1.4a	4.9 ± 1.5b	5.0 ± 1.4b	4.7 ± 1.4ab	4.7 ± 1.5bc	4.1 ± 1.7a	4.5 ± 1.2b	4.2 ± 1.6c	4.3 ± 1.6b
60/40TA	5.2 ± 1.3a	4.6 ± 1.3a	4.9 ± 1.3b	4.8 ± 1.4b	4.5 ± 1.5b	4.5 ± 1.5c	4.0 ± 1.1b	4.0 ± 1.8b	4.2 ± 1.6c	4.2 ± 1.5b

†Values are mean of fifty measurements ± SD. Means followed by different letters in the same column are significantly different at p < 0.05. *Control-100% teff, 90/10TA-90% teff + 10% amaranth, 80/20TA-80% teff + 20% amaranth, 70/30TA-70% teff + 30% amaranth, 60/40TA-60% teff + 40% amaranth.

3.7.5. Conclusion

Most of children under-five years in Ethiopia consume, injera prepared from different cereals as a complementary food. Blending different cereals to prepare such complementary foods has been found to be one way of tackling malnutrition using food based approaches. Thus this study strongly recommends the utilization of amaranth, a nutrient rich cereal, as an ingredient for the formulation of complementary food especially for fermented products like injera due to the very high level of phytic acid in raw amaranth which could significantly be decreased during fermentation. Due to the inherent nature of amaranth grain flour, preparation of injera solely from amaranth with acceptable sensorial attributes was not possible unless further modification of the characteristic nature of the flour and/or modification of the overall process followed to prepare teff injera. Generally this study shows that up to 20% amaranth blending in to teff has been found to be equally acceptable as injera prepared from teff grain only. Moreover, the current price of amaranth in the place where the sample was collected was about three fold less than that of teff and thus found to be a good means to reach underprivileged societies.

4. Chapter Four: General Conclusion and Recommendation

4.1. Conclusion

The study evaluates the nutritional qualities and health benefits of three different types of *Amaranthus caudatus* grain cultivated under the agroecological conditions of Ethiopia. The macro- and micronutrient contents, mineral absorption inhibitors (IP6 and Iron binding polyphenols, galloyls and catechol), free and total amino acid profile, protein fractions and digestibility, starch fractions and digestibility, bioactive compounds (total polyphenol, total flavonoid and GABA) and antioxidant activity using DPPH and ABTS assays were determined. The effect of commonly utilized processing methods for cereal food preparation, popping and fermentation, on the overall nutritional qualities and health benefits was evaluated. Moreover, combining treatments such as soaking (at optimum pH and temperature for endogenous phytase enzyme) and malt addition was applied in order to see the quality changes consequent to phytate degradation and starch hydrolysis.

The study showed that all the three types amaranth are rich sources of protein and fat compared to most commonly utilized cereals like teff, barley, wheat, maize and rice etc. They are also a good sources of iron, calcium and magnesium. Moreover, due to the small size of the grain, amaranth is not able to be decorticated and thus maintains micronutrients and dietary fibers concentrated in the bran. Therefore, amaranth could potentially complement both cereals and legumes to narrow down deficiencies existing in those commonly utilized cereals and legumes especially during complementary food formulation.

Although formulation of complementary food from a single ingredient doesn't promote dietary diversity and thus fails to ensure nutrition security, the attempt made to compare a sole amaranth based complementary food using popped amaranth, as served in the study area, with the fortified and non-fortified commercial complementary food referring to the adequacy of iron, zinc, protein, fat and energy to the daily requirement for 6-23 month old children showed that amaranth based complementary foods better contributes to the requirement for

protein, fat, energy. Nevertheless, both the commercial and amaranth based CFs contain high levels of phytic acid that could hamper the absorption of minerals and digestibility of macronutrients.

The amino acid profile shows that all the three amaranth types meet the WHO/FAO recommendation for infants and young children. As a result, the crop could potentially substitute other more common cereals used in complementary food formulation for young children, and thereby allowing a reduction in the proportion of legumes which generally have high antinutritional content. In general, in developing countries, where animal source food are not easily accessed and not regularly consumed, amaranth protein could serve as a good source of essential amino acids and therefore help to enrich protein deficient or low quality protein diets for all age groups. However, estimation of the contribution amino acids to daily requirement should take into account the digestibility of the protein which in the present study, caused a maximum of 30% reduction in essential amino acid contribution in all raw and processed samples.

Due to the small granular size and low amylose content, more than 80% of the starch in amaranth porridge was hydrolyzed and improved after popping and fermentation. This is a good attribute for individuals requiring quick energy from the meal ingested like athletes and children. The limited gastric capacity of children could only allow them to consume small amount of food. However, if the food is highly digestible, it helps them to satisfy their energy requirement by increasing their meal frequency.

The content of bioactive compounds in amaranth especially total polyphenols and total flavonoids are better than some common cereals like wheat, rice and oats but lower than their pseudocereal counterparts: quinoa and buckwheat. Nevertheless, it could be used as an ingredient in functional food formulation especially for celiac patients. The content of the above bioactive compounds, predominately known for their antioxidant action, and γ -aminobutyric acid, that impart a role in lowering blood pressure was increased after germination and fermentation. Therefore, the use of malted and fermented amaranth as ingredient in food formulation could enhance the health benefits of amaranth as demonstrated

by higher antioxidant activity of germinated and fermented amaranth than the raw in the present study.

The nutritional limitation with the studied amaranth types, especially for the formulation of complementary foods, was the presence of high levels of phytic acid that could potentially decrease the absorption of minerals and digestibility of starch and protein. Processing methods applied in the present study, popping and fermentation, decreased the content of phytic acid but the amount left after the process is much higher than what is recommended to demonstrate minimal effect on bioavailability of iron and zinc. Although the extent of phytic acid degradation was better after fermentation than popping, the type of fermentation does not allow complete degradation of phytic acid as observed in other cereal fermentation for the preparation of injera.

The attempt to degrade phytic acid in raw and popped amaranth using endogenous amaranth phytase at optimum pH and temperature by combining two treatments: soaking and malt addition caused a decrease in the content of phytic acid to a larger extent. The treatment also improves the energy and nutrient density of porridge, prepared from the slurry, due to hydrolysis of starch by amylases of added malt especially in popped amaranth. Nevertheless, the treatment is still not enough to convey nearly complete degradation of phytic acid. Therefore, in order to get better phytic acid degradation it is good to combine fermentation along with the above treatments.

Amaranth has been used to prepare different types of foods like pasta, cake, bread, cookies, crackers etc in many countries. But due to the presence of high level of phytic acid, the use of amaranth to prepare the aforementioned foods may adversely affect mineral absorption. Therefore, preparation of injera could be one preferred way to alleviate this effect as the preparation involves fermentation as a major processing method that could degrade the level of phytic acid much better than other heat treatment techniques. The attempt to prepare teff-amaranth injera was successful and blending 20% amaranth into teff provides good quality injera. Moreover, it also adds variety to the diet and may have useful health promoting properties, particularly antioxidant activity.

4.2. Recommendation

Amaranth is a reliable and low risk cereal that grows on a wider ecology under moisture stress and waterlogged areas with few plant diseases and grain storage pest problems. However, due to the high content of phytic acid in the studied amaranth samples, the promotion to formulate complementary food for infants and young children should be done with great caution involving best processing methods to degrade phytic acid. The selection and breeding of other low phytic acid containing amaranth is also strongly recommended.

Further treatments like inoculation of high phytase producing microorganisms prior to the preparation of amaranth porridge could also be an alternative approach to exploit the potential of the crop for complementary feeding. Moreover, the use of amaranth protein concentrate might lower the level of IP6 and could be a best strategy to exploit amaranth application.

In general, the dephytinization process should be combined with enrichment with animal-source foods and/or fortification with appropriate levels and forms of mineral fortificants as the amount and bioavailability of the major problem nutrients are low in plant foods.

Experimental result on the nutrient content of commercial complementary foods also revealed that the design and formulation of those complementary foods should be done with a good knowledge of raw material selection and application of best processing methods. Moreover, selection of the type of fortificant and level of fortification should also be done with great concern to satisfy the nutrient needs of children.

Limitation of the study

The three different types of *Amaranthus caudatus* grains were collected from a single kebele and may not represent other types of amaranth grown in a different agro-ecology than the current study area in the country.

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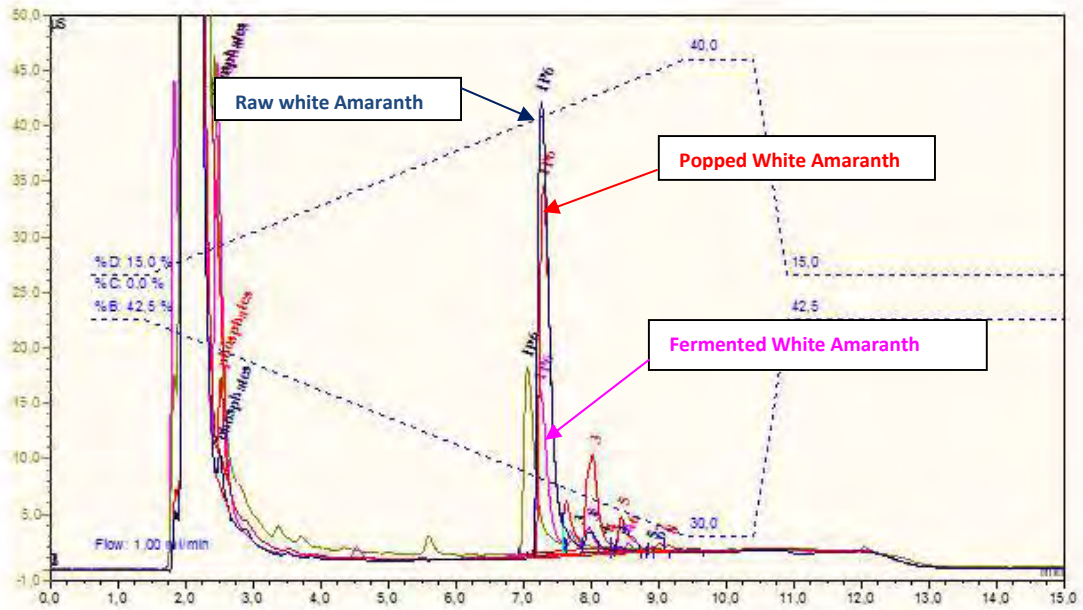
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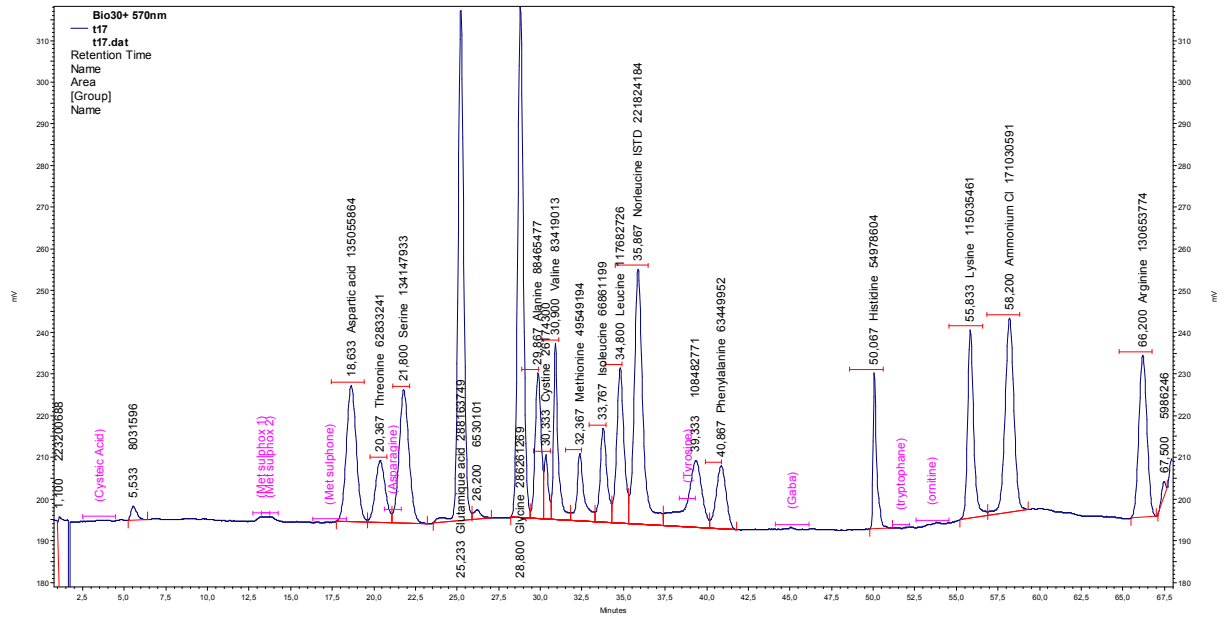
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Annexes

Annex 3.1.1 Chromatogram showing the effect of processing on IP6 degradation



Annex 3.4.1 Chromatogram of total amino acid profile in amaranth



Annex 3.7.1 Sensory Evaluation Acceptability Test Score Sheet

You are given servings of Injera to test and express your degree of liking of each sensory attributes provided. Test each sample and indicate your response by marking (x) in the corresponding acceptances. Please rinse your mouth between tastes to remove after tastes.

Thank you for your participation.

Name _____

Sex _____

Date _____

Acceptance with scale		Sensory Attribute									
		Color	Surface gas holes	Suppleness	Appearance		Texture	Mouth feel	Taste	Flavor	Over all acceptability
					Back	Fore					
Like Extremely	7										
Like very much	6										
Like Moderately	5										
Neither like nor dislike	4										
Dislike moderately	3										
Dislike very much	2										
Dislike Extremely	1										

Comment

Thank you
