INSTITUTO TECNOLÓGICO Y DE ESTUDIOS SUPERIORES DE MONTERREY

CAMPUS MONTERREY

ESCUELA DE INGENIERÍA Y TECNOLOGÍAS DE INFORMACIÓN

EFFECTS OF SORGHUM DIGESTIBILITY, ENDOSPERM TEXTURE AND TYPE AND PHENOLIC PROFILE ON POSTHARVEST RESISTANCE TO MAIZE WEEVIL AND FUEL ETHANOL PRODUCTION

THESIS SUBMITTED

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR ON PHILOSOPHY

WITH A MAJOR IN BIOTECHNOLOGY

BY

CRISTINA ELIZABETH CHUCK HERNÁNDEZ

MONTERREY, N. L. December 2012

INSTITUTO TECNOLÓGICO Y DE ESTUDIOS SUPERIORES DE MONTERREY

CAMPUS MONTERREY

ESCUELA DE INGENIERÍA Y TECNOLOGÍAS DE INFORMACIÓN

EFECTOS DE LA DIGESTIBILIDAD, TEXTURA, TIPO DE ENDOSPERMO Y PERFIL DE FENOLICOS DE SORGO EN RESISTENCIA POSTCOSECHA AL GORGOJO DEL MAIZ Y PRODUCCION DE BIOETANOL

PRESENTADA COMO REQUISITO PARCIAL PARA OBTENER EL GRADO ACADÉMICO DE:

DOCTORA EN CIENCIAS DE LA INGENIERÍA CON

ESPECIALIDAD EN BIOTECNOLOGÍA

POR

CRISTINA ELIZABETH CHUCK HERNÁNDEZ

MONTERREY, N. L. Diciembre 2012

ACKNOWLEDGMENTS

I would like to wholeheartedly thank to my thesis director, Dr. Sergio Serna Saldívar for the trust and inspiration, for his support, encouragement and for his always contagious energy and enthusiasm. I´m deeply in debt with you.

I would like also to wholeheartedly thank to my thesis co-director, Dr. Silverio García Lara for his patience, inspiration, help, guidance and the opportunity to work with him. Without you, this project wouldn´t be possible. Many thanks.

I would like to extend my sincere gratitude to my thesis committee: Dr. Jorge Welti Chanes, Dr. José G. Ríos Alejandro and Dr. Luis Damas Buenrostro for their time, comments, manuscript corrections and for their very punctual advises. Your point of view was very valuable for me.

I´m very grateful with Dra. Esther Pérez, Mayeli Peralta, Erick Heredia, Ana Chew, Isela Baez, Gabriela Bando, Mariano Vera, Nallely Gaxiola, Ranses Tamayo, Feliznando Cardenas, for their help and always kind support.

Many thanks to Dr. Manuel Zertuche, Dra. Janet Gutiérrez, Dr. Daniel Jacobo and Dr. Hugo Mújica for their support and counseling.

Special thanks to Jessica González and Ricardo Luna for their help, friendship and lab support.

I would like to express my gratitude to George Mahuko, Emiliano Gutiérrez and Carlos Muñoz (CIMMYT Entomology and Phytopathology Laboratory) for their technical support, as well as Beatriz Moreno, Alejandra Castañeda and Enrique García (Centro de Biotecnología, Tecnológico de Monterrey). Many thanks to Dr. Dirk Hays for sorghum cultivars provided.

This research was possible thanks to the financial support of: Research Chair Fund CAT-005 from Tecnológico de Monterrey and the PhD scholarship by CONACyT and Tecnológico de Monterrey. Thanks so much for the sponsorship.

My deepest gratitude to my family: my mother, sisters and brother for their lovely support and to Fernando for his encouragement in the moments when I needed the most, for being in good and bad times with me. Because of you, this goal in my life was achieved. You are my strength.

DEDICATION

To Betty, Norma, Selina, Linda, Cuqui, Anuar and Fernando.

"Atravesando muros, atmósferas, edades,

tu rostro […] viene desde la muerte, desde antes

del primer día que despertara al mundo"

Jaime Sabines

GENERAL CONTENT

GENERAL CONTENT i

LIST OF FIGURES viii

LIST OF TABLES ix

ABBREVIATIONS xi

KEYWORDS xii

ABSTRACT xiii

1. BACKGROUND 1.1

1.1. Importance of Sorghum 1.2

1.2. Physicochemical Characteristics of Sorghum Grain 1.3

1.2.1. Anatomical Portions 1.3

1.2.2. Proximate Composition 1.5

1.2.2.1. Starch 1.5

1.2.2.2. Protein 1.7

1.2.2.3. Fiber 1.8

1.2.2.4. Lipids 1.8

1.2.2.5. Ash 1.9

1.3. Phenolics in Sorghum Grain 1.9

1.4. Digestion Properties of Sorghum Grain 1.15

1.5. Postharvest Losses in Sorghum Grain 1.17

1.6. Pest Resistance Mechanisms in Sorghum Grain During Storage 1.18

1.7. Fuel Ethanol from Sorghum Grain 1.20

1.8. Sorghum Grain as Part of the Productive Chain 1.22

1.9. HYPOTHESIS 1.24

1.10. OBJECTIVES 1.24

1.10.1. General Objective: 1.24

1.10.2. Specific Objectives: 1.24

2. EFFECTS OF ADDITION OF RED SORGHUM (*SORGHUM BICOLOR* L. MOENCH) PHENOLICS OR SPENT BRAN ON THE PERFORMANCE OF DECORTICATED KERNELS BIOCONVERTED INTO ETHANOL* 2.1

2.1. ABSTRACT 2.2

2.2. INTRODUCTION 2.3

2.3. MATERIALS AND METHODS 2.4

2.3.1. Grain Sources 2.4

2.3.2. Decortication, Milling and Particle Size Distribution 2.5

2.3.3. Extraction of Phenolic Compounds and Spent-Bran Production 2.5

2.3.4. Analytical Methods 2.5

2.3.5. Treatments 2.6

2.3.6. Liquefaction 2.6

2.3.7. Saccharification 2.7

2.3.8. Fermentation 2.7

2.3.9. Statistical Analysis 2.7

2.4. RESULTS AND DISCUSSION 2.8

2.4.1. Grain Physical Properties and Particle Size Distribution. 2.8

2.4.2. Chemical Composition 2.8

2.4.3. Sugars during Enzymatic Treatments 2.11

2.4.4. Total Soluble Sugars throughout Saccharification. 2.14

2.4.5. Sugars and Ethanol Profile during Fermentation 2.16

2.5. CONCLUSIONS 2.20

3. CONVERSION INTO BIOETHANOL OF INSECT (*SITOPHILUS ZEAMAIS* MOTSCHULSKY), MOLD (*ASPERGILLUS FLAVUS* LINK) AND SPROUT-DAMAGED MAIZE (*ZEA MAYS* L.) AND SORGHUM (*SORGHUM BICOLOR* L. MOENCH)* 3.1

3.1. ABSTRACT 3.2

3.2. INTRODUCTION 3.3

3.3. MATERIALS AND METHODS 3.4

3.3.1. Grain Sources and Evaluated Treatments 3.4

3.3.2. Sample Preparation 3.4

3.3.2.1. Insect Infestation 3.5

3.3.2.2. Mold Inoculation 3.5

3.3.2.3. Grain Sprouting 3.5

3.3.2.4. Milling 3.6

3.3.3. Physical and Chemical Characterization 3.6

3.3.4. Liquefaction 3.6

3.3.5. Simultaneous Saccharification and Fermentation (SSF) 3.7

3.3.6. Analysis of Hydrolyzates and Fermented Mashes 3.7

3.3.7. Statistical Analysis 3.7

3.4. RESULTS AND DISCUSSION 3.8

3.4.1. Physical and Chemical Analysis 3.8

3.4.2. Reducing Sugars (RdS) and FAN during Liquefaction 3.14

3.4.3. FAN, Glucose, Glycerol, Ethanol and Fermentation Efficiency during

SSF 3.17

3.5. CONCLUSIONS 3.20

4. SUSCEPTIBILITY OF DIFFERENT TYPES OF SORGHUMS DURING STORAGE TO *SITOPHILUS ZEAMAIS* MOTSCHULSKY 4.1

4.1. ABSTRACT 4.2

4.2. INTRODUCTION 4.3

4.3. MATERIALS AND METHODS 4.4

4.3.1. Grain Varieties 4.4

4.3.2. Sample Preparation 4.4

4.3.3. Physical Characterization 4.5

4.3.4. Chemical Characterization 4.5

4.3.5. Extraction and Determination of Free and Bound Phenolics 4.6

4.3.6. Antioxidant Capacity (AOXC) 4.6

4.3.7. Susceptibility Tests 4.6

4.3.7.1. Insect Culture 4.6

4.3.7.2. Confinement and Dobie Index (DI) Assays 4.6

4.3.8. Statistical Analysis 4.7

4.4. RESULTS AND DISCUSSION 4.7

4.4.1. Physical and Anatomical Traits for the Evaluated Sorghums 4.7

4.4.2. Chemical and Nutraceutical Composition of Sorghum Samples 4.11

4.4.3. Parameters Obtained from the Different Susceptibility Tests and their Correlation with Physical and Chemical Kernel Properties. 4.15

4.5. CONCLUSIONS 4.19

5. CHANGES ELICITED BY *SITOPHILUS ZEAMAIS* IN STARCH AND PROTEIN FRACTIONS OF FLOURS OBTAINED FROM DIFFERENT SORGHUM CULTIVARS 5.1

5.1. ABSTRACT 5.2

5.2. INTRODUCTION 5.3

5.3. MATERIALS AND METHODS 5.4

5.3.1. Grain Varieties 5.4

5.3.2. Sample Preparation 5.5

5.3.3. Chemical Characterization 5.5

5.3.4. Extraction of Phenolic Compounds for Resistant Starch Evaluation 5.5

5.3.5. Susceptibility Tests 5.6

5.3.6. Statistical Analysis 5.6

5.4. RESULTS AND DISCUSSION 5.6

5.4.1. Starch Characteristics Before and After Confinement 5.6

5.4.1.1. Total Starch (TS) 5.6

5.4.1.2. Damaged Starch (DS) 5.7

5.4.1.3. Resistant Starch (RS) 5.7

5.4.1.4. Effect of Phenolic Extraction in RS. 5.8

5.4.1.5. Amylose 5.8

5.4.1.6. Differences in Starch during Storage and Insect Infestation 5.9

5.4.2. Protein Characteristics Before and After Confinement (and Fractions of Protein) 5.9

5.4.2.1. Crude Protein (CP) 5.9

5.4.2.2. Free Amino Nitrogen (FAN) 5.11

5.4.2.3. Protein Digestibility (PD) 5.13

5.4.3. Correlations among Starch and Protein Fractions Remained in Samples after Confinement 5.14

5.5. CONCLUSIONS 5.15

6. COMMON CHARACTERISTICS OF SORGHUM GRAIN INVOLVED IN FUEL ETHANOL PRODUCTION AND ITS RESISTANCE TO MAIZE WEEVIL (*SITOPHILUS ZEAMAIS*). 6.1

6.1. ABSTRACT 6.2

6.2. INTRODUCTION 6.3

6.3. MATERIALS AND METHODS 6.4

6.3.1. Grain Varieties. 6.4

6.3.2. Sample Preparation. 6.5

6.3.3. Susceptibility Tests. 6.5

6.3.4. Ethanol Production. 6.5

6.3.4.1. Liquefaction. 6.5

6.3.4.2. Simultaneous Saccharification and Fermentation (SSF). 6.5

6.3.5. Sugars and Ethanol Determination. 6.6

6.3.6. Physical and Chemical Characteristics. 6.6

6.3.7. Extraction and Determination of Free and Bound Phenolics and Antioxidant Capacity (AOXC). 6.7

6.3.8. Statistical Analysis 6.7

6.4. RESULTS AND DISCUSSION 6.8

6.4.1. Physical and Chemical Analysis 6.8

6.4.2. Reducing Sugars (RdS) during Liquefaction 6.10

6.4.3. Ethanol Production (TEthanol), Fermentation Efficiency (FF) and Yield 6.13

6.4.4. Correlations among Hydrolysis and Fermentation Performance with Sorghum Physicochemical Characteristics and Susceptibility to *S. zeamais* 6.16

6.4.5. Principal Component Analysis (PCA) 6.21

6.5. CONCLUSIONS 6.24

7. PRINCIPAL CONCLUSIONS 7.1

8. STATEMENT OF ADDITIONAL COLABORATION 8.1

9. APPENDIX 9.1

10. REFERENCES 10.1

LIST OF FIGURES

Figure 1.1. *Sorghum bicolor* L. Moench 1.2

Figure 1.2. First five global sorghum producers in 2010 1.3

Figure 1.3. Sorghum kernel structure 1.4

Figure 1.4. A. Benzoic and B. Ferulic Acid 1.11

Figure 1.5. Condensed tannins 1.14

Figure 1.6. *Sitophilus zeamais* and its damage on maize and sorghum kernels 1.19

Figure 2.1. A) Glucose; B) Maltose; C) Maltotriose and D) Fructose during saccharification of all evaluated treatments 2.14

Figure 2.2. Effect of grain type and addition of red sorghum bran and its phenolic extract on the relative percentage of fermentable sugars obtained after liquefaction and saccharification 2.15

Figure 2.3. A) Fructose, B) Maltose and C) Maltotriose profile during fermentation for all evaluated treatments 2.17

Figure 2.4. Glucose consumption and ethanol production profile during fermentation for all evaluated treatments 2.18

Figure 3.1. Free Amino Nitrogen (FAN) concentration (mg/L) at the beginning and at the end of SSF. 3.18

Figure 4.1. Scatter plots for Dobie Index, Ash, Amylose, Alpha Amino Nitrogen, Endosperm percentage and Flotation Index, indicating the correlation among these variables. 4.18

Figure 5.1. Scatter plots for Resistant Starch, Total Starch, Crude Protein and Protein Digestibility, depicting the correlation among these variables. 5.14

Figure 6.1. Scatter plots for Endosperm Percentage, Crude Protein, Total Starch, Reducing Sugars during liquefaction at 100 and 200 minutes of reaction, Fermentation Efficiency, Ethanol yield from Flour and from Starch, depicting the correlation among these variables. 6.18

LIST OF TABLES

Table 1.1. Anatomical percentage and proximate composition of sorghum 1.6

Table 1.2. Annual fuel ethanol production per country (million liters) 1.21

Table 2.1. Physical and chemical properties of maize, sorghum (red, white and decorticated) and red sorghum bran (whole and spent) 2.10

Table 2.2. Percent particle-size distribution of maize and sorghum (red, white and decorticated) ground in a Wiley Mill equipped with a 2-mm screen 2.11

Table 2.3. Effect of grain type and addition of phenolic extract or spent red sorghum bran to decorticated kernels on FAN during liquefaction with α-amylase 2.12

Table 2.4. Effect of grain type and addition of phenolic extract or spent red sorghum bran to decorticated kernels on ethanol yield and fermentation efficiency 2.19

Table 3.1. Effects of insect, mold and sprout-damages on physical properties and color of maize and sorghum 3.9

Table 3.2. Effects of insect, mold and sprout -damages on the chemical properties of maize and sorghum 3.11

Table 3.3. Effects of insect, mold and sprout -damages on the amount of reducing sugars [RdS] (g/L) generated at 0, 100 and 200 minutes of liquefaction 3.16

Table 3.4. Effects of insect, mold and sprout -damages on the free amino nitrogen production during liquefaction (mg/L) 3.17

Table 3.5. Effects of insect, mold and sprout -damages of maize and sorghum on glucose (before fermentation) and ethanol, glycerol and ethanol yields and efficiencies obtained after 72 hours fermentation 3.22

Table 4.1. Physical characterization for the different sorghum cultivars 4.9

Table 4.2. Anatomical fractions for each different sorghum cultivar (% of tip, pericarp, germ and endosperm), kernel size and Color Index (CI) 4.10

Table 4.3. Chemical and nutraceutical composition for the different types of sorghums 4.13

Table 4.4. Susceptibility parameters obtained from the confinement and Dobie Index tests using *S. zeamais* 4.16

Table 4.5. Pearson correlations for physical, chemical and susceptibility parameters of the different sorghum genotypes 4.20

Table 5.1. Starch characteristics of sorghum cultivars before and after *S. zeamais* damage: total, damage and resistant starch and amylose percentage for all evaluated sorghum and maize samples 5.10

Table 5.2. Crude protein, alpha amino nitrogen and digestibility of protein (before and after *S. zeamais* damage) for all evaluated cultivars 5.12

Table 5.3. Correlations among content of starch and protein fractions after confinement with *S. zeamais* (F_) and original physicochemical (Ph_) properties of the evaluated sorghum cultivars 5.16

Table 6.1. Main physical and chemical characteristics of sorghum used for ethanol production 6.10

Table 6.2. Reducing sugars [RdS] (g/L) at 0, 100 and 200 minutes of liquefaction and ratio between final RdS and total theoretical glucose based on initial starch of sample used 6.11

Table 6.3. Ethanol production (TEthanol), fermentation efficiency (FF) and final yield of the evaluated sorghum cultivars after 72 hours fermentation 6.14

Table 6.4. Pearson correlations for physicochemical, susceptibility to *S. zeamais* and fermentation parameters of different sorghum genotypes 6.20

Table 6.5. Eigenvalues, percent of variance and cumulative percent for each principal component (PC) 6.21

Table 6.6. Variables with the most significant correlations within each of the principal components 6.23

ABBREVIATIONS

AMY, Amylose;

ANOVA, Analysis of Variance;

AOXC, Antioxidant Capacity;

AOXCB, Antioxidant Capacity of Bound Fraction;

AOXCF, Antioxidant Activity of Free Fraction;

CI, Color Index;

CP, Crude Protein;

DI, Dobie Index;

DNS, Reducing Sugars using Dinitrosalicylic Acid Method;

DS, Damaged Starch;

ET, Endosperm Texture;

FAN, Free Amino Nitrogen;

FF, Fermentation Efficiency;

FI, Flotation Index;

FID, Flame Ionization Detector;

g, grams;

GAE, Gallic Acid Equivalent;

HD, High Digestible;

HL, Hectoliter;

HTW, Heterowaxy;

k, velocity constant;

Kg, Kilogram;

MDT, Median Development Time;

ml, milliliter;

PCA, Principal Component Analysis;

PC, Principal Component;

PD, Protein Digestibility;

RdS, Reducing Sugars;

RH, Relative Humidity;

RS, Resistant Starch;

SSF, Simultaneous Saccharification and Fermentation

TADD, Tangential Abrasive Dehulling Device;

TD, True Density;

TDF, Total Dietary Fiber;

TEI, Total Emerged Insects;

TEthanol, Total Ethanol;

TKW, Thousand Kernel Weight;

TS, Total Starch;

TW, Test Weight;

WL, Weight Loss

KEYWORDS

Bioethanol; Bran; Decortication; Digestibility; Maize; Phenolics; Postharvest losses; Protein; *Sitophilus zeamais*; Sorghum; Starch; Susceptibility; Resistance.

ABSTRACT

The use of sorghum for fuel ethanol production has been reported as not equally efficient as other cereals such as maize, being protein availability, endosperm type, gelatinization temperature, viscosity of the mashes and phenolic compounds the most important detrimental factors. Furthermore, some of the higher ethanol producing sorghums has been reported as more prone to pest damage during storage. The objective of this thesis was to study fermentation efficiency of different types of sorghums altogether with their resistance to insect infestation through the analysis of their physical characteristics, endosperm type and texture, protein digestibility and phenolic profile. First, the role of phenolics and endosperm texture associated to red sorghum was evaluated in liquefaction, saccharification and fermentation in terms of reducing sugars and fermentation efficiency. Phenolics did not significantly affect ethanol productivity. Secondly, the viability of using damaged sorghum for ethanol production was assessed, detecting no difference in fermentation efficiency, but a more stable response of sorghum in terms of physical changes, reducing sugars and free amino nitrogen level after insect, mold and sprout damage compared with maize. Then, the susceptibility parameters for sorghum during infestation using maize weevil (one of the most detrimental insects in developing countries) were obtained. Despite the wide range of physical, chemical and phenolic characteristics of evaluated sorghums, all genotypes have high susceptibility to the maize weevil based on the calculated Dobie Index (>13). The most susceptible materials have low endosperm percentage, soft texture, contained high free amino nitrogen and low amylose levels. Next, the effect of maize weevil in the starch (total, damaged and resistant starch as well as amylose) and protein (crude protein, free amino nitrogen and digestibility) within sorghum endosperm after confined storage was further evaluated. Maize weevil affected the integrity of sorghum protein, but not the quantity measured as crude protein. This insect also reduced total and resistant starch content (2.3% average) and increased amylose concentration (2.8%). At this point, the consumption of starch (mainly amylopectin fraction), by maize weevil was evidenced and also the interference of the sorghum endosperm proteins in insect development. Next, the fermentation performance of the same sorghum cultivars was studied obtaining a range from 79 to 89 %. Reducing sugars was the common parameter correlated with ethanol productivity.

Reducing sugars in turn was positively associated with starch and endosperm percentage and negatively to protein and tannins, indicating at the same time an inverse relation among insect resistance and ethanol productivity. Thus in sorghum, traits associated with endosperm characteristics (type and texture) were common among both evaluated processes. Protein digestibility *per se* was neither related to ethanol production nor with resistance to maize weevil. The phenolic profile, specifically tannin content was deleterious for bioethanol production but yielded no effect on sorghum resistance during infestation with maize weevil. These results depict the role of sorghum endosperm characteristics, namely the relationship between starch and proteins, standing insect infestation and producing bioethanol. These findings are specific for sorghum compared with other cereals such as maize.

This is the first study about pest resistance in sorghum grain associated with an industrial process as fuel ethanol production. Common chemical, physical and nutraceutical traits are described and this information allows further research to the better understanding of sorghum insect resistance mechanisms and nutrient availability for industrial use.

1. BACKGROUND

1.1. IMPORTANCE OF SORGHUM

Sorghum (*Sorghum bicolor* L. Moench) is a crop from the *Poaceae* family well adapted to tropical areas and dry environments. Sorghum has an outstanding performance in marginal lands, where water and nutrients are scarce, with an excellent CO_2 turnover and well adapted to biotic stresses such as the one caused by insects (Chanapamokkhot and Thongngam, 2007; Kim and Day, 2010; Yan et al., 2009). Sorghum grain (Figure 1.1) and its different cultivars (sweet and forage sorghums) need only one third of the fertilizer used for other crops such as sugarcane or maize (Kim and Day, 2010) and yields after a relatively short agronomic cycle (3 to 5 months). Furthermore it has also a high genetic variability and this characteristic is the key to design new cultivars, with better adaptability to different world regions (Zhang et al., 2010).

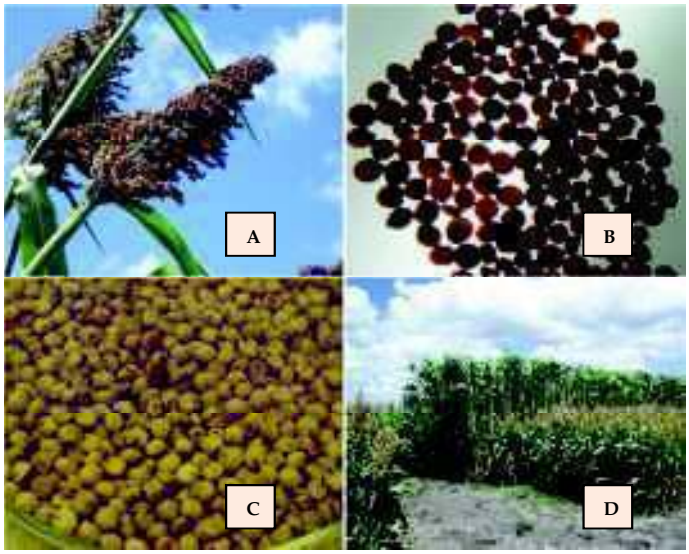


Figure 1.1. *Sorghum bicolor* L. Moench.

A: sorghum panicle; B. red sorghum grain; C. white sorghum grain; D. sweet and grain sorghum

These characteristics made sorghum a highly successful crop in Semi-Arid regions from Africa and Asia (Duodu et al., 2002), but also in some regions in North and Central Mexico. Sorghum is used to obtain a wide array of products, as beer (malt and starch source), tortilla, bread and traditional African and Asian foods (injera, tô, ogi, couscous, chapatis) (Serna-Saldivar, 2010; Rooney and Serna-Saldivar, 2000). The grain has also been used for starch refining and bioethanol production (Chuck-Hernández et al., 2012; Taylor et al., 2006; Wang et al, 2008; Wu et al., 2007; Yan et al., 2011).

Sorghum, after wheat, rice, maize and barley, is the fifth cereal produced worldwide with a yearly output of 55 million ton and a cultivated surface of 41 million hectares (FAOSTAT, 2012). The United States of America, Mexico, India, Nigeria and Argentina (Figure 1.2) are the main producers and altogether provide almost half of the global output. From the geographical point of view, Africa accounts for 39% of the whole production, followed by Asia and North America with 26% each (Taylor and Belton, 2002).

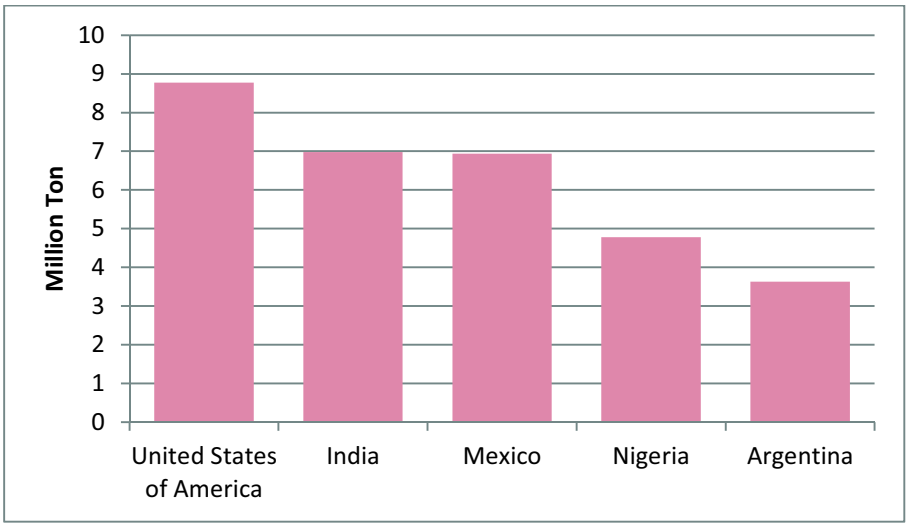


Figure 1.2. First five global sorghum producers in 2010 (FAOSTAT, 2012).

In 2011, sorghum production in Mexico was 6.94 million ton and in 2012, an equivalent harvest is expected with a productivity of 3.6 ton / Ha (FAOSTAT, 2012; USDA, 2011). In Mexico, 99% of the sorghum is used for feed purposes and the rest as seedlings (FR, 2011).

After this brief introduction about the importance of sorghum in terms of production, uses and agronomic stability, in the next subsection the physical and chemical characteristics of sorghum kernel are described.

1.2. Physicochemical Characteristics Of Sorghum Grain

1.2.1. Anatomical Portions

Sorghum is a naked caryopsis, spherical and slightly flat in one side. Its general dimensions are: 3.0 to 5.0 mm length, 2.5 mm wide and 1.2 mm thickness (Gélinas and McKinnon, 2000). Similar to other grains, its structure can be separated in three major anatomical parts (Table 1.1):

pericarp, endosperm and germ. Moreover, the pericarp can be divided into: epicarp, mesocarp and endocarp where the testa or seed coat is attached. Condensed tannins can be detected mainly in the latter structure, based on the genetic characteristics of the sorghum (Hoseney, 1998; Serna-Saldivar, 2010). Condensed tannins impart to sorghum protection against birds and pests, mainly because of their impact in palatability (tannins impart astringency). This astringency is related with the ability of tannins to bind to and precipitate endogenous and exogenous proteins (including digestive enzymes), which in turn reduces the nutritional contribution of the grain. As a result, the intake of high-tannin sorghums reduces weight gains and efficiency of feed conversion in animals and consumers (Klopfenstein and Hoseney, 1995). According to Dykes et al. (2005), tannin in sorghums is not specifically toxic to birds and animals, neither to humans. In other words, high tannin sorghums are not *bird resistant*, but this characteristic make animals prefer other sorghum than high-tannin genotypes.

The second physical structure of sorghum is the endosperm (Figure 1.3), the largest anatomical fraction of the whole grain (yield around 84%). The endosperm is considered as the second storage tissue of the grain and is divided into four subsections: 1) aleurone layer, 2) peripheral, 3) vitreous and 4) floury endosperms (Rooney and Serna-Saldivar, 2000).

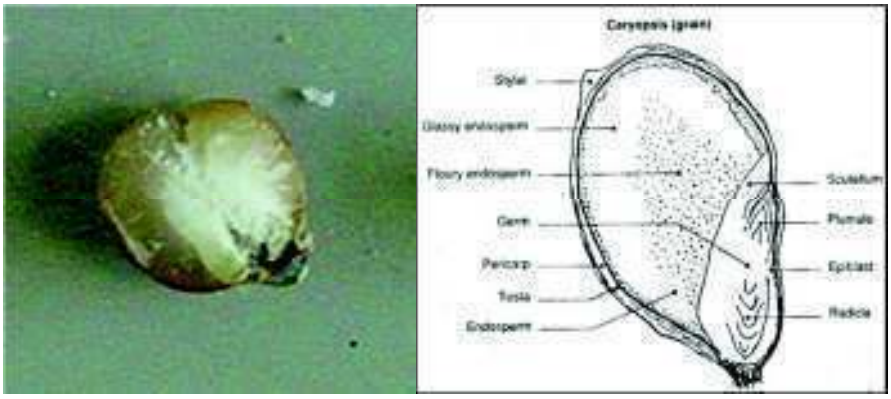


Figure 1.3. Sorghum kernel structure (Modified from: Sautier and O'Déyés, 1989).

Vitreous and floury endosperms are the main source of starch, which is stored inside the starch granules. The endosperm cell is composed of a thin cell wall, numerous starch granules associated to protein bodies and a continuous protein matrix. The floury part of the endosperm has a "chalky" appearance and in this portion, starch granules are bigger and rounder compared with the vitreous (or corneous) counterpart. This appearance is related to the protein quantity

and form (protein bodies and matrix) (Waniska and Rooney, 2000). Furthermore, the cell wall is composed by soluble and insoluble fiber. The ratio among vitreous and chalky or floury endosperm, which in turn is an indicator of the relative proportion of protein and starch in sorghum, is designated as endosperm texture, the most important factor affecting grain hardness and apparent and true densities (Rooney and Serna-Saldivar, 2000).

The third physical structure is the germ (around 10% of total kernel weight) which in turn is divided into the embryonic axis and scutellum. The embryo upon germination will form the rootlets and plumulae that will originate the new plant whereas the cotyledon or scutellum is the first reserve tissue. The scutellum stores lipids, minerals, enzymes, vitamins and proteins for the development of the future sorghum plant.

1.2.2. Proximate Composition

Sorghum proximate composition varies due to genetics and environment, being starch its main component with a range from 55.6 to 75.2%, followed by protein (7.3-15.6%), fiber (1.2-6.6%), lipids (0.5-5.2%) and ash (1.1-2.5%) (Serna-Saldivar and Rooney, 1995; Waniska and Rooney, 2000). The average proximate composition for sorghum is depicted in Table 1.1. The sorghum chemical composition is similar to dent corn, but sorghum usually contains slightly more protein and less fat (Waniska and Rooney, 2000).

1.2.2.1. Starch

Starch represents one-half to three-fourths of the grain weight and is the main source of energy during germination (Serna-Saldivar, 2010). It is composed of linear chains of glucose units linked by α-1,4 and α-1,6 glycosidic bonds, yielding two types of molecules: amylose and amylopectin. These are held together by hydrogen bonding in granules within the endosperm (Serna-Saldivar and Rooney, 1995). Amylopectin, compared to amylose, is a larger (and branched) polymer, a chain with α 1-4 linked glucoses and α-1-6 branching points every 20 to 25 glucose residues (Rooney and Pflugfelder, 1986).

Most sorghum starches contain 70 to 80% branched amylopectin and 20-30% amylose whereas waxy or glutinous sorghum contains starch with nearly 100% amylopectin. This starch has special properties for industrial and feed purposes (Rooney and Serna-Saldivar, 2000). According with its pasting properties, waxy starch depicts a rapid cooking, high peak viscosity,

low stability during cooking, paste clarity, high water-binding capacity and especially resistance to gel formation and retrogradation (Rooney and Serna-Saldivar, 2000; Serna-Saldivar and Rooney, 1995; Serna-Saldivar, 2010). These differences in pasting properties are related to subtle changes in starch granule structure. Waxy starches heated in water have a high swelling power compared to non-waxy, indicating that amylose has a role in restricting granule swelling. According to Rooney and Plugfelder (1986), the orientation of amylose molecules within starch granules, allows an increase in intermolecular hydrogen bonding, limiting both swelling and enzymatic hydrolysis. Also, according to same authors, amylose is located primarily in amorphous regions, forming a complex with lipids. These slightly differences affect the processing properties of grains and the starch accessibility (Rooney and Pflugfelder, 1986).

Table 1.1. Anatomical percentage and proximate composition of sorghum[1]

	Anatomical Parts (%)			Proximate composition (%)					
	Pericarp	Endosp.	Germ	Protein	Fat	Fiber[2]	Minerals	NFE[3]	Starch
Range	4.3-8.7	81.7-86.5	8.0-10.9	7.3-15.6	0.5- 5.2	1.2-6.6	1.1-4.5	68.1-89.9	55-6-75.2
Average	6.5	84.2	9.4	11.0	3.2	2.7	1.8	81.3	71.8

[1] With data from: Serna-Saldivar (2010); Waniska and Rooney (2000); [2] Crude fiber values; [3] Nitrogen Free Extract.

A type of starch usually analyzed in flours for industrial purposes is the damaged fraction. It can be defined as the most susceptible to hydration and enzymatic attack (mainly susceptible to α amylase) and is located in starch granules mechanically damaged mostly by milling operations. The milling process damages partially the native structure of starch, with the formation of small dimension fragments. These structural changes modify some properties of flour such as its susceptibility to amylases and its ability to absorb water (Mariotti et al., 2006). In bread making, this type of starch affects the product quality characteristics and during grain storage could also be related with its own susceptibility to mold and insects. There are several methods for damaged starch determination, but regularly enzymatic hydrolysis is preferred because of its specificity.

Other interesting starch type is the resistant portion, considered one of the components of Total Dietary Fiber (TDF). Resistant starch (RS) is the fraction that escapes enzymatic hydrolysis in the

small intestine and consequently passes to the colon (Yadav et al., 2010). RS has been categorized into four types (RS1, RS2, RS3, and RS4). RS1 represents starch molecules that are physically inaccessible to digestive enzymes –physically protected-. RS2 includes compact and non-hydrated starch molecules, non-gelatinized, slowly hydrolyzed by α amylase. RS3 is the retrograded or re-crystallized starch occurring mostly in heat-processed foods and RS4 is the chemically modified starch due to cross-linking with chemical reagents (Sajilata et al., 2006; Yadav et al., 2010).

RS content in cereals is related to several factors influencing the formation RS and they can be divided in: 1) inherent as crystallinity of starch, its granular structure, amylose and amylopectin ratio, amylose retrogradation, influence of amylose chain length, also the 2) extrinsic as: heat and moisture during storage and treatment, processing conditions, storage conditions and process as milling, germination and fermentation (Sajilata et al., 2006). In general terms, cold storage increases RS content, but this depends on the type of raw material and storage conditions. According to Platel and Shurpalekar (1994), dry heat treatments affect more than wet heat to cereals, legumes and tubers, yielding more RS in these products.

1.2.2.2. PROTEIN

Protein, the second more important component in sorghum after starch, varies widely due to genotype, water availability, soil fertility, temperatures and environmental conditions during grain development (Serna-Saldivar and Rooney, 1995). Sorghum cultivars with high protein content usually have lower yields. Furthermore, agronomical factors such as drought reduce starch synthesis during grain development and increases protein concentration in the kernel (Waniska and Rooney, 2000). On the other hand, nitrogen fertilization significantly increases the amount of protein due to accumulation of prolamins (Warsi and Wright, 1973). Prolamins or kafirins constitute the major protein fraction in sorghum followed by glutelins. Kafirins, hydrophobic, rich in proline, aspartic and glutamic acids and poor in lysine, comprise 50% or more of the proteins. This protein fraction is located in the protein bodies arranged within the sorghum endosperm. Moreover, glutelins, the second most important protein fraction and the most difficult to extract, has a high molecular weight and shape the protein matrix that provides structure to the endosperm. Albumins (water-soluble) and globulins (salt-soluble) are the third and fourth sorghum protein fractions, located mainly in the germ. They have the highest

quantities of lysine, the limiting essential amino acid in all cereals (Klopfenstein and Hoseney, 1995; Serna-Saldivar and Rooney, 1995; Rooney and Serna-Saldivar, 2000; Wong et al., 2009).

1.2.2.3. FIBER

After protein, fiber is the next most important chemical fraction of sorghum. Fiber consists of components resistant to regular enzyme digestion in the stomach and upper gastrointestinal tract of monogastrics (Serna-Saldivar, 2010). The major individual components are cellulose, hemicellulose, lignin and pectins, mainly located in the pericarp and endosperm cell walls (Serna-Saldivar and Rooney, 1995; Waniska and Rooney, 2000). In sorghum, crude fiber (determined as subsequent steps of acid and base hydrolysis), ranges between 1.2 to 6.6%, whereas dietary fiber (determined as the residue of consecutive enzymatic hydrolysis with amylases and protease) from 8.3 to 15.3% (Serna-Saldivar, 2010). Dietary fiber is divided into water soluble and insoluble, being the latter the most abundant in sorghum and all cereals (around 90% of total fiber). The fiber is mainly located in the pericarp, where it fulfills a protective and structural function (Serna-Saldivar and Rooney, 1995; Waniska and Rooney, 2000). Typical insoluble and soluble fiber content in sorghum is 6.5 to 7.9% and 1.1 to 1.2 respectively. *Beta* glucans, comprise most of the soluble fiber (Waniska and Rooney, 2000).

1.2.2.4. LIPIDS

Lipids are relatively minor constituents in sorghum and most of them are associated to the germ and aleurone layer. Within the germ, the scutellar area contains most of this fraction and provides 80% of the total sorghum oil (Rooney and Serna-Saldivar, 2000). The typical fatty acid composition of sorghum oil is similar to maize, being dominated by linoleic, oleic and palmitic acid (18:2, 18:1, 16:0) (Rooney, 1978). The sorghum lipids can be classified as polar, nonpolar and nonsaponifiable, being the nonpolar the most abundant fraction (70 to 80%) and triacylglycerides the most important within nonpolar lipids (85%), followed by sterols and diglycerides (around 4% each). Triacylglycerides are an important source of energy during sorghum germination (Waniska and Rooney, 2000). The less abundant polar lipids have important biochemical functions, being glycolipids and phospholipids the most common (2.5 to 6.2% and 17 to 25% respectively). The most abundant nonsaponifiable compounds are carotenoids, phytosterols and tocopherols (3 to 5% total). These compounds are viewed as

important nutraceuticals nowadays (Serna-Saldivar and Rooney, 1995; Waniska and Rooney, 2000).

1.2.2.5. ASH

As the smallest fraction in the proximate analysis of sorghum, ash is a measure of the total amount of minerals. It is the inorganic residue remaining after removal of water and organic matter by ashing at high temperatures (550°C in a muffle furnace) (Marshall, 2010). Sorghum is an important source of minerals, being the pericarp, aleurone and germ the main reservoirs. According to Serna-Saldivar and Rooney (1995), phosphorous is the most abundant mineral, but its availability is negatively correlated with the amount bound by phytates. Its bioavailability increases when activity of phytases is enhanced during germination or malting. According to Waniska and Rooney (2000), sorghum as most of the cereals, is a good source of potassium, but poor in calcium and sodium.

After review the physicochemical and anatomical characteristics of sorghum kernel, an important group of secondary metabolites found in sorghum is described in the next subsection. Its importance within sorghum use as well as nutraceutical, resistance and digestion properties is also depicted.

1.3. PHENOLICS IN SORGHUM GRAIN

Phenolic stands for a compound with a benzene ring and one or more hydroxyl group. All plant-based materials have phenolics, which in turn alter its composition, appearance, color, taste and oxidative stability (Dykes and Rooney, 2007). Cereal grains are not the plant exception, being a particular source of phenolics. Dykes and Rooney (2007) screened several cereals for total phenolics and antioxidant capacity evaluation, being sorghum (high-tannin cultivar), the one with the highest phenolic concentration (18 mg gallic acid equivalent/g) followed by black rice (around 10 mg gallic acid equivalent/g). High-tannin sorghum also exerted the highest antioxidant capacity contrasted with wheat, rice, barley and maize. Sumac and high-tannin genotypes had an antioxidant activity between 300 to 350 μmol Trolox Equivalent /g, whereas the Chinese black rice slightly higher than 100 μmol Trolox Equivalent /g (Dykes and Rooney, 2007; Guajardo-Flores et al., 2006).

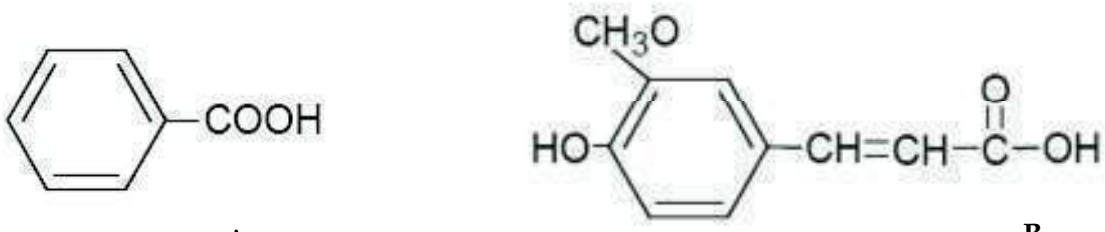
According to Dykes et al. (2005), several health benefits have been reported in sorghum and associated to its phenolic content: *cholesterol lowering, anticarcinogenic properties, cardiovascular disease reduction and slow digestibility*. Stepping apart the last concept, much of the described health benefits could be linked to the antioxidant capacity of phenolics. Reactive oxygen species (i.e. superoxide anion, hydroxyl and peroxide radicals) are implied in diseases as cancer, atherosclerosis, rheumatoid arthritis, inflammatory bowel disease and cataracts. Phenolics lower the amount of free radicals in organisms, and this capacity has been associated to positive health-related activity (Dykes et al., 2005; Dykes and Rooney, 2007).

Whereas the latter health claim listed by Dykes et al. (2005) (*slow digestibility*), indeed should be interpreted according the concern about obesity in some western countries and not from the negative side of retarded (or low) energy generation, as described in the next subsections. To paraphrase Dykes et al. (2005), *slow digestibility* is the longer sense of satiety when some types of sorghums are consumed (high-tannin mainly), a desired characteristic in some medical conditions.

Before obesity and diabetes were seen as an increasing health problem in the western world, there were plenty of reports about the role of sorghum phenolics, specifically tannins, in the reduced weight gain of animals (rats, pigs, rabbits, poultry). According to Awika and Rooney (2004), high calorie consumption and reduced physical activity account for the mass of obesity cases. Therefore, the use of high-tannin sorghum could be a strategy to reduce the energy ingestion and to regulate plasma glucose levels. The mechanisms by which tannin sorghums reduce nutritive value include binding of food proteins and carbohydrates into insoluble complexes that cannot be hydrolyzed by digestive enzymes, and a characteristic already described in subsection 1.2. : direct binding of digestive enzymes including invertases, amylases, trypsin, chymotrypsin and lipases, inhibiting thus their activity (Awika and Rooney, 2004; Dicko et al., 2006; Dykes et al., 2005; Dykes and Rooney, 2007; Klopfenstein and Hoseney, 1995; Serna-Saldivar, 2010; Serna-Saldivar and Rooney, 1995; Rooney and Serna-Saldivar, 2000; Waniska and Rooney, 2000). Inhibition of intestinal brush-border bound amino acid transporters by tannins has also been reported as one of the factors reducing sorghum nutritive value (Awika and Rooney, 2004; King et al., 2000).

Phenolic compounds are classified into three categories: phenolic acids, flavonoids and condensed tannins (Dykes and Rooney, 2006; Serna-Saldivar and Rooney, 1995).

In sorghum, phenolic acids are mainly located in the pericarp, testa, and endosperm´s aleurone layer and consist of two classes: hydroxybenzoic and hydroxycinnamic acids. Hydroxybenzoic acids are directly derived from benzoic acid (Figure 1.4 A.) and include gallic, *p*-hydroxybenzoic, vanillic, syringic and protocatechuic. The hydroxycinnamic acids have a C6–C3 structure and include coumaric, caffeic, ferulic, and sinapic (Dykes and Rooney, 2006 and 2007).



A **B**

Figure 1.4. A. Benzoic and B. Ferulic Acid (From Serna-Saldívar, 2010).

According to Dykes and Rooney (2007), sorghum and millets are the cereals with the widest variety of phenolic acids. These compounds are detected in: 1) free form, extractable with organic solvents and located in the outer parts of the kernel (pericarp), as well as 2) bound and esterified to cell wall polymers (Awika and Rooney, 2004; Dicko et al., 2006; Dykes and Rooney, 2006). Bound phenolics are released through acidic or basic hydrolysis, being ferulic the most abundant in sorghum (as in most of the cereals) (Figure 1.4 B.) followed by *p*-coumaric (Dykes and Rooney, 2007). According to Awika and Rooney (2004), phenolic acids in sorghum seems to play a role in defense against pest and pathogens and because of their high antioxidant capacity may be associated with the health benefits previously described.

Flavonoids have a C6-C3-C6 skeleton (two aromatic rings joined by a three-carbon link) (Dykes and Rooney, 2007). This flavylium unit is the base of the largest class of phenolic compounds in nature with more than three to five thousand different structures (Dicko et al., 2006; Dykes and Rooney, 2007). Flavonoids are present in pericarp of all cereals, being sorghum the one with the widest array of reported flavonoids (Dykes and Rooney, 2006). According to Dicko et al. (2006), flavonoids presents in sorghum are: flavanols (flavan-3-ols and flavan-4-ols), flavanones,

flavones and anthocyanins. In turn, anthocyanins are the most studied sorghum (and cereal) flavonoids, because of their antioxidant activity and the potential use as natural food colorants (Awika and Rooney, 2004). Anthocyanins are water-soluble pigments that contribute to the blues, purples and reds in plant foods and the six common in nature are: 1) cyanidin; 2) delphinidin; 3) malvinidin; 4) pelargonidin; 5) petunidin and 6) peonidin (Dykes and Rooney, 2007). In the case of sorghum, the most abundant anthocyanins are 3-deoxyanthocyanidins (3DA), which in turn have a small distribution in nature (Awika and Rooney, 2004). These compounds do not contain the hydroxyl group in the 3-position of the C-ring, being thus called 3-deoxyanthocyanidins (Dykes and Rooney, 2006). They are detected in nature mainly as aglycones (Awika and Rooney, 2004), being apigeninidin and luteolinidin the two main 3DA reported in sorghum (Awika et al., 2004a). The distinctive 3DA chemical structure increases the stability at high pH, without affecting the antioxidant properties (similar to their hydroxylated counterpart), suggesting thus a potential use of sorghum as a commercial source of food colorants (Awika et al., 2004a, 2004b; Dicko et al., 2006). According to Dicko et al. (2006), 3DA are abundant in red sorghum grain, whereas Dykes and Rooney (2006 and 2007) reported the highest level of 3DA in black sorghum. Total anthocyanin in black sorghum bran is 10.1 mg/g, at least twice than the red or brown sorghums bran (2.8 to 4.3 mg/g) and eight to twenty fold times the concentration in purple and blue wheat bran (Awika et al., 2004b; Awika and Rooney, 2004). Furthermore, anthocyanin in black sorghum bran is three to four times higher than whole black sorghum grain (Awika et al., 2004b; Dykes and Rooney, 2006), indicating that pericarp is the main reservoir of these compounds. The 3DA are also related with the sorghum phytoalexin response to mold invasion and other related biotic stresses (Dykes and Rooney, 2006). According to Lo et al. (1999), 3DA are the main phytoalexins in sorghum in response to fungal infection. The response of sorghum resistant cultivars to *Colletotrichum sublineolum*, fungus causing the anthracnose disease, was compared with susceptible cultivars. A complex phytoalexin mixture was detected in resistant sorghums, including luteolinidin and 5-methoxyluteolinidin, different from the mixture produced in susceptible genotypes after fungal inoculation.

Tannins, the third phenolic group detected in sorghum (but not present in all cultivars), have a genetic component: sorghums with B1_B2_ gene display tannins in the testa (Dicko et al., 2006;

Serna-Saldivar and Rooney, 1995). Sorghum tannins, also known as proanthocyanidins or procyanidins are polymerized flavan-3-ol or pro-3-deoxyanthocyanidin units (Figure 1.5; Dicko et al., 2006; Dykes and Rooney, 2006). Tannin levels are different among sorghum cultivars, and based on these compounds the most widely use sorghum classification is made. Type 1 sorghums do not contain condensed tannins whereas type II and III have tannin levels of 0.02-0.19 and 0.4-3.5 mg catechin equivalent/100 mg, respectively (Dykes and Rooney, 2006). Awika and Rooney (2004) describe sorghum types based on tannin extractability: type I sorghums has no significant levels of tannins extracted by 1% acidified methanol, type II has tannins extractable in 1% acidified methanol but not pure methanol and type III, where tannins are extractable in both acidified methanol and pure methanol, as in the Sumac genotype.

As previously described, sorghum proanthocyanidins consist of flavan-3-ol units linked mainly by C-C bonds (C4 to C8 interflavan bonds, type B proanthocyanidins) and occasionally also by C-O-C bonds (C2 to C7 ether bond, type A proanthocyanidins) ranging from 1 to 15 units (Dicko et al., 2006; Dykes and Rooney, 2006). Type A proanthocyanidins have been mostly identified in other natural sources as cranberries but not in sorghum (Dykes and Rooney, 2006). According to Dykes and Rooney (2006), polymerized flavan-3-ols up to 8 to 10 units have been extracted and quantitatively analyzed from sorghum, but there are difficulties to resolve the high molecular weight compounds, the most abundant forms of tannins in sorghum (Awika and Rooney, 2004).

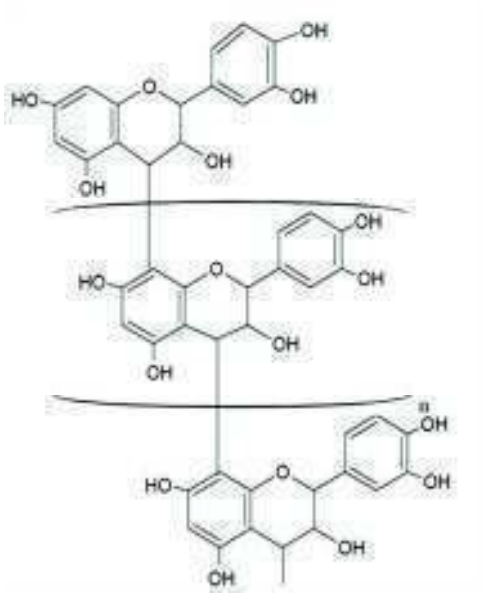


Figure 1.5. Condensed tannins (From Serna-Saldívar, 2010).

Beyond methodological troubles, condensed tannins give consistently the highest antioxidant activity in sorghum with pigmented testa (Awika and Rooney, 2004; Dykes and Rooney, 2007), yielding genotypes with a potential application as nutraceuticals. Furthermore and as previously described, tannins are related with the protective scheme of sorghum against insects, birds and weathering (Serna-Saldivar and Rooney, 1995). Besides, high-tannin sorghums depict a good agronomic yield, being thus a high desirable crop (Yan et al., 2012).

From the nutritional point of view, the presence of tannins in sorghum is detrimental, because its tendency to bind proteins, reducing its bioavailability. There are at least four binding reported mechanisms among sorghum tannins and proteins: 1) hydrogen bonds; 2) ionic bonds among anionic groups within tannins and cationic residues in proteins; 3) hydrophobic interactions and 4) covalent bonds (Butler et al., 1984; Van Buren and Robinson, 1969). This characteristic reduces the nutrient availability of sorghum, enhancing worldwide the use of low tannin sorghums. In the United States of America, more than 99% of the cultivars are tannin free (Dykes and Rooney, 2006).

Besides the influence of tannins in the nutritional performance of sorghum, there are some other mechanisms affecting the nutrient bioavailability. These mechanisms, altogether with the role of phenolics are further described in the next subsection.

1.4. DIGESTION PROPERTIES OF SORGHUM GRAIN

Compared with other cereals, sorghum has a poor performance in terms of caloric contribution. The reduced energy could be associated, first of all to its protein properties. Protein digestibility can be defined as the susceptibility of the protein to proteolysis and therefore as the availability of amino acids for absorption (Duodu et al., 2003). Sorghum gruels from tannin-free cultivars have an apparent protein digestibility of 46%, while rice, maize and wheat gave values of 66, 73 and 81% respectively (MacLean et al., 1981). The apparent digestibility is considered the value obtained as the ratio of the difference of ingested and fecal nitrogen to the ingested nitrogen whereas true digestibility in turn, makes allowance for nitrogen in feces of non-dietary origin (metabolic nitrogen), which is subtracted from total fecal nitrogen (Greaves, 1963). Besides, this low digestibility is exacerbated by wet-cooking (Duodu et al., 2003), process where the availability of the sorghum prolamins or kafirins is comparatively more affected compared to

proteins in maize or wheat (Hamaker et al., 1987; Wong et al., 2009). Prolamins in sorghum account for the 70 to 80% of total protein content in the grain, and their characteristics are so different from the other within endosperm and germ that even a classification based on these proteins is recommended (Wong et al., 2009**).** Despite kafirins have a high homology with the maize prolamins or zeins, the differences in digestibility can be associated to the higher hydrophobicity and their tendency to form disulfide-cross linkages (Wong et al., 2009). Factors affecting sorghum protein digestibility are numerous and can be separated in two main groups: 1) exogenous, or those related with non-protein components of sorghum grain, as polyphenols, starch, non-starch polysaccharides, phytates and lipids; 2) endogenous, related with the nature of the sorghum proteins and their organization within the grain (high hydrophobicity and higher susceptibility to disulfide cross-linking) (Duodu et al., 2003).

Kafirins, in turn, could be classified according to its molecular weight: α-(23 and 25kDa), β-(20kDa) and γ-(28kDa), being the first the most abundant with 80% of the total, followed by γ-(15%) and β- (5%). The protein bodies within sorghum endosperm are basically composed by kafirins. A typical protein body contains γ- and β-kafirins enclosing the more digestible α-kafirin, through a disulfide bond polymer network. Protein bodies are surrounded by a protein matrix, mainly glutelins, responsible of the union of protein bodies with the starch granules. This net, altogether with protein bodies, contributes to the low protein digestibility and even the reduced starch digestibility because it represents a barrier to starch gelatinization and to its own proteolysis (Wong et al., 2009). Sorghum grains rich in protein bodies also have a lower capacity to starch gelatinization (Ezeogu et al., 2005) and according to Rooney and Pflugfelder (1986), the structure and composition of cereal starches and their interaction with protein play a major role in the digestibility of the grain. Specifically, the starch digestibility is affected by its own composition, but also by protein-starch interactions, cellular integrity of the starch granules and presence of anti-nutritional factors such as tannins (Rooney and Pflugfelder, 1986).

Besides nutrition, differences in the protein digestibility of sorghum impact the industrial process of the grain. In sorghum wet milling, separation of starch and protein is more difficult compared to maize. For this reason, the (few) available commercial sorghum starch is higher in protein compared with other commercial starches (Rooney and Pflugfelder, 1986). The higher residual protein affects its functional properties such as gelatinization (Ezeogu et al., 2005).

Thereby sorghum caloric contribution is not only related with protein and its availability to hydrolysis, but also to the protein-starch interaction. Sorghum digestibility should therefore be studied as the availability of energy and nutrients for different purposes and uses.

It should be noted that not all sorghums cultivars possess a low digestibility. Some genotypes, as waxies (up to 5% amylose), have a better performance and is related to differences in susceptibility of both endosperm proteins and starch to digestive enzymes (Rooney and Pflugfelder, 1986). The waxy trait is a genetic related characteristic and in order to obtain a completely waxy sorghum, the triploid endosperm must be homozygous recessive (Serna-Saldivar and Rooney, 1995). In waxy sorghums, protein bodies are uniformly distributed into vitreous endosperm, avoiding the pronounced peripheral protein layers of regular sorghum, exposing themselves to protease activity and therefore to starch uncover. Waxy starch granules are also more susceptible to enzyme hydrolysis compared to regular counterparts (Rooney and Pflugfelder, 1986).

Despite that the presence of the waxy trait in sorghum improves its food value, it has also some agronomical disadvantages: poorer seedling emergence and vigor, low harvest yield and more rapid deterioration before harvest (Rooney, 2009). Besides, sorghums with high digestibility could be more prone to weathering and molding and for this reason Rooney (2009) recommend the use of these cultivars only in dry places. Thus, the digestion characteristics of sorghum could be linked with the postharvest performance of this cereal; a stage usually underestimated yielding important losses in cereals. More information about postharvest losses in sorghum as well as biotechnology efforts to increase resistance of genotypes is described next.

1.5. Postharvest Losses in Sorghum Grain

According to Ramputh et al. (1999) cereal postharvest losses in small-farm tropical agriculture usually exceeds 30% and according to FAO (1993), the range of worldwide losses is between 10 to 37%. If the losses reported by FAO (1993) were quantified, the damage in the United States of America in 2011 ranged from 31 to 115 and 1 to 3 million tons of maize and sorghum, respectively representing economic losses of $190 million to $37.0 billion dollars (FAOSTAT, 2012).

One of the main biotic factors associated with postharvest losses are insect such as weevils (Teetes and Pendleton, 2000). There are at least 150 insect species infesting sorghum varieties worldwide (Guo et al., 2011), being *S. zeamais* the main pest of sorghum in tropical agroecologies and also the most harmful (Abebe et al., 2009). *S. zeamais* infests kernels in the field, deposit eggs in stored kernels and the larva feeds inside and damages the inner part of the kernel. Despite the importance of this insect in postharvest stage of sorghum, for more than 20 years neither resistance of sorghum genotypes nor the use of sorghum resistant as an alternative to insecticides has been evaluated (Pendleton et al., 2005). Besides insect damage, fungal infections are also a major postharvest problem causing undesirable effects such as discoloration, off-odors, loss of germination capacity and contamination with harmful mycotoxins (Serna-Saldivar, 2010; Waniska and Rooney, 2000).

Biotechnology, altogether with traditional breeding programs, has been recognized as an important tool for controlling insect pests and increasing sorghum production (Guo et al., 2011). Genetic research has revealed the existence of resistant traits in insect tolerant sorghum cultivars. Satish et al. (2009) reported QTLs for resistance to sorghum shoot fly. They discovered 29 QTLs by multiple QTL mapping. According to the authors, the insect resistance QTLs are located in syntenic maize genomic regions, showing conservation of insect resistance loci between maize and sorghum. Wu and Huang (2008) explored sorghum resistance to greenbug and discovered two QTLs on chromosome 9 that account for 55-80% and 1 to 6% of the resistance, respectively. Klein et al. (2001) identified five QTLs for mold resistance from a cross between Sureño (resistant) and RTx430 (susceptible) on a sorghum chromosome map using molecular markers.

Attempts to increase resistance to pathogens and pests in sorghum through genetic engineering have been also reported (Chandrashekar and Satyanarayana, 2006). The first work of transgenic sorghum was published by Girijashankar et al. (2005), who achieved the expression of an insect resistance gene (Bt *cry1Ac*) under the control of a wound inducible promoter from maize. Transformation was made through particle bombardment of shoot apices. Krishnaveni et al. (2001) reported both the insertion of a rice chitinase gene (*chill)* into sorghum genome and the resistance of this cultivar to stalk rot. There is also published work about transformation of millets (from the same sorghum *Poaceae* family): Girgi et al. (2006), Latha et al. (2006) and

O´Kennedy et al., (2006). Transgene silencing in sorghum seems to be the major obstacle in its successful modification, specifically methylation-based silencing has been reported (Emani et al., 2002; O'Kennedy et al., 2006).

Beyond transgenic cultivars is necessary to stress the fact that research in sorghum resistance to pests is a step behind other cereals such as maize. Even susceptibility mechanisms facing insect infestation is scarce and some of the available information is described in the next section.

1.6. PEST RESISTANCE MECHANISMS IN SORGHUM GRAIN DURING STORAGE

Sorghum utilizes a vast array of physical and chemical defense mechanisms against pathogens and pests. The systems used to impart resistance against insects and fungi, seem to be linked (Chandrashekar and Satyanarayana, 2006). Chandrashekar and Mazhar (1999) described the importance of grain hardness to deter pests and pathogens. The harder kernels depict a high quantity of protein bodies in the endosperm compared with soft counterparts. Thickness of the seed coat has been also associated with sorghum resistance to maize weevil (Figure 1.6; Pendleton et al., 2005). Related to grain hardness, nutrients availability may determine suitability to insect infestation (Dobie, 1977). Singh and McCain (1963) reported corn genotypes with low carbohydrate levels as resistant to *S. oryzae*. Nevertheless, in sorghum, Torres et al (1996) found no-direct relationship among susceptibility index (to *S. oryzae*) neither with nitrogen nor carbohydrate content.

An exception to the grain hardness mechanism could be the high-tannin, high phenolic cultivars (v.gr. high ferulic content, presence of phenolic acid amides) because despite the softness, they are resistant to molds and pests infections (Burt, 2003; García-Lara et al., 2004; Ramputh et al., 1999; Rooney, 2009). In fungal infections, the presence of flavan-4-ols has been reported as the most important defense mechanism in sorghum, even more than endosperm texture (Chandrashekar and Satyanarayana, 2006; Dykes and Rooney, 2006).

In maize, it has been demonstrated the role of phenolics bound to cell walls in its stability against *S. zeamais* (Arnason et al., 1992), but in sorghum this mechanism has not been fully explored yet. At this time, only one study correlating soluble phenolic content with

susceptibility to *S. zeamais* has been published (Ramputh et al., 1999). The effect of sorghum tannins in birds and digestibility is also well known (Dicko et al., 2006; Serna-Saldívar and Rooney, 1995), but there are scarce reports about their role in insect resistance.

Figure 1.6. *Sitophilus zeamais* and its damage on maize and sorghum kernels.

Besides endosperm texture and phenolic content, the presence of pathogenesis related proteins, has also been reported. In maize, the presence of a ribosome inactivating protein (molecular weight 32kDA) with antifungal activity has been documented. Likewise, an increase in *beta* amylases and chymotrypsin inhibitors in barley lines have been associated to insect resistance (Chandrashekar and Mazhar, 1999 and Chandrashekar and Satyanarayana, 2006). According to Chandrashekar and Satyanarayana (2006), the pathogenesis related proteins in sorghum are similar to maize and other cereals, but there is yet room for exploring.

In this subsection, the most important pest resistance mechanisms in sorghum have been described, roughly grain hardness, phenolics and pathogenesis related proteins. The first two factors have also been associated with sorghum performance in industrial application as starch production and fuel ethanol fermentation. The latter represents a case on its own, because of the importance of the renewable energy for transportation at this time and the widely use of starchy materials, mainly maize, as a source of fermentable sugars. The current role of ethanol for fuel and the special features of sorghum influencing fuel production are described in the next subsection.

1.7. FUEL ETHANOL FROM SORGHUM GRAIN

Besides human consumption, sorghum is also used as feed, construction material, among other industrial purposes. In the United States, around 3 to 5% of fuel ethanol is obtained from sorghum grain and is one of the most promising crops for lignocellulosic production.

In countries like Mexico, where the use of maize for fuel ethanol production is limited by the federal law, the use of other starchy materials as sorghum becomes a real industrial alternative. Even more, the use of pest damaged material, not suitable for human consumption becomes also a good alternative, mainly if the hydrolysis and fermentation stages are not affected by these raw materials.

Ethanol for fuel has become important in the last decades, mainly because of the worldwide concerns on energy security, as well as environmental and economic interests. In 2008, 65 thousand million liters of ethanol were produced globally and by 2010 this figure was more than 85 thousand million liters (Table 1.2). Fuel ethanol can also be used as a substitute of MTBE (Methyl Terbutyl Ether), gasoline oxygenate highly toxic and one of the main water contaminants worldwide. The main advantage of ethanol from starchy materials is the use of renewable sources, being an opportunity to the economic growth of rural areas in developing countries. The most obvious disadvantage is the use of resources related with direct food production and also the calorific content of ethanol which is estimated to be only 63% of the gasoline.

Despite the interest in the use of sorghum for industrial purposes, as in starch and ethanol production, the physicochemical characteristics (and differences compared with other cereals) restrict its use. As previously described, the special association among proteins and starch in sorghum hinders utilization.

The main factors that influence sorghum productivity during industrial fermentation processes are: "protein and starch digestibility, protein-starch interactions, viscosity of flour-water slurries, phenolic concentration, possible presence of tannins and the ratio amylose-amylopectin, as well as the amylose-lipid complexes formed during hydrolysis" (Wang et al., 2008). All these factors, could be indeed described as digestibility related-issues (as previously described in subsection 1.4.) and as Wang et al. (2008) points directly in the first element of the previous array of factors.

Definitely starch content, as in all starchy materials for fermentation, has a direct impact in final ethanol yield but in sorghum, the role of protein has a paramount importance. Amylose ratio, has been inversely related to sorghum fermentation efficiency (Wu et al., 2006), as well as the presence of phenolics (mainly tannins related with sorghum type III) (Wang et al., 2008).

Table 1.2. Annual fuel ethanol production per country (million liters)[1]

Country	2009	2010	2011
United States of America	40,068	51,865	54,437
Brazil	24,864	26,163	21,066
European Union	3,929	4,448	4,413
China	2,047	2,047	2,097
Canada	1,098	1,348	1,747
Thailand	1,645	--	
Colombia	315	--	
India	347	--	
Australia	215	249	329
Others	934	868	4,261
Total	**75,463**	**86,988**	**88,350**

[1] Chuck-Hernández et al. (2011) and Serna-Saldívar (2012)

These polymers, as described in subsection 1.6. , are part of the grain resistance mechanism against birds, insects and fungi but at the same time bind proteins, including digestive enzymes (Dykes and Rooney 2007) as described in subsections 1.3. and 1.4. The influence of phenolics and protein arrangement in the α-amylase activity during liquefaction is one of the areas where more research is needed in order to the fully use of kernel's energy content. The role of tannins during enzymatic hydrolysis for ethanol production has been already studied and the higher the tannin concentration, the lower the fermentation efficiency (Wu et al., 2007; Zhao et al., 2008). According to Yan et al. (2011), tannins could enhance sorghum protein cross-linking during heating, retarding starch hydrolysis or enzymatic degradation, nevertheless, because of the ability of tannins to bind proteins (around 12 times its own weight), the formation of indigestible protein-tannin complexes is the most accepted explanation for the reduced activity

of α- amylases (Duodu et al., 2003; Wang et al., 2008; Wu et al., 2007). Tannin's role has been previously described in subsections 1.3. and 1.4. under a different context. The detrimental effect of tannins specifically during fermentation is linked to the same mechanisms affecting liquefaction, because mashes with low α- amylase hydrolysis rate, increase their viscosity lowering the fermentation efficiency and increasing energy required for transportation during process (Duodu et al., 2003; Salunke et al., 1982; Wu et al., 2007).

1.8. SORGHUM GRAIN AS PART OF THE PRODUCTIVE CHAIN

Despite the importance of sorghum as one of the main cereals produced globally, information about resistance mechanisms during storage and its utilization for food and industrial purposes, compared with other cereals as maize or wheat, is yet scarce. Nowadays, food production is a priority (FAO, 2009; 2012) and the agricultural, chemical and physical profiles of sorghum hold a big potential for a wider global use. The analysis of different stages of its productive chain can lead to holistic research efforts in order to increase the amount of grain arriving to final and industrial consumers. Therefore the study of physical and chemical characteristics in sorghum, its resistance mechanism to insects and the link of that information with industrial processes as fermentation could lead to a better understanding of the common structures affecting all these stages at one time.

1.9. HYPOTHESIS

Sorghum cultivars highly efficient in ethanol production are the most susceptible to insect damage during storage. The kernel physical properties, endosperm type and texture, protein digestibility and phenolic profile affect both the kernel resistance to *S. zeamais* and efficiency of bioethanol production.

1.10. OBJECTIVES

1.10.1. GENERAL OBJECTIVE:

To study fermentation efficiency of sorghum grain during fuel ethanol production, altogether with its resistance to insect infestation through the description of its physical properties, endosperm type and texture, protein digestibility and phenolic profile.

1.10.2. SPECIFIC OBJECTIVES:

- To evaluate the deleterious effects of addition of red sorghum bran or its extracted phenolics to decorticated sorghum on the sequential steps of ethanol production (liquefaction with thermoresistant α-amylase, saccharificacion with glucoamylase/pullulanase and fermentation with *Saccharomyces cerevisiae*). The effects of these factors studied in terms of fermentable sugars and free amino nitrogen production, ethanol yield and fermentation efficiency.
- To investigate the behavior of insect (*Sitophilus zeamais*), mold (*Aspergillus flavus*) and sprout-damaged lots of maize and sorghum during fuel ethanol production, in terms of susceptibility to enzyme hydrolyses and fermentation efficiency.
- To study the susceptibility of sorghum grain cultivars to the presence of maize weevil (*Sitophilus zeamais*) during storage for further association with their own physical properties, endosperm type and texture, protein digestibility and phenolic profile.
- To assess the effect of *S. zeamais* in starch and protein attributes of flour obtained from different sorghum grain genotypes to understand the influence of this insect on the main endosperm components.
- To evaluate fermentation efficiency of different sorghum grain cultivars following a dry-grind process and to obtain the common physical, chemical and nutraceutical traits underlying fuel ethanol productivity and sorghum resistance during infestation with *Sitophilus zeamais*.

2. EFFECTS OF ADDITION OF RED SORGHUM (*SORGHUM BICOLOR* L. MOENCH) PHENOLICS OR SPENT BRAN ON THE PERFORMANCE OF DECORTICATED KERNELS BIOCONVERTED INTO ETHANOL*

Chuck-Hernández, C., Peralta-Contreras, M., Bando-Carranza, G., Vera-García, M., Gaxiola-Cuevas, N., Tamayo-Limón, R., Cárdenas-Torres, F., Pérez-Carrillo, E., Serna-Saldívar, S.O. 2012. Bioconversion into ethanol of decorticated red sorghum (*Sorghum bicolor* L. Moench) supplemented with its phenolic extract or spent bran. Biotechnology Letters 34, 97–102.

2.1. ABSTRACT

The effects of extracted phenolics or red sorghum spent bran added to decorticated kernels during fuel ethanol production were studied and compared to maize and whole red and white sorghums. Maize contained the highest starch followed by the decorticated red and whole white sorghums (68.0, 67.0 and 66.5% respectively). The whole red sorghum contained 2.25, 2.80 and 3.57 more phenolics, flavonoids and free ferulic acid compared to maize and decortication of red sorghum reduced the amount of total phenolics by 30%. White sorghum had similar phenolic acid composition compared to maize but harder endosperm texture and coarser granulation after grinding. After liquefaction free amino nitrogen (FAN) concentration ranged from 65 to 101 mg/L and at the end of saccharification all mashes had approximately 80 g glucose and 2 to 5 g maltose/100 g meal (dwb). Saccharified worts were fermented and yielded 50 to 90 mL ethanol/L. The lowest fermentation efficiency (76%) was obtained in the white sorghum. Results indicate that sorghum bran or its associated phenolics did not significantly affect the efficiency of the sequential steps involved in ethanol production. Apparently, the sorghum endosperm protein structure and composition and the nature of the starch granules are the main reasons why this crop yields lower bioethanol compared to maize.

2.2. INTRODUCTION

The production of fuel ethanol reached more than 19 billion gallons in 2009 (EPI 2010; RFA 2010), being the United States of America the chief producer with approximately 53% of the output. More recent statistics indicate that the USA biorefineries produced 13 billion gallons in 2010 (RFA 2011). The USA ethanol industry is based on maize and this feedstock accounts for 75 to 80% of total final unitary cost (McAloon et al 2000). The use of starchy materials for ethanol production is attractive because employs a mature technology and represents an excellent outlet for agricultural production surplus, but in some countries such as Mexico, maize as source of biofuels is not economically neither socially feasible (Perez-Carrillo and Serna-Saldivar 2007) and its use is even limited by a federal law (CD 2008).

Sorghum represents an excellent alternative to maize for fuel ethanol production because is comparatively cheaper and contains almost the same amount of starch. Agronomically, it can be grown in drier and harsher lands where maize could not be planted (Taylor et al 2006). This cereal is one of the main crops in the USA and Mexico, being the first and fourth world producers, respectively (FAO 2010). Despite these advantages, nowadays only about 2.5% of fuel ethanol is produced from grain sorghum (Zhao et al 2008). A drawback of sorghum in ethanol production is the lower yield compared to maize and its comparatively higher starch gelatinization temperature as well as the reduced protein and starch digestibility or susceptibility to hydrolysis (Duodu et al 2002; Duodu et al 2003; Ezeogu et al 2008; Hamaker and Bugusu 2003). Several investigations have been aimed to study the most critical factors that negatively affect sorghum starch and protein digestibility (Duodu et al 2002; Duodu et al 2003; Ezeogu et al 2008; Rooney and Pflugfelder 1986; Zhang and Hamaker 1998), being the phenolic content and disulfide cross-linking among endosperm storage proteins the two most relevant factors (Awika and Rooney 2004; Cheynier 2005; Daiber 1975; Duodu et al 2003; Makkar et al 2007; McDougall et al 2005; Rooney and Pflugfelder 1986; Wang et al 2008; Zhan et al 2003; Zhao et al 2009). In order to overcome these disadvantages and improve grain sorghum ethanol yield, physical and chemical treatments have been devised. The main strategies include particle size reduction, decortication, steam-flaking and use of proteases (Chuck-Hernández et al 2009; Corredor et al 2006; Perez-Carrillo and Serna-Saldivar 2007; Perez-Carrillo et al 2008; Wang et al 2008).

In the specific case of decorticated red sorghum, the increase in yield apparently is related to the reduction in phenolic compounds found in the bran and to the increase of starch concentration resulting from the mechanical removal of the kernel´s outer layers (Corredor et al 2006). Abrasive decortication reduces the levels of fiber, fat and phenolics (Perez-Carrillo and Serna-Saldivar 2007) and can lead to an increase as much as 10% of starch and improve protein digestibility (Corredor et al 2006). The effect of the selective removal of fiber from maize to improve ethanol fermentation and yield has also been investigated. Ponnampalam et al (2004) obtained significantly higher ethanol yield when endosperm rich fractions were used instead of the whole ground grain. Recently, it was demonstrated that the use of steam-flaked sorghum, commonly used in feedlots, also improved ethanol production to the level observed in both whole and steam-flaked maize. The significant improvement is mainly attributed to the physical disruption of the peripheral endosperm layer that allowed a better starch hydrolysis and production of fermentable sugars (Chuck-Hernández et al 2009). These investigations indicate that the physical and chemical properties of sorghum grain affect in different degrees ethanol production. Nevertheless, the effect of sorghum bran and its extracted phenolic compounds has not been investigated. This research was undertaken to evaluate the deleterious effects of addition of red sorghum bran or its extracted phenolics to decorticated sorghum on the sequential steps of ethanol production (liquefaction with thermoresistant α-amylase, saccharificacion with glucoamylase/pullulanase and fermentation with *Saccharomyces cerevisiae*). The effects of these factors were studied in terms of fermentable sugars and free amino nitrogen production, ethanol yield and fermentation efficiency.

2.3. MATERIALS AND METHODS

2.3.1. GRAIN SOURCES

Grains used were commercial regular soft-endosperm yellow dent maize, a commercial red sorghum with a relatively soft endosperm texture and an experimental white sorghum (ATX631* TX436) with intermediate endosperm texture. The white sorghum was kindly donated by the Texas A&M University Sorghum Breeding Program. Grain physical characteristics were determined using standard procedures: test weight according to Official US Grain Standard

Procedures (AACC Method 55-10); thousand-kernel weight by weighing 100 randomly selected kernels and endosperm texture according to the subjective procedure reported by Chuck-Hernández et al (2009).

2.3.2. Decortication, Milling and Particle Size Distribution

Maize and both sorghums were cleaned by air aspiration and sieves. Four kilograms of red sorghum were decorticated for 8 minutes using a minihull dehuller (Nutana Machine, Saskatoon, Canada) equipped with five 30-cm diameter carbodurum disks to remove 10% of the kernel weight. The abraded bran and decorticated sorghum were separated using a screen with triangular orifices (3mm each side). The decorticated bran was placed in a plastic bag and stored in a freezer (-20°C). Whole and decorticated kernels were ground using a Wiley mill (Arthur Thomas, Philadelphia, PA, USA) equipped with a 2 mm screen. The particle-size distribution was determined by sifting 100 g of meal through a set of sieves (U.S # 20, 35, 60, 80, 100 and 140 mesh) on a Rotap (Duratap Model DT 168, Advantech Mfg., New Berlin, WI, USA) operated for 10 min. The resulting fractions were expressed as percentages of the original weight.

2.3.3. Extraction of Phenolic Compounds and Spent-Bran Production

Two hundred grams of the red sorghum abraded bran were used for phenolic extraction. The bran was extracted with 500 mL of 1% HCl/Methanol 80% (v/v) for 12 hours at room temperature and 100 rpm. The extract was first centrifuged during 15 min at 4,000 rpm (Centra MP4R centrifuge, International Equipment Company, Needham, Heights, MA) and then decanted. The resulting extract (260 mL) was concentrated in a rota-evaporator (Buchi Model R-210) at 60°C and vacuum (-22'' Hg) during 15 min. The spent-bran was dried in a convection oven set at 50°C during 2 hr.

2.3.4. Analytical Methods

Moisture content was determined using a gravimetric method AACC 44-15A. Total and damaged starch contents were determined using commercially available kits (Megazyme International Ireland Ltd., Wicklow, Ireland) according to AACC Approved Method 76-13. Protein (N * 6.25) was determined using the micro-Kjeldhal method AACC 46-13. Crude fiber, crude fat, and ash were determined according to approved AACC methods 32-10, 30-20 and 08-

01, respectively (AACC 2000). Total phenols were determined according to the procedure of Vinson et al (2001) and free ferulic acid by HPLC UV-Vis equipped with a C18 column (Zorbax Sb Aq). Water acidified with triflouroacetic acid (pH 2) was used as the mobile phase (0.5 mL/min) and > 99% pure ferulic acid (Fluka, Switzerland) as external standard. Flavonoids were determined according to Wolfe et al (2003). The amount of reducing sugars generated during liquefaction was determined with the dinitrosalicylic acid method described by Miller (1959) and free amino nitrogen by reaction with ninhydrin according to Official Method 945.30 (AOAC, 1980). Fructose, glucose, maltose and maltotriose were analyzed in a HPLC-IR chromatograph (Waters 2114, Milford, MA, USA) furnished with an ion-exchange column (Aminex HPX-87H, Bio-Rad®, Hercules, CA, USA). The operation conditions were set according to Chuck-Hernández et al (2009). Ethanol was determined through GC (Agilent 6850) with a flame ionization detector at 250°C. The column used was HP-INNOWAX column (30m x 0.53 mm x 1μm) with helium as carrier gas (4mL/min).

2.3.5. TREATMENTS

Six treatments were evaluated: 1) whole red sorghum; 2) decorticated red sorghum with spent-bran free of phenolics; 3) decorticated red sorghum supplemented with phenolic extract; 4) decorticated red sorghum; 5) whole yellow maize and 6) whole white sorghum. In treatment 2, solids were composed of 90% decorticated sorghum meal and 10% ground spent-bran whereas in treatment 3 the phenolic extract was added to reach the concentration originally found in the red whole sorghum.

2.3.6. LIQUEFACTION

Ground meals (150 g db) were first mixed with water to obtain mashes with 30% (w/v) solids and then the pH adjusted to 6.5 with 0.1 N HCl. The temperature of the slurry was increased to 60°C in a shaking water bath (Hot Shaker, BellCo Glass, Inc. Vineland, NJ, USA) and 225 μL of Liquozyme SC DS (240 KNU-S/g, Novozymes, Bagsvaerd, Denmark) was added. The temperature was gradually increased to 90°C and the total time of hydrolysis was 195 minutes. Aliquots were taken at different times in order to determine the progressive extent of starch hydrolysis.

2.3.7. SACCHARIFICATION

Mashes, previously treated with α-amylase, were allowed to cool down to 60 ˚C in preparation for addition of Dextrozyme DX (Novozymes, Bagsvaerd, Denmark) at a rate of 1 mL/200mL of liquefied slurry. Dextrozyme DX is a mixture of glucoamylase (EC 3.2.1.3) and pullulanase (EC 3.2.1.41) produced from genetically modified strains of Aspergillus and Bacillus, respectively. The declared optimum activity of this enzyme mix is at pH 4.1-4.5 and 60-63°C. Reaction vessels were maintained for 16 h in an incubator-shaker (VWR International, Model RF1575) set at 60°C and 100 rpm. Mashes were filtrated using a plastic sieve (1mm) to remove spent grains. The insoluble were washed with 200 mL of distilled water.

2.3.8. FERMENTATION

Saccharified worts were adjusted to 13°Plato and enriched with 150 mg/L of FAN (Yeast Nutrient Yeastex 1433 Probamex, Naucalpan, Mexico). Total volume of worts was recorded. Before yeast pitching, worts (125 mL) were pasteurized at 70°C for 30 min and cooled down to 35°C. Worts were inoculated with a yeast (Nevada, Safmex, Toluca, Mexico) suspension containing 1.3 x 107cells/mL. The yeast suspension was prepared by mixing one gram of dry yeast with 100 mL distilled-sterile water 20 minutes before pitching. Flasks were sealed and incubated for 72 hr in a shaker (Lab-Line 3526 Model) set at 30°C with 125 rpm agitation.

2.3.9. STATISTICAL ANALYSIS

Data of at least three replicates for all determinations were analyzed using analysis of variance (ANOVA) with a confidence level of 95%. Residual analysis was also performed in order to review the compliance of statistical assumptions (normality, independence and homoscedasticity). When means resulted different (null hypothesis rejected, $Pvalue<0.05$), multiple means comparison was performed (Fishers's least significant difference test) with $alpha= 0.05$ (Minitab® 14, Minitab Inc.). Homogeneous mean´s groups were indicated with the same alphabetic sub-indices within tables.

2.4. RESULTS AND DISCUSSION

2.4.1. GRAIN PHYSICAL PROPERTIES AND PARTICLE SIZE DISTRIBUTION.

Physical properties of grains indicated that test and thousand kernel weights were within ranges previously reported by Dicko et al (2006a), Perez-Carrillo and Serna-Saldivar (2007) and Serna-Saldivar (2010). Particularly, the red and white sorghums were in the upper range for test weight and maize for thousand kernel weight (Table 2.1). These results indicated that kernels were sound for dry milling and ethanol production. As expected, the decorticated red sorghum had higher test weight or apparent density and lower thousand kernel weight compared to the whole counterpart because of the selective removal of less dense pericarp and germ tissues rich in fiber and fat, respectively. The particle size distribution of ground meals is depicted in Table 2.2. Maize and white sorghum had the highest proportion of coarse fractions whereas red sorghum the highest percentage of fine fractions. These differences can be attributed to the harder endosperm texture of maize and white sorghum compared to red sorghum. According to Naidu et al (2007) the granulation of maize meals influences ethanol yields.

2.4.2. CHEMICAL COMPOSITION

The contents of crude protein, crude fiber, crude fat and ash depicted in Table 2.1 are within values reported in the literature (Perez-Carrillo and Serna-Saldivar 2007; Rooney and Serna-Saldivar 2000; Serna-Saldivar 2010). As expected, the red sorghum kernels contained higher protein compared to maize. According to Rooney and Serna-Saldivar (2000), regular sorghum usually contains between 1 to 2 % more protein compared to maize and up to 70% of the proteins are associated to the different types of kafirins (Dicko et al 2006b; Hamaker and Bugusu 2003). These alcohol soluble proteins have a reduced digestibility due to their high tendency to interact with other proteins, carbohydrates and (poly)phenols (Dicko et al 2006a).

Crude fat was slightly higher in maize compared to both whole sorghums. Rooney and Serna-Saldivar (2000) indicate that up to 1% less fat is found in sorghum compared to maize. Decorticated sorghum had the lowest fat because of the partial removal of the germ and aleurone tissues (Serna-Saldivar 2010). Maize contained the highest total starch content (68%) followed by white, decorticated red and whole red sorghums. Maize total starch content is within the range reported by Serna-Saldivar (2010) and the red sorghum starch is in the lower

limit reported by Dicko et al (2006a). It is well-known that starch contents vary according to genotype and environment and is related to 1000 kernel and test weights (Serna Saldivar 2010). White sorghum had a 1000 kernel weight and starch content 6 and 10% respectively higher compared to the whole red sorghum counterpart. The kernel size is positively related to the size of the endosperm. Large kernels usually contain a higher percentage of endosperm whereas smaller counterparts a higher ratio of pericarp-germ to endosperm.

The percent of damaged starch was slightly higher in decorticated red sorghum probably due to mechanical treatment used during milling. Decorticated sorghum contained less fiber, crude fat and minerals and more starch in contrast with its whole counterpart (Table 2.1; Serna-Saldivar 2010; Rooney and Serna-Saldivar 2000). The fiber mainly associated to the pericarp and cell walls contains most of the phenolic compounds as ferulic and caffeic acids (Rooney and Serna-Saldivar 2000). These compounds are generally bound or esterified to cell walls, being ferulic acid the most abundant in sorghum and other cereals (Awika and Rooney 2004).

Phenols are found in all sorghums genotypes and affect the color, appearance and nutritional quality of the grain (Rooney and Serna-Saldivar 2000). According to Awika and Rooney (2004), the phenolic composition and content in sorghums varies widely and is related mainly to genetics and to a lesser degree to environmental factors. Total phenols, flavonoids and ferulic acid contents (Table 2.1) were below values indicated by Perez-Carrillo and Serna-Saldivar (2007) and Dykes et al (2009). Red sorghums did not contain significant amounts of condensed tannins but contained extractable phenols. On the other hand, white or "food-type" sorghums do not contain tannins and have low total extractable phenols levels (Awika and Rooney 2004). As expected, the white sorghum kernels contained lower levels of total phenols, flavonoids and ferulic acid compared to the red counterpart. Actually, the phenolic profile in white sorghum was more similar to maize than to red sorghum (Table 2.1). The phenolic content in decorticated sorghum was below the level detected in whole red sorghum because in cereals, flavonoids and free phenolics are mainly associated to the kernel´s outer layers (Dykes and Rooney 2007).

Table 2.1. Physical and chemical properties of maize, sorghum (red, white and decorticated) and red sorghum bran (whole and spent)[1]

Treatment	Physical Properties			Proximate Composition[b]							Phenolic compounds		
	Test Weight, kg/hL	1000 Kernel Weight, g	Endosperm Texture[a]	Crude Protein (N x 6.25), %	Crude Fiber, %	Crude Fat, %	Ash, %	Nitrogen Free Extract[c], %	Total Starch, %	Damaged Starch, %	Total Phenols, mg gallic ac. eq./g	Free Ferulic Acid, mg/g	Flavonoids, mg catechin eq./g
Red Whole Sorghum	74.7±0.3b	26.8±0.9b	1.4±0.4b	9.8±0.5ab	2.2±0.3c	3.3±0.1ab	1.2±0.0de	84.6±0.0	60.6±1.5b	3.2±0.1bc	0.59±0.013	0.025±0.005	0.70±0.009
Decorticated Red Sorghum	77.6±0.4a	22.9±0.8b	1.4±0.4b	9.5±0.5ab	1.7±0.4c	3.0±0.4b	1.1±0.0e	85.4±0.5	67.0±0.7ab	4.6±0.6a	0.41±0.021	0.017±0.002	0.40±0.031
Maize	73.2±0.0c	334.7±3.8a	1.9±0.5b	8.5±0.2b	2.0±0.3c	3.6±0.1ab	1.3±0.1d	84.8±0.2	68.0±3.7a	2.9±0.0bc	0.26±0.022	0.007±0.001	0.25±0.036
White Whole Sorghum	77.9±0.8a	28.4±1.3b	2.5±0.4a	9.5±0.7ab	2.0±0.5c	1.5±0.3c	1.6±0.1c	85.3±0.7	66.5±4.1ab	3.5±0.1b	0.29±0.006	0.002±0.001	0.28±0.010
Red Sorghum Spent-Bran	-	-	-	10.3±0.8a	6.9±0.5b	2.8±0.2b	1.9±0.1b	78.1±0.6	29.2±0.8c	2.6±0.1cd	1.49±0.030	0.140±0.010	3.00±0.070
Red Sorghum Bran	-	-	-	11.0±0.8a	7.9±0.3a	4.2±0.7a	2.3±0.1a	74.5±0.9	33.5±1.3c	2.1±0.0d	2.25±0.130	0.190±0.010	4.80±0.410

[1] All values are the average of at least three replicas ± standard error of the mean. [a] Subjectively determined by viewing the ratio of soft to hard endosperm on dissected kernels. 5=totally vitreous or hard and 1= totally soft or chalky. [b] All values are expressed on dry matter basis. [c] Calculated by difference 100 - % crude protein – crude fiber – crude fat – ash.

2.4.3. SUGARS DURING ENZYMATIC TREATMENTS

During liquefaction, the reducing sugar concentration gradually increased until it reached the maximum at the end of the programmed hydrolysis (195 min) with thermostable α-amylase. At the end of liquefaction a lower total reducing sugars in the red whole sorghum hydrolyzate compared to the other five treatments was obtained. However, the difference between the whole red and white sorghums was not significant (0.54±0.08 vs 0.57±0.09 g/g flour –data not shown) despite the notorious difference in original starch content. Thus, the harder endosperm texture of the white sorghum kernels (Table 2.1) and the comparatively coarser meal granulation (Table 2.2) negatively affected rate of hydrolysis. Naidu et al (2007) concluded that the coarser granulation of maize meals negatively influenced fermentation and ethanol yields but these authors did not examine the effect of this factor during the stages of liquefaction and saccharification. In relation to the endosperm texture, starchy cells located in the floury and vitreous regions of the endosperm have similar components (cell walls, starch granules, matrix and protein bodies) and chemical composition. However, starch granules present in the vitreous endosperm are tightly bound to the protein matrix and therefore are more difficult to hydrolyze.

Table 2.2. Percent particle-size distribution of maize and sorghum (red, white and decorticated) ground in a Wiley Mill equipped with a 2-mm screen[1]

Particle Size μm	Maize	Whole White Sorghum	Whole Red Sorghum	Decorticated Red Sorghum
> 841	34.6±4.4	43.4±3.4	38.7±3.7	39.3±4.3
500-841	26.7±1.8	24.7±2.8	29.9±4.5	29.5±6.6
250-500	24.1±3.8	20.8±4.0	16.4±3.8	24.1±3.2
177-250	10.9±4.5	5.4±0.3	11.8±7.5	4.8±1.0
149-177	0.90±0.86	0.08±0.01	0.53±0.58	0.20±0.17
105-177	0.96±1.50	0.64±0.25	1.26±1.38	0.56±0.25
< 105	0.83±0.76	0.27±0.19	0.80±0.96	0.13±0.05

[1] All values are the average of at least three replicas ± standard error of the mean.

The FAN content for all treatments ranged from 65 to 101 mg/L at the end of liquefaction (Table 2.3). The maize hydrolyzates contained the highest FAN (101 mg/L). These results are similar to values

reported by Perez-Carrillo and Serna Saldivar (2007) who found higher concentration of FAN in maize compared to whole sorghum. The variation between sorghum and maize is related to the susceptibility of the sorghum proteins to denaturation especially during high temperature cooking and proteases. It is well-known that cooking reduces protein digestibility of sorghum and appears to be correlated with formation of disulfide-bonded oligomeric proteins and not to the total polyphenol content (Duodu et al 2002; Wu et al 2007). Despite the high homology between zeins and kafirins, the sorghum prolamins have more susceptibility to crosslink during cooking due to the higher content of hydrophobic sequences (Belton et al 2006; Duodu et al 2002; Duodu et al 2003; Ezeogu et al 2008; Rooney and Pflugfelder 1986; Wong et al 2009). Previous studies have reported improvements in fermentation performance in decorticated sorghum samples (Corredor et al 2006) and decorticated sorghum treated with proteases during liquefaction (Perez-Carrillo et al 2008). During hydrolysis with α-amylase, Perez-Carrillo and Serna-Saldivar (2007) reported the highest reducing sugar yield with the use of protease in decorticated samples followed by whole sorghum. However, the treatments without protease depicted a similar final level of reducing sugars. Apparently, protein content and its digestibility are the most important factors affecting starch and protein hydrolysis during liquefaction rather than the presence of fiber or phenolics (Wu et al, 2006; Wang et al, 2008).

Table 2.3. Effect of grain type and addition of phenolic extract or spent red sorghum bran to decorticated kernels on Free Amino Nitrogen during liquefaction with α-amylase[1]

Treatment	FAN (mg/L)		
	Initial	**After 65 min hydrolysis**	**After 195 min hydrolysis**
Whole Red Sorghum	63.3 ±5.85	69.37 ±5.17	79.36 ±20.02
Decorticated Red Sorghum + Spent Bran	64.37 ±6.36	56.24 ±8.34	76.34 ±5.73
Decorticated Red Sorghum + Phenolic Extract	63.29 ±7.82	53.13 ±15.35	65.82 ±14.39
Decorticated Red Sorghum	43.01 ±4.8	57.31 ±5.84	77.78 ±16.78
Maize	82.53 ±11.97	97.35 ±16.44	101.67 ±4.93
Whole White sorghum	43.14 ±16.79	47.4 ±9.79	65.93 ±10.27

[1] All values are the average of at least three replicas ± standard error of the mean.

Figure 2.1 depicts changes in glucose, maltose, maltotriose and fructose throughout saccharification with glucoamylase/pullulanase. There were no significant differences among treatments in terms of glucose (Figure 2.1A). The highest rate of production was achieved during the first 60 min hydrolysis. This initial stage was followed by a steady state where the highest concentration of glucose was reached. The curve showed a biphasic kinetic behavior consistent with product inhibition and enzymatic inactivation reported by several authors (Cepeda et al 2001; Perez-Carrillo et al 2008; Saville et al 2006; Åkerberg et al 2000).

Maltose concentrations for the different hydrolyzates were slightly higher compared to maltotriose at the start of saccharification (Figure 2.1B and Figure 2.1C). At the end of the programmed hydrolysis, maltose was around 10 to 20 mg/mL (2 to 5 g/100 g meal) higher compared to maltotriose. The decorticated red sorghum, maize and white sorghum hydrolyzates contained the highest maltose contents. The residual maltose at the end of process can be due to glucoamylase inhibition by product (Cepeda et al 2001; Perez-Carrillo et al 2008; Saville et al 2006; Åkerberg et al 2000). Maltotriose showed the highest rate of hydrolysis during the first 30 minutes followed by a slight increase in concentration. The highest concentration (approximately 6 g/100 g meal) was observed in the decorticated red sorghum plus spent bran. Profiles for this carbohydrate were similar among treatments ($P>0.05$). The increase of maltotriose before minute 100 could be linked to the gradual hydrolysis of existing dextrins. The hydrolysis of maltotriose in the first stage of saccharification had an effect in glucose and maltose concentrations (Figure 2.1A and Figure 2.1B). The lowest amount of maltotriose was reached at the fifth hour and this trisaccharide disappeared by the end of saccharification. Thus, glucoamylase hydrolyzed maltotriose with a good efficiency even though this enzyme is known to have higher affinity for longer chain oligosaccharides and preference for α-1,4 rather than α-1,6 bonds (Findrik et al 2008; Lee et al 1992). In some fermentation processes such as beer production, maltotriose, dextrins and other oligosaccharides account up to 38% of total sugars present in wort (Garcia-Garibay and Lopez-Munguia 1998). Nevertheless, in bioethanol production a higher level of monosaccharides is desired because regular yeast cannot metabolize linear and branched dextrins. Maize and white sorghum hydrolyzates contained the highest fructose concentration during saccharification (Figure 2.1D). Fructose concentration was in all treatments less than 1.0 g/100 g of original meal and can be related to the amount associated to original grains or to

the hydrolysis or inversion of the small quantity of sucrose present in cereal grains. Mature kernels contain small amounts of mono and disaccharides and most of these soluble sugars are associated to the germ (Serna-Saldivar 2010). According to the same author, sorghum and maize have roughly the same level of soluble sugars (1.5 to 1.9%), being the average of sorghum slightly lower compared to maize.

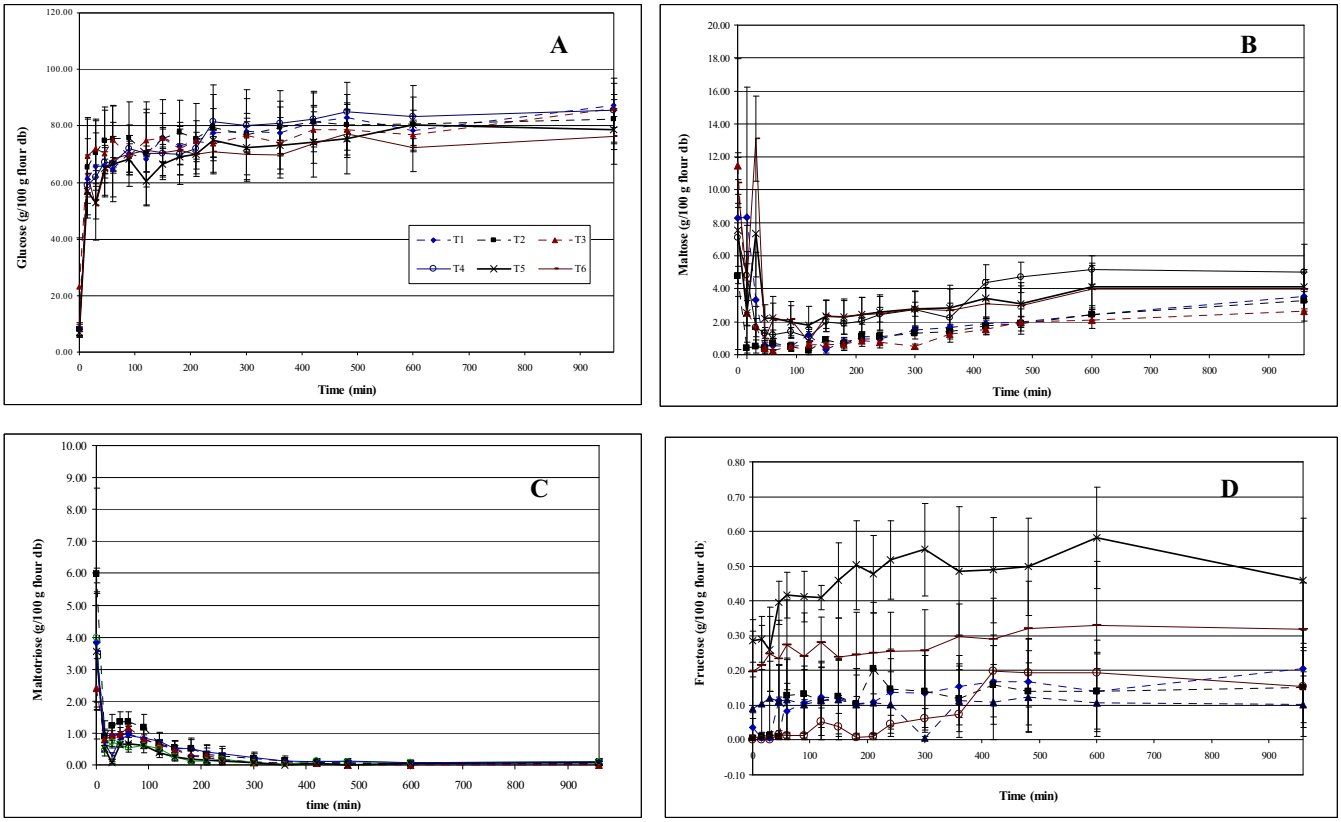


Figure 2.1. A) Glucose; B) Maltose; C) Maltotriose and D) Fructose (g/100 g flour) during saccharification of T1) whole red sorghum, T2) decorticated red sorghum + spent-bran, T3) decorticated red sorghum + phenolic extract, T4) decorticated red sorghum, T5) whole maize and T6) whole white sorghum.

2.4.4. Total Soluble Sugars Throughout Saccharification.

At the start of saccharification the percentage of fermentable carbohydrates, calculated as glucose, fructose, maltose and maltotriose, was between 22 to 46 % of the total reducing sugars. The decorticated red sorghum + phenolic extract hydrolyzate produced the highest amounts of fermentable species. Figure 2.2 depicts the relative percentage of total soluble sugars at the end of

saccharification. As expected, glucose was the principal sugar reaching 96% in relative abundance. The maltose content of hydrolyzates prepared from decorticated red sorghum, maize and white sorghum were slightly higher compared to the other treatments, although these dissimilarities were not statistically different ($P > 0.05$). Wang et al (2005) reported that saccharified mashes from maize processed by the dry grind process contained 80, 7, 4, 1 and 5% of glucose, fructose, maltose, maltotriose and higher sugars or dextrins, respectively. Zhao et al (2009), working with a small-scale mashing procedure for predicting ethanol yield from sorghum, reached after saccharification a level of 47% of glucose, followed by sugars of degree of polymerization higher than three (35%).

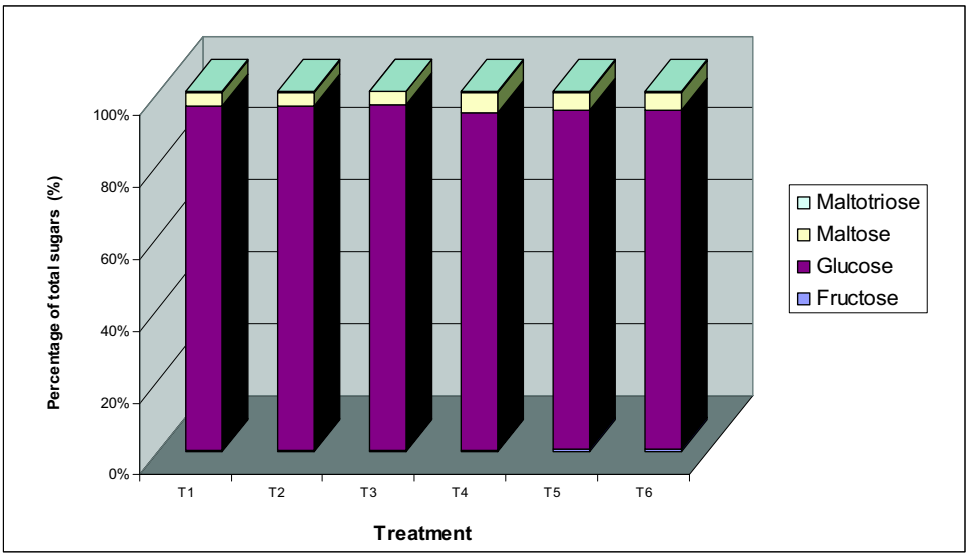


Figure 2.2. Effect of grain type and addition of red sorghum bran and its phenolic extract on the relative percentage of fermentable sugars obtained after liquefaction and saccharification[1,2]

[1] T1= Whole Red Sorghum; T2= Decorticated Red Sorghum + Spent Red Sorghum Bran; T3= Decorticated Red Sorghum + Phenolic Extract; T4= Decorticated Red Sorghum; T5= Whole Maize and T6= Whole White Sorghum

[2] Carbohydrates with a higher degree of polymerization than maltotriose are not included

The type and concentration of carbohydrates at the end of saccharification can be related to the type (and concentration) of enzyme used in the process, to the time and conditions of catalysis and undoubtedly to the type of starch (Zhao et al 2009). In the case of sorghum, higher starch gelatinization temperature (compared to maize) as well as the tendency of kafirins to form a web-like matrix during mashing reduces the efficiency of commercial glucoamylase. Zhao et al (2009) concluded that the protein matrix held starch granules, oligosaccharides and polysaccharides which

limited the ability to predict ethanol yield from total sorghum starch. In this research, there were no significant differences in soluble carbohydrate profile at the end of saccharification as affected by the type of raw material (Figure 2.2).

The use of Dextrozyme DX [mixture of glucoamylase (E.C. 3.2.1.3) and pullulanase (E.C. 3.2.1.41)] instead of just glucoamylase is recommended in order to overcome the factors that reduce the efficiency of catalysis such as relative slow debranching action of glucoamylase (15 times slower than the α-1, 4 hydrolysis) and formation of reversible products such as isomaltose (Findrik et al 2008; Lopez-Munguia 1998; Roy and Gupta 2004; Sears 1995; Slominska et al 2003).

The high glucose concentration at the end of saccharification is desired in ethanol production because yeast has preference for this substrate, followed by maltose and maltotriose (D'Amore et al 1989). These preferences are related with the easiness of transport of carbohydrates across the yeast cell membrane and the type of molecular regulation (Barnett 1997).

2.4.5. Sugars and Ethanol Profile During Fermentation

Fructose consumption during fermentation is depicted in Figure 2.3(A). The maize wort contained the highest initial fructose concentration whereas all sorghum treatments had similar amounts. After 24 hr fermentation, fructose was completely consumed in all treatments. Consumption of fructose and glucose (Figure 2.3A and Figure 2.4) showed similar patterns and are congruent with results previously reported for hydrolyzed starchy materials (Chuck-Hernández 2009). According to D'Amore et al (1989) after glucose consumption, fructose is the preferred substrate for *S. cerevisiae* and is directly oxidized in the glycolysis (Emden-Meyerhof pathway) by means of facilitated diffusion (Ingledew 1995). On the other hand, higher sugars such as maltose and maltotriose, require systems with more energy requirements. For example maltose is transported to the interior of the yeast by a proton co-transporter system and then is hydrolyzed by an intracellular maltase (E.C 3.2.1.20) in two units of glucose (Barnett, 1997; Boulton and Quain, 2001; Novak et al, 2004).

The worts used for fermentation had a high concentration of simple sugars, mainly glucose, facilitating the process (Figure 2.2 and Figure 2.4). Maltose and maltotriose contents were relatively low (Figure 2.3B and Figure 2.3C). The former sugar was around 6 to 8 g/L for all treatments and the latter less of 1 g/L. In the case of maltose, treatment 4 (decorticated red sorghum) reached the lowest concentration after 72 hours and the higher level between 30 to 50 hours when maltotriose was

present at the lowest concentration (Figure 2.3C). At the end of fermentation, residual maltose can be related to glucoamylase inhibition by product or to glucose inhibition of maltose transport in yeast, a dominant factor for the control of maltose catabolism to ethanol during fermentation (Rautio and Londesborough 2003). Besides maltose, maltotriose concentration throughout fermentation is also showed in Figure 2.3 (C). Most of this carbohydrate was consumed after 30 hours and the relatively slow uptake can be explained for the permease specificity in *Saccharomyces* cells. If a higher expression of AGT1 permease is present in cell then, an increase in preference of maltotriose respect to maltose consumption could be accomplished (Alves et al 2007).

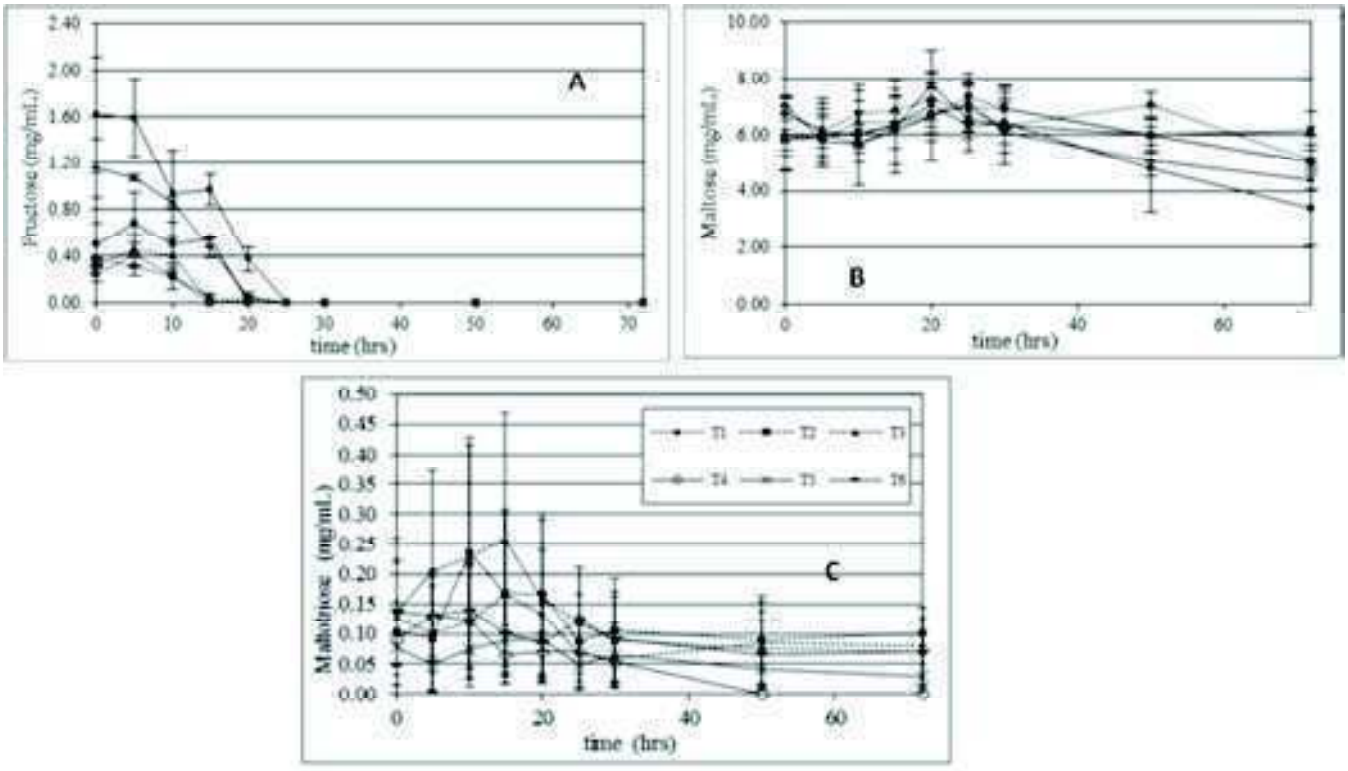


Figure 2.3. A) Fructose, B) Maltose and C) Maltotriose profile during fermentation for all evaluated treatments (T1 whole red sorghum, T2 decorticated red sorghum + spent-bran, T3 decorticated red sorghum + phenolic extract, T4 decorticated red sorghum, T5 whole maize and T6 whole white sorghum)

As stated before, the principal substrate present in worts was glucose (Figure 2.4), which in turn was totally consumed through the first 25 hours of fermentation when the highest amount of ethanol was also produced.

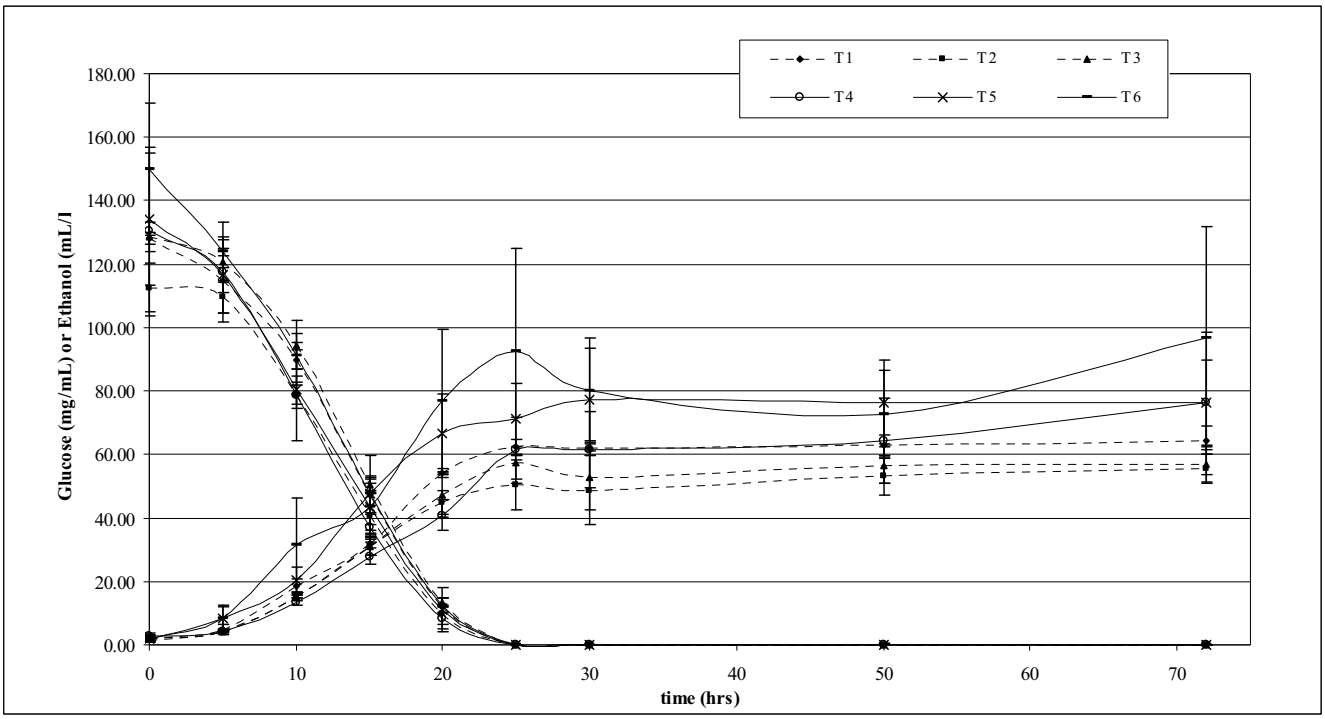


Figure 2.4. Glucose consumption and ethanol production profile during fermentation for all evaluated treatments (T1 whole red sorghum, T2 decorticated red sorghum + spent-bran, T3 decorticated red sorghum + phenolic extract, T4 decorticated red sorghum, T5 whole maize and T6 whole white sorghum)

The consumption and production profile observed was similar to previous reports (Chuck-Hernández et al 2009; Perez-Carrillo et al 2008). The highest concentration of glucose at the time of inoculation was for the white sorghum treatment. Glucose was also the substrate with the highest rate of consumption mainly compared to maltose and this result is related to transport mechanisms of *S. cerevisiae* as previously mentioned (D'Amore et al 1989; Leiper et al 2006).

In Table 2.4 ethanol yields and fermentation efficiencies for the six treatments are depicted. Yields for maize and red sorghum were 0.362 and 0.352 mL/g respectively whereas for the decorticated red sorghum 0.365 mL/g. These results are in the range reports previously by Perez-Carrillo et al. (2008) (0.279 – 0.402 mL/g flour), being the highest the obtained with decorticated sorghum using protease during liquefaction. The yield per flour depicted in Table 2.4 is also within the values described by Chuck-Hernandez et al. (2009) with steam flaked sorghum (0.276 to 0.398 mL/g flour). In terms of ethanol yield expressed per unit of starch, the decorticated with spent bran treatment (T2) was the

highest followed by red sorghum (T1) with 0.607 and 0.581 mL/g. The higher fermentation efficiency of T2 (98.2%) was probably due to the presence of starch in the bran after phenolic extraction. On the other hand, the lowest yield and efficiency was observed in white sorghum treatment due to its harder endosperm texture: 0.313 mL/g flour and 76.06% respectively (Table 2.1) which also negatively affected performance during liquefaction. This efficiency is similar to the previously reported by Wu et al. (2006) (74.34%), higher than the 69.8% obtained with whole red sorghum for Chuck-Hernandez et al. (2009) but lower than the ranges reported by Wu et al. (2007, 2008 and 2010) that goes from 85.2 to 93.9%. The latter fermentation efficiency range is comparable with results obtained for T1 to T5 (Table 2.4), describing thus that with sorghum, values around 90% of bioconversion can be expected during ethanol production. Despite the differences in yield and fermentation efficiencies, the statistical analysis showed no significant discrepancies among treatments (P>0.05).

Table 2.4. Effect of grain type and addition of phenolic extract or spent red sorghum bran to decorticated kernels on ethanol yield and fermentation efficiency[1]

Treatment	mL ethanol/ g flour	mL ethanol/g starch	Fermentation efficiency
	mb	mb	%
Whole Red Sorghum	0.352 ± 0.04a	0.581 ± 0.06a	94.01 ± 9.48a
Decorticated Red Sorghum + Spent Bran	0.384 ± 0.04a	0.607 ± 0.06a	98.18 ± 9.57a
Decorticated Red Sorghum + Phenolic Extract	0.347 ± 0.08a	0.517 ± 0.12a	83.7 ± 19.73a
Decorticated Red Sorghum	0.365 ± 0.04a	0.545 ± 0.06a	88.11 ± 9.46a
Maize	0.362 ± 0.05a	0.532 ± 0.07a	86.08 ± 11.93a
Whole White sorghum	0.313 ± 0.04a	0.470 ± 0.06a	76.06 ± 10.24a

[1] All values are the average of at least three replicas ± standard error of the mean.

2.5. CONCLUSIONS

The effect of sorghum bran and phenolic compounds was studied in sorghum meals subjected to liquefaction with thermostable α-amylase, saccharification with glucoamylase/pullulanase and fermentation with *Saccharomyces cerevisiae*. Red sorghum contained the lowest starch content (60.6±1.5) whereas the white counterpart and maize the hardest endosperm textures (2.5 and 1.9, respectively). No significant differences were found in FAN content among treatments, nevertheless, maize was slightly higher compared to all sorghum treatments (101.7 mg/L versus 73 mg/L average in the rest of treatments). No significant differences were found in maltotriose and glucose profiles during saccharification of liquefied starch. Glucose was the main fermentable sugar which accounted for up to 96% of the total sugars. All treatments had residual maltose after saccharification and fermentation. At the end of process, *S. cerevisiae* produced from 0.313 to 0.384 mL ethanol/ g meal, being the white sorghum treatment the one which had the lowest fermentation efficiency (76.06 ± 10.24%). The harder endosperm texture and coarser particle size distribution of ground meals observed in white sorghum negatively affected hydrolysis during liquefaction and saccharification and ethanol yield. Treatments supplemented with spent red sorghum bran (without phenolics) and with added phenolic were not significantly different compared to the decorticated red sorghum in terms of glucose production after liquefaction and saccharification. Thus, results clearly demonstrate that the red sorghum bran and its phenolics did not affect performance of α-amylase during conventional liquefaction, glucoamylase/ pullulanase during saccharification or *S. cerevisiae* during fermentation.

Once that the influence of red sorghum bran and its phenolics in fuel ethanol production was studied, there was also an interest to evaluate the bioconversion performance of maize and sorghum kernels damaged by biotic agents into ethanol. The use of mold, insect and sprout-damaged caryopsis in industrial fermentation could reduce farmer losses, at least in some extent and the environmental impact because the use of cereals for fuel transportation production.

3. CONVERSION INTO BIOETHANOL OF INSECT (*SITOPHILUS ZEAMAIS* MOTSCHULSKY), MOLD (*ASPERGILLUS FLAVUS* LINK) AND SPROUT-DAMAGED MAIZE (*ZEA MAYS* L.) AND SORGHUM (*SORGHUM BICOLOR* L. MOENCH)*

Chuck-Hernández, C., García-Lara, S., Serna-Saldívar, S.O. 2012. Conversion into bioethanol of insect (Sitophilus zeamais Motschulsky), mold (Aspergillus flavus Link) and sprout-damaged maize (Zea mays L.) and sorghum (Sorghum bicolor L. Moench). Journal of Cereal Science 55, 285-292.

3.1. ABSTRACT

The bioconversion into ethanol of insect (*Sitophilus zeamais*), mold (*Aspergillus flavus*) and sprout-damaged maize and sorghum was investigated. Kernel test weight losses due to insect damage in maize were almost twice as much compared to sorghum (18.6 *vs.* 10.7%). All damaged kernels loss some of the starch and increased soluble sugars, ash and crude fiber. The mold-damaged sorghum contained approximately five times more FAN compared to the control. The sprout-damaged kernels contained the highest amounts of reducing sugars both prior (11 g/L) to and at the end (146.5 g/L) of liquefaction with α-amylase. Ethanol yields based on the already damaged grain indicated that sprout-damaged kernels yielded similar amounts compared to sound kernels (381.1 vs 382.6 L/ton and 376.6 vs 374.8 L/ton of sorghum or maize grain used respectively). The insect-damaged maize and sorghum kernels have reduced ethanol yields compared with the controls (29 and 23% respectively), and this negative result was mainly due to dry matter losses incurred during the inadequate storage. Despite differences in ethanol yield, all treatments have similar conversion efficiencies (76.1 to 89.9%) indicating the robustness of yeast facing biotic-damaged feedstock. This research clearly demonstrates that the use of already damaged insect, mold or sprouted kernels is feasible and a good alternative for biorefineries.

3.2. INTRODUCTION

Worldwide ethanol production is growing at a remarkable pace derived from the rising concern of fuel sustainability and due to environmental problems associated to fossil energy. In 2010, bioethanol production in the USA reached 13 billion gallons, 30% more than the previous year (RFA, 2011), whereas in Brazil 9.7 billion gallons are expected to be distilled in 2012 (Walker, 2011). This unprecedented ethanol production has been supported mainly with maize and sugar cane, respectively.

In Latin America and some areas of Africa, maize is the main source of caloric intake and for this reason its use for biofuels is not socially feasible. In the specific case of Mexico, maize for bioethanol is limited by a federal law, where is established that only surplus domestic production can be channelled to bio refineries (CD, 2008). However there are other options to produce biofuels such as agricultural wastes and insect, fungi and sprout-damaged kernels which are not fitted for human consumption or industrial processing.

In the postharvest system of cereals some qualitative and quantitative losses occur. Overall postharvest losses in maize can be around 29%, mainly during drying in the field and storage, and can predominantly be attributed to biotic factors such as insects, molds, rodents and sprouting. According to FAO (1993), the range of worldwide postharvest losses is between 10 to 37%. Considering these values, the USA losses are between 33 to 123 and 1 to 3 million tons of maize and sorghum, representing economic losses of $5.5 to 20.1 billion dollars.

One of the main biotic factor associated with these losses are insects, being *S. zeamais* the main pest in tropical agro ecologies and also the most harmful causing 20 to 40% of losses in stored grain (García-Lara and Bergvinson, 2007). Besides insect damage, fungal infections is also a major postharvest problem causing undesirable effects such as discoloration, off-odors, loss of germination capacity and contamination with harmful mycotoxins. According to Bandyopadhyay et al. (2000), the estimated annual losses due to molds in semi-arid tropical areas of Asia and Africa reached US$130 million. Fungi affecting cereal grains are diverse, but the genus *Aspergillus* and *Fusarium* are the most widespread in storage (Serna-Saldívar, 2010). In tropical areas, field and storage sprouting also causes direct and indirect losses due to the activation of degrading intrinsic enzymes that breakdown proteins, carbohydrates and lipids into simpler forms.

The use of insect, fungi and sprout-damaged grain for human consumption is not always possible and its utilization in other industrial processes could reduce, at least in some extent, the producer losses. Regarding the use of damaged kernels in ethanol production, Yan et al. (2010) tested field-sprouted sorghum and concluded that the use of these kernels significantly reduced fermentation time and yielded higher ethanol. The detrimental effect of aflatoxins associated to maize mashes was studied by Murthy et al. (2005). These authors concluded that maize containing 775 ppb or less aflatoxin did not affect ethanol yields.

The present study was undertaken to compare the bioethanol conversion efficiency of insect (*Sitophilus zeamais*), mold (*Aspergillus flavus*) and sprout- damaged lots of maize and sorghum. These comparisons were made to investigate the behaviour of damaged kernels with different chemical compositions in terms of susceptibility to enzyme hydrolyses and efficiency of ethanol production.

3.3. MATERIALS AND METHODS

3.3.1. GRAIN SOURCES AND EVALUATED TREATMENTS

Grains used were commercial yellow dent maize and type II red sorghum, obtained from a local market (Monterrey, N.L. Mexico). The undamaged maize and sorghum kernels had a soft and intermediate endosperm textures rated as 1.5 and 2.5, respectively (in a subjective scale where 1 is a totally soft and 5 is totally hard endosperm). These grains were not treated with insecticides or fungicides. Four subsamples with three replicas were taken from each type of cereal grain. One was kept as control (sound kernels) whereas the others were purposely damaged with *Sitophilus zeamais, Aspergillus flavus* and sprouting.

3.3.2. SAMPLE PREPARATION

Original grains were cleaned by air-aspiration and sieves, tempered to 14% moisture, using the formula ([(100 - % Initial Moisture)/(100 – % Final Moisture)] – 1] * Sample Weight) and one kilogram stored in closed polyethylene terephtalate containers stored at 27°C and 75% relative humidity (RH), using a saturated NaCl solution.

3.3.2.1. INSECT INFESTATION

For insect infestation, kernels were stored in glass jars and equilibrated for 30 days at 27 ± 1°C and 70 ± 5% RH (using a saturated NaCl solution). Then, grains were infested with 200 unsexed adult weevils (*Sitophilus zeamais*) no older than 7 days and then stored under controlled conditions (27°C, 70% RH) with a photoperiod of 12:12 h (dark/light). The infestation experiments were run by triplicate during 7 weeks or until the level of damage reached 17.6 and 14.8% of dry matter losses for maize and sorghum (53 and 18% of damaged kernels, respectively). Mesh sieves (No. 10 and 16) were used to sort grain and adult weevils. Grain weight loss was calculated by subtracting the final from the initial dry grain weight. Damaged kernels were separated and counted based on visible tunneling or emergence holes. These losses and extent of damage are typically observed in grain elevators in northern Mexico.

3.3.2.2. MOLD INOCULATION

Aspergillus flavus isolate from experimental field station at Agua Fria, Puebla, Mexico (19° North latitude, 60 m above sea level) was used to inoculate kernels. The fungus was grown on V8 juice agar plates (5% juice -vol/vol- pH 5.2 and 2% agar -wt/vol-) at 30°C in the dark. Conidia from 7-day old cultures suspended in deionized water were used as inoculums. Then, the infection experiment was run by triplicate during 7 weeks at 27°C, 75% RH and a dark/light period of 12:12 h. The level of infection was visually evaluated and when the fungi mycelia reached 75% of the grain-container, simulation was finished and grain used for further tests. Both insect and mold infestations were performed at the Entomology and Phytopathology Laboratory facilities at CIMMYT (Texcoco, Estado de Mexico, Mexico) and samples sent to the Biotechnology Center at ITESM (Monterrey, N.L., Mexico) for further analysis and processing.

3.3.2.3. GRAIN SPROUTING

Kernels were steeped in distilled water (1:4) with 0.01% (vol/vol) of formaldehyde during 36 h at 22°C with constant aeration. In order to avoid mold growth, a 30 ppm sodium hypochlorite water wash was applied. Then, the soaked kernels were spread onto trays covered with wet paper towel and placed in a germination cabinet (Seedburo Equipment Co., Chicago IL, USA) set at 27°C and >75% RH. Maize and sorghum kernels were sprouted for 36 and 12 h, until they

reached 40 and 60% of visually germinated kernels, respectively. Then, the sprouted kernels were dehydrated at 50°C during 12 h and stored at 4°C.

3.3.2.4. MILLING

Control and induced-damaged kernels were ground in a Wiley mill equipped with 2 mm round orifices screen. The particle size distribution of resulting meals was determined by the Rotap sieving method reported for Chuck-Hernández et al. (2009).

3.3.3. PHYSICAL AND CHEMICAL CHARACTERIZATION

Grain physical characteristics were determined by triplicate using standard procedures: test weight according to Official US Grain Standard Procedures (AACC Method 55-10); thousand-kernel weight by weighing 100 randomly selected kernels and endosperm texture according to the subjective procedure reported by Chuck-Hernández et al. (2009). Flotation index (FI) was determined according to Gutiérrez-Uribe at al. (2010) and expressed as a percentage of floating kernels on an aqueous solution of sodium nitrate (1.25 g/cm^3 specific weight at 35°C). L*, a*, b*, and other CIE color parameters of ground samples were determined using a colorimeter (Minolta CR-300, Osaka, Japan). Moisture content was assayed using a gravimetric method AACC (2000) 44-15A. Total starch was calculated using a commercially kit (Method 76-13, AACC 2000, Megazyme International, Wicklow, Ireland). Protein (N*6.25) was determined using the micro-Kjeldhal method 46-13 whereas crude fiber, fat, and ash were assayed according to methods 32-10, 30-20 and 08-01, respectively (AACC, 2000).

3.3.4. LIQUEFACTION

Ground meals (15 g dry basis) with 0.02% of calcium hydroxide were mixed with distilled water to obtain mashes with 30% (wt/vol) solids. pH was a initially adjusted to 5.6 with 0.1N HCl and temperature was increased to 85°C in a shaking water bath (BellCo Glass, Vineland, NJ). When slurries reached 80°C, 25μL of Liquozyme (240 KNU-S/g, Novozymes, Bagsvaerd, Denmark) was added. Mashes were maintained at 90°C during 200 min. In order to determine the progressive extent of starch hydrolysis, aliquots were taken before enzyme addition, and after 100 and 200 min hydrolysis. All treatments were performed by triplicate.

3.3.5. Simultaneous Saccharification and Fermentation (SSF)

Mashes were cool down from 80°C to 30˚C, adjusted to 15°Plato and pasteurized (65°C for 30 min). A commercial mixture (Dextrozyme DX, Novozymes) of glucoamylase (EC 3.2.1.3) and pullulanase (EC 3.2.1.14) produced from genetically modified strains of *Aspergillus* and *Bacillus* was added altogether with dry yeast (*Saccharomyces cerevisiae*) (Nevada, Safmex, Toluca, Mexico). Dextrozyme was added at a rate of 1 mL/100mL of liquefied slurry whereas the yeast was previously suspended in sterile water and pitched at a cell concentration of 1.5 x 10^7 cells/mL according to Chuck-Hernández et al. (2009). Reaction vessels were sealed and maintained for 72 h in an incubator-shaker (VWR International, Model RF1575) set at 30°C with agitation (100 rpm) during the first 24 h. All treatments were performed by triplicate.

3.3.6. Analysis of Hydrolyzates and Fermented Mashes

Free amino nitrogen (FAN) during liquefaction and SSF was determined by triplicate using the ninhydrin method 945.30L (AOAC, 1980). Total reducing sugars during liquefaction were determined with the dinitrosalicylic acid method (DNS) of Miller (1959). Ethanol concentration after fermentation was determined by gas chromatography (GC Agilent 6850) with Flame Ionization Detector (FID) at 220°C. The GC was furnished with an HP Innowax column (30 m x 0.53 mm x 1.0 μm) and helium at 4.0 mL/min was used as carrier. Injection volume was 0.2 μL at a split rate of 1:50. Maltotriose, maltose, glucose, fructose and glycerol were quantified throughout 72 h SSF by HPLC-IR (Waters 2114, Milford, MA) furnished with an ion-exchange column (Aminex HPX-87H, Bio-Rad®, Hercules, CA) at 60°C using 5 mM sulfuric acid at a rate of 0.6 mL/min as mobile phase (Chuck-Hernández et al., 2009).

3.3.7. Statistical Analysis

All determinations were performed by triplicate and data analyzed with ANOVA (Minitab® 14, Minitab Inc). The variables studied were type of grain and damage. Additionally, the generation of FAN throughout liquefaction was statistically analyzed. Residual analysis was performed in order to review the compliance of statistical assumptions (normality, independence and homoscedasticity). When means resulted different (null hypothesis rejected, $Pvalue<0.05$), multiple means comparison was performed (Tukey's test) with $alpha=0.05$.

Homogeneous mean´s groups were indicated with the same alphabetic sub-indices within tables.

3.4. RESULTS AND DISCUSSION

3.4.1. PHYSICAL AND CHEMICAL ANALYSIS

The initial test weights of maize and sorghum were within ranges specified by Serna-Saldívar (2010), indicating that both types of kernels were initially sound and healthy. As expected, all sorts of damaged kernels had a lower bulk density and average kernel weight (Table 3.1). Test weight losses due to insect damage in maize were higher (18.6%) compared to sorghum (10.7%). Insect damage and sprouting were more detrimental in maize compared to sorghum. The observed difference could be associated to the softer endosperm texture of maize and to the higher resistance of the sorghum pericarp which contains more phenolics. These compounds are related to kernel resistance through two main mechanisms: physical (hydroxycinnamic acids bound to cell wall) and toxicological (due to the effect of phenolic acid amides in the invasive organism) (Bergvinson and García-Lara, 2011; García-Lara et al., 2004). According to Dicko et al. (2006) fifty different sorghum varieties contained from 0.5 to 3% (w/w) of total phenolics and these compounds were mainly associated to pericarp cell walls. Phenolics have an important role in protecting kernels against biotic and abiotic stresses (Serna-Saldívar, 2010). Ramputh et al. (1999) reported a positive relationship between phenolic content of sorghum and *Sitophilus oryzae* resistance.

The FI was also greatly affected by the different sorts of damages. This parameter estimates the relative density of the grain and therefore is an indicator inversely related to grain hardness (Roselena et al., 2009). In ethanol production, an intermediate or soft endosperm texture is desired because these kernels usually have a higher proportion of starch (Serna-Saldívar, 2010). In all treatments, an increase of FI was observed because of the reduction of density due to endosperm damage or starch consumption or degradation (Table 3.1 and Table 3.2). According to Lozano-Alejo et al. (2007), soft and intermediate-textured maize and sorghum, such as the ones used herein, had FI of 85 and 17.5%, respectively.

Table 3.1. Effects of insect, mold and sprout-damages on physical properties and color of maize and sorghum[1]

Samples			Test weight, kg/hL[2]	Flotation index [3], %	1000 kernel weight [3], g	Color values[4]			
						Color Difference, ΔE[3]	Hue (h°)[3]	Chroma (C)[3]	Color index (CI)[3]
Maize	Control (without damage)		72.43±0.33a	85.00±2.88a	287.50±1.99a	NA[5]	-1.42±0.00a	35.15±0.00a	-2.70±0.00b
	Damaged with:	*A. flavus*	62.26±0.40b	100.00±0.00b	292.18±2.16a	6.49±2.06	-1.30±0.05a	30.70±1.76ab	-5.16±0.89c
		S. zeamais	58.91±0.40c	100.00±0.00b	245.34±2.71c	7.45±2.34	-1.39±0.01a	40.44±1.88a	-3.01±0.03bc
		Sprouted	58.49±0.15c	100.00±0.00b	257.73±1.31b	14.2±0.53	1.38±0.00b	25.97±0.81b	3.27±0.09a
Sorghum	Control (without damage)		75.68±0.28d	17.50±2.50c	24.67±0.52d	NA[5]	1.39±0.00c	24.12±0.00c	4.90±0.00d
	Damaged with:	*A. flavus*	67.58±0.23e	63.33±6.67d	24.78±0.29d	6.32±1.05	-0.48±1.02c	21.87±0.29d	-1.00±0.91e
		S. zeamais	67.54±0.91e	44.43±7.28d	21.39±0.24e	2.36±0.90	1.39±0.02c	21.91±0.86d	4.98±0.52d
		Sprouted	72.91±0.12a	63.33±1.67d	20.17±0.52e	11.61±0.34	0.95±0.02c	27.02±0.31e	18.67±1.05f

[1]Means are the average of at least three replicas ± standard error of the mean. [2] Means with different letter(s) within column are statistically different (P<0.05). [3] Means with different letter(s) within kernel type are statistically different (P<0.05). [4]Color values were estimated on ground samples. Color difference $(\Delta E)=(\Delta L^2+\Delta a^2+\Delta b^2)^{1/2}$; Hue (h°)= arctangent(b/a); Chroma (C)= $(a^2+b^2)^{1/2}$; Color index(CI)= (a*1000/L*b). [5]Not Apply, NA.

A subjective endosperm texture evaluation was also performed and the results were in agreement with the observed FI and test weights. The difference in endosperm texture also affected the particle-size distribution after milling (data not shown). The sorghum-control treatment yielded a flour with higher amounts of the coarse fraction (>850 μm) compared to maize (45 versus 35% respectively).

The thousand kernel weight was severely affected by the different types of damages. This parameter is an important quality criterion because a larger grain usually contains a higher proportion of endosperm and starch. Both types of kernels were within ranges reported previously (240 to 370 g for maize and 23 to 35 g for sorghum) (Serna-Saldívar, 2010). The loss of weight for insect and sprout-damaged maize and sorghum was between 8.5 to 17.6%, indicating the effect of nutrient consumption because of the insect infestation and the activation of grain metabolism during germination. Sprout-damaged maize and sorghum lost 11 and 8.5% of the kernel weight compared to sound counterparts whereas insect-damaged maize and sorghum, 17.6 and 14.8%, respectively. The mold-infested kernels were heavier due to the purposely added conditioning moisture even tough maize and sorghum lost 3.8 and 7.9% of their dry matter weight, respectively.

The color difference, obtained as a distance in relation to controls treatments in the CIE L*a*b* space color, was higher in the meals from sprouted-kernels (Table 3.1). These results can be related to changes in carbohydrate composition during germination. During this physiological process part of the starch is hydrolyzed into reducing sugars and subsequently these sugars react with simpler nitrogenous compounds forming color species (Maillard reactions). These changes are exacerbated by thermal treatments such as the ones used for drying and liquefaction.

Color changes in insect and mold-damaged treatments were not as severe as the observed for sprouted kernels. However, ground meals contaminated with mycelia from *A. flavus* had color indexes of -5.16 and -1.00 for maize and sorghum, respectively. According to Vignoni et al. (2006), these values are within the range of green to yellow-greenish, close to the characteristic or typical coloration of *A. flavus* conidia.

Table 3.2. Effects of insect, mold and sprout -damages on the chemical properties of maize and sorghum.[1]

Samples			Moisture, %	Crude Protein (N x 6.25), %	Crude Fiber, %	Crude Fat, %	Ash, %	Nitrogen Free Extract[2], %	Total Starch, %
Maize	Control (without damage)		14.19±0.08a	7.91±0.40a	1.57±0.24a	2.51±0.02a	1.43±0.03a	86.58±0.30a	72.31±3.57a
	Damaged with:	*A. flavus*	18.76±0.02b	9.41±0.25b	2.80±0.45ab	2.45±0.07a	1.65±0.03b	83.69±0.46b	71.91±1.04a
		S. zeamais	17.11±0.07c	9.93±0.15bc	3.33±0.46b	3.50±0.14b	1.86±0.05b	81.38±0.71c	68.19±1.29a
		Sprouted	8.84±0.07d	8.59±0.1abc	2.43±0.24ab	3.16±0.12b	1.32±0.05a	84.50±0.46ab	68.09±0.10a
Sorghum	Control (without damage)		14.09±0.06ef	11.51±0.04c	1.36±0.18c	1.98±0.41c	1.58±0.05c	83.57±0.5d	73.65±0.06b
	Damaged with:	*A. flavus*	21.23±0.17e	11.56±0.15c	2.27±0.21d	1.64±0.20c	1.44±0.06c	83.09±0.36d	69.96±0.81b
		S. zeamais	15.64±0.02f	12.04±0.25cd	3.24±0.16de	2.61±0.08c	1.41±0.03c	80.72±0.22e	70.17±1.54b
		Sprouted	7.95±0.01g	10.45±0.39ce	2.42±0.05de	1.27±0.43c	1.05±0.02d	84.82±0.75d	68.84±0.28b

[1] All values are expressed on dry matter basis and are the average of at least three replicas ± standard error of the mean. Means with different letter(s) within kernel type were statistically different ($P<0.05$). [2] Calculated by difference 100 - % crude protein – %crude fiber – %crude fat – %ash.

All hue or tone values, except the one observed for sprouted ground maize, were similar (Table 3.1). In contrast, chroma values were significantly lower for mold and sprout-damaged maize. In the case of sorghum, all treatments were significant different compared to the control. Sprouting promoted the highest difference especially after drying likely due to Maillard reactions.

Regarding to color indexes, all maize treatments had similar values ranging between green to yellow. The sprouted-maize was the only treatment with a positive color index (in the range of yellow to orange). Likewise, the control and insect-damaged sorghums had color indexes similar to the sprouted-maize. The color index of mold-infested sorghum went down to a green-yellow tone because of the presence of mycelium. The largest color index change was observed in the ground sprouted-sorghum. The corresponding value indicated colorations between orange to intense red rather than the original orange coloration.

As expected, the chemical composition was also affected by insects, molds and sprouting (Table 3.2). Moisture, by far the most important factor influencing the rate of deterioration, increased from 14% in the controls to 18 and 21% in mold-damaged maize and sorghum, respectively. The insect-damaged maize and sorghum contained 17 and 15% moisture. In the case of sprouting, moisture was purposely increased to 41 and 35% in maize and sorghum respectively in order to activate gibberellins and enzyme activity. After achieving the desired level of sprouting, kernels were dehydrated to stop metabolic processes and stabilized kernels (Table 3.2). The moisture increment observed in insect or mold-infested kernels was mainly due to respiration. Serna-Saldívar (2010) indicates that insects proliferate when the grain moisture exceeds only 1.5% of the critical moisture content (14% in cereals). The degree of insect infestation is mainly affected by the grain moisture, storage conditions (temperature, RH and controlled atmosphere), length of storage and extraneous material among other factors. Insect-infested sorghum contained significantly lower moisture compared to the maize counterpart. Thus, *S. zeamais* presumably had less available water compared to counterparts developing in maize.

In the case of *A. flavus* infested kernels, both sorghum and maize, showed all the typical signs of contamination such as discoloration, formation of kernel clusters trapped with a web-like mycelium and an increase in moisture due to its own metabolic activity. It is well-known that the growth of storage fungi occurs when the grain moisture exceeds 3.5% above the critical

moisture. It is likely that *A. flavus* exacerbated its growth and activity due to the generation of metabolic energy (temperature) and moisture (product of respiration).

The crude protein was higher in sound sorghum compared to maize (11.5 versus 7.9%). The protein is substrate for the production of simpler nitrogenous or FAN. Despite a significant increase in FAN concentration (Table 3.4) in all damaged kernels, the protein content remained stable. In maize, all damaged treatments had almost twice the initial level of simpler nitrogenous compounds whereas the mold-damaged sorghum contained approximately five times more FAN compared to sound kernels. This difference is attributed to the potent mold proteolytic enzymes that hydrolyzed to a higher extent sorghum proteins compared to maize.

In relation to crude fiber (Table 3.2), the control kernels were within the ranges reported (2.4 to 3.5% in maize and 1.2 to 6.6% dry basis in sorghum) (Serna-Saldívar, 2010). The insect-damaged kernels contained higher crude fiber due to the reduction in starch indicating that *S. zeamais* had preference for the starchy endosperm.

The control and mold-damaged maize treatments contained similar amounts of crude fat. These amounts are below the range indicated in the literature (3.9 to 5.8%) and can be related to the relative size of germen, where about 80 to 85% of total fat content is stored (Serna-Saldívar, 2010). The similar amounts of fat found in the mold infested maize compared with control treatment, indicate that this microorganism had preference for starch rather than fat as nutrient source. However, the crude fat slightly increased in insect and sprout-damaged maize kernels (3.5 and 3.1 % respectively) and in both cases, these compositional changes can be associated to the analogous reduction in starch (Table 3.2). The crude fat in control and damaged sorghum was approximately 2%, values within the range of 0.5 to 5.2% reported by Serna-Saldívar (2010).

Most of the minerals are located in the pericarp, aleurone layer and germ; thus, the ash content in insect and mold-damaged kernels was expected to increase. The ash in sound maize and sorghum was around 1.5% corresponding to values previously reported (Serna-Saldívar, 2010). The insect and mold-damaged maize contained higher amounts of minerals. However, this effect was not observed in the sorghum counterparts. The higher dry matter loss and damage observed in maize is the most reasonable explanation for the observed differences. As expected, the nitrogen free extract (NFE), highly related to starch content in cereal grains, was significantly

lower in both insect-damaged maize and sorghum kernels. The highest NFE concentrations were observed in the controls and sprouted-kernels.

Starch is the most important component of the grain for ethanol production and the main substrate consumed by contaminating microflora and insects and is also an indicator of intrinsic enzyme activity due to germination. For both types of kernels, all sorts of damages significantly reduced starch. The sprout-damaged kernels were the most affected with 5.8 and 6.5% less starch compared to the maize and sorghum controls, respectively. Interestingly, the starch content was inversely related to the reducing sugar concentration observed prior to liquefaction (Table 3.3).

3.4.2. Reducing Sugars (RdS) and FAN During Liquefaction

At the start of liquefaction, maize contained higher RdS compared to sorghum. As expected, the sprouted-maize originally contained the highest amounts of RdS (approximately seven times more compared to sound kernels). This phenomenon was not observed in sprouted-sorghum (Table 3.3) and can be associated with the differences in metabolic activation and sequence of the appearance of amylases during germination. Both α and β-amylases appear at the end of sprouting (after phytases, lipolytic, fibrolitic and proteolitic enzymes) (Serna-Saldívar, 2010). Yan et al. (2009) germinated high-tannin sorghum during three and four days and reported an increase of 2.2 and 4.2 times more fermentable sugars. The notorious difference between sprouted maize and sorghum can be attributed to the low β-amylase concentration and activity generally associated to sorghum (Beta et al., 1995). This enzyme is mainly synthesized at the end of malting and its activity is mainly associated to the acrospires.

A significant increase in RdS at minute 100 and 200 of liquefaction of sprouted sorghum mashes occurred due to the intrinsic amylases or to the better accessibility of the exogenous α-amylase. The difference after 100 min liquefaction was not observed in maize, implying that sorghum sprouting can be a good pretreatment for bioethanol production.

A ratio of RdS versus total theoretical glucose was calculated as a parameter of liquefaction efficiency. The most effective treatments were the sprouted-sorghum and maize followed by insect-damaged sorghum (26, 8 and 6% more RdS compared to the controls). The lowest efficiency was observed in mold-damaged maize with 11% less RdS (Table 3.3).

The FAN was also evaluated at the beginning, half and last part of liquefaction. This parameter is a good indicator of the nitrogenous compounds available for yeast growth during fermentation. Nitrogen deficiencies can lead to sluggish or incomplete fermentations and in the case of maize and sorghum mashes, the main FAN source are hydrolyzed endosperm storage proteins (Thomas and Ingledew, 1990). FAN is also an indicator of protein matrix proteolysis, event that increases the available surface area of starch granules to amylases improving efficiency of liquefaction and saccharification (Vidal et al., 2011). Despite having the highest protein, the control sorghum contained the lowest FAN concentration both at the start and end of liquefaction. The difference in relation to maize has been already observed by other authors (Pérez-Carrillo and Serna-Saldívar, 2007) and is attributed to the kafirin cross-linking during thermal treatments and to the higher phenol concentration generally observed in sorghum. These compounds have a high affinity for kafirins decreasing rate of protein hydrolysis. It is well-known that sorghum is the cereal with the lowest protein digestibility and that this problem is intensified by wet-cooking similar to liquefaction. Besides the interactions of protein with non-protein components such as polyphenols, the endogenous factors such as the nature of sorghum proteins and their organization within the endosperm cells seems to play an important role in the rate of digestibility. This condition can be surmounted with the use of external proteases during liquefaction (Pérez-Carrillo and Serna-Saldívar, 2007). Despite no external proteases were added in this research, the use of protease-producers organisms (*A. flavus* and *S. zeamais)*, and proteases generated during sprouting, is the most logical explanation for the higher FAN assayed in damaged kernels (Table 3.4). The maize proteins were more prone to hydrolysis and produced more FAN compared to the sorghum treatments, except for the mold-damaged sorghum. This exception is noteworthy because indicates the strong proteolytic activity of *Aspergillus* that together with a higher initial protein content, allowed an elevated FAN concentration which is correlated with fermentation efficiency (Pérez-Carrillo and Serna-Saldívar, 2007). At the end of liquefaction, mashes produced from mold-damaged sorghum contained the highest FAN followed by the insect and sprouted-maize samples. This finding is noteworthy for industrial processes because feedstocks with high FAN levels prevent nitrogen supplementation before fermentation. Generally, the recommended FAN level is 150 mg/L (Thomas and Ingledew, 1990).

Table 3.3 Effects of insect, mold and sprout -damages on the amount of reducing sugars [RdS] (g/L) generated at 0, 100 and 200 minutes of liquefaction[1]

Sample			Hydrolysis time (min)			Reducing sugars obtained at the end of liquefaction / Total theoretical glucose based on initial starch (%)
			0	100	200	
Maize	Control (without damage)		1.44±0.18b	121.03±10.51ab	122.59±12.89a	40.73±4.28ab
	Damaged with:	*A. flavus*	1.32±0.26b	112.14±5.98b	108.90±7.09a	36.38±2.37b
		S. zeamais	4.44±1.56ab	102.89±3.23b	108.78±6.16a	38.32±2.17ab
		Sprouted	11.05±4.93a	131.06±5.87ab	125.48±8.46a	44.27±2.98ab
Sorghum	Control (without damage)		0.84±0.52b	124.34±5.44ab	125.78±6.94a	41.03±2.26ab
	Damaged with:	*A. flavus*	0.54±0.45b	107.64±11.36b	112.62±12.22a	38.67±4.20ab
		S. zeamais	0.96±0.69b	114.90±4.68ab	126.92±7.42a	43.45±2.54ab
		Sprouted	0.54±0.36b	146.56±2.68a	149.02±4.36a	52.01±1.52a

[1] All values are expressed on dry matter basis and are the average of at least three replicas ± standard error of the mean. Means with different letter(s) within columns were statistically different ($P<0.05$).

Table 3.4. Effects of insect, mold and sprout -damages on the free amino nitrogen production during liquefaction (mg/L)[1]

Sample			Hydrolysis time (min)[2,3]		
			0	100	200
Maize	Control (without damage)		111.67±4.86d	97.46±3.68b	101.58±5.55cd
	Damaged with:	*A. flavus*	191.67±12.63c*	135.18±13.6bc	131.75±8.89c
		S. zeamais	250.88±6.94b*	174.91±9.14cd	191.23±11.06b
		Sprouted	248.25±14.52b*	183.42±6.22d	173.33±7.40b
Sorghum	Control (without damage)		81.84±3.93d	72.54±2.36a	76.84±1.46d
	Damaged with:	*A. flavus*	389.47±4.87a*	293.07±7.75e	314.74±5.66a
		S. zeamais	130.26±4.64d	106.31±2.39abc	121.84±8.01cd
		Sprouted	96.05±11.23d	76.84±8.85ab	93.77±5.90cd

[1] All values are expressed on dry matter basis and are the average of at least three replicas ± standard error of the mean. [2] Means with different letter(s) within columns were statistically different (P<0.05). [3] Means within rows marked with an asterisk (*) are statistically different (P<0.05).

3.4.3. FAN, Glucose, Glycerol, Ethanol and Fermentation Efficiency During SSF

The mashes used in SSF had an original pH of 5.6 to 5.9, except for the mold-damaged sorghum which was slightly more acidic (pH 5.45). This is below the average of the other treatments probably because of the metabolites produced by *A. flavus*. This mold has potent lipases that hydrolyze stored triglycerides to fatty acids. Thus, both FAN and pH assays can be useful to evaluate the extent of grain damage.

During SSF of mashes adjusted to 15°Plato, FAN was assayed at the beginning and at the end of fermentation (Figure 3.1). In the case of maize, mashes produced from damaged kernels showed a higher FAN compared to the control. This difference was not observed in sorghum except in mold-damaged kernels which contained 110 mg/L (almost twice the amount detected in the rest of the sorghum treatments). At the end of SSF, all treatments had almost the same leftover FAN (10 to 20 mg/L), indicating that yeast metabolized these nitrogenous compounds. Despite the differences in initial FAN this parameter was not positively correlated with fermentation efficiencies.

The concentrations of glucose and ethanol at the beginning and end of SSF are depicted in Table 3.5. The initial glucose in all worts was between 130 to 140 g/L. After 72 h fermentation all the glucose was metabolized by the fermenting yeast. This indicates a complete and high efficient fermentation and the effectiveness of the liquefaction step that rendered dextrins highly susceptible to amyloglucosidase and pullulanase. Therefore, the presence of *A. flavus*, *S. zeamais* and intrinsic enzymes did not affect yeast and α-amylase or amyloglucosidase/pullulanase activities.

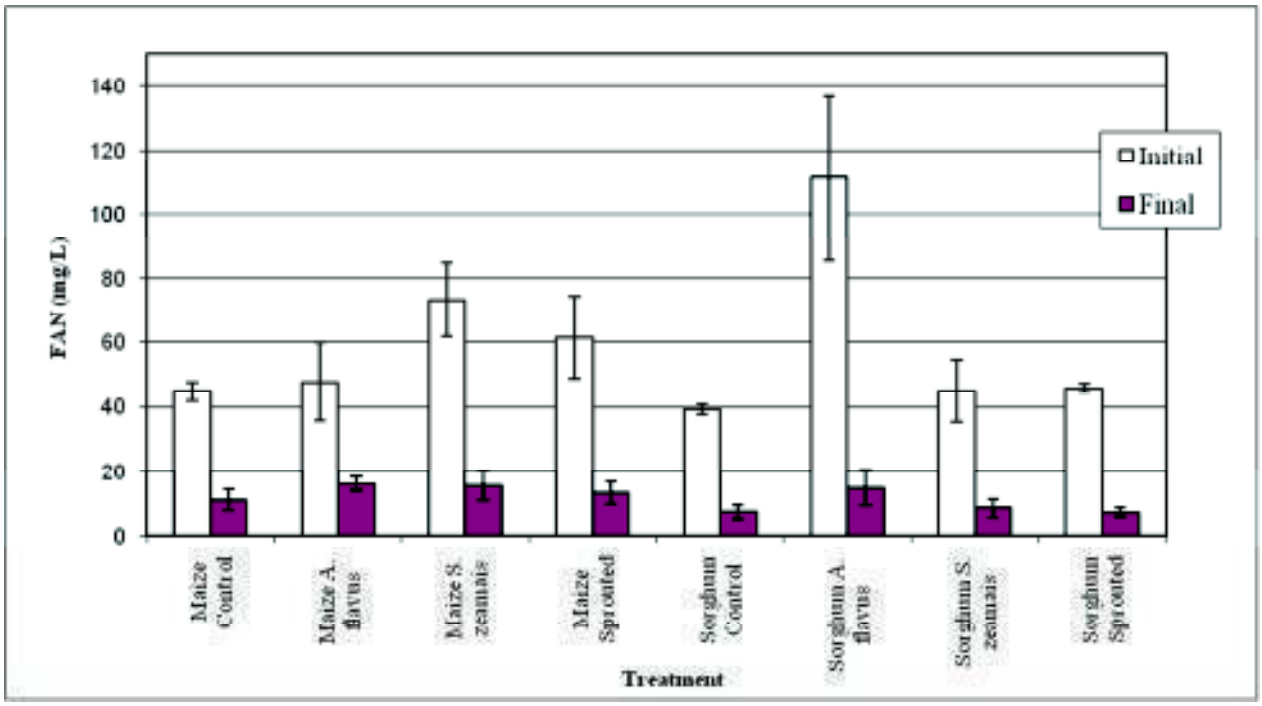


Figure 3.1. Free Amino Nitrogen (FAN) concentration (mg/L) at the beginning and at the end of SSF.

The final ethanol concentration varied from 81.43 mL/L in mold-damaged maize to 92.31 and 95.49 mL/L in the sound maize and sorghum, respectively. The sprout-damaged kernels had the highest fermentation efficiencies followed by the controls, indicating the positive effect of intrinsic enzymes generated during sprouting. The processing of sprout-damaged kernels also saved liquefaction time (Table 3.3), increased FAN (Table 3.4) and produced similar amounts of ethanol compared to sound kernels. The similar fermentation efficiencies between sorghum and maize differed from previous research works (Pérez-Carrillo and Serna-Saldívar, 2007). These authors obtained 26% less ethanol from red sorghum compared to maize (279 versus 380 L/ton, respectively). Fermentation efficiencies are indeed tightly related to grain composition. Wu et al.

(2007), working with different sorghum cultivars obtained efficiencies from 85.2% for high-tannin varieties to 90.2% for waxy and "high yield" samples. In maize, the detrimental influence of amylose content in fermentation has been reported. Wu et al. (2006) achieved 88.7% conversion for normal starch maize while for a fermentation media with 35, 55 and 70% of amylose, efficiencies were 80.4, 61.8 and 52.9% respectively. Thus, the similar conversions between maize and sorghum can be related to grain composition (Table 3.5).

Interestingly, fermentation efficiencies (Table 3.5) seem to be slightly lower compared to previous reports. This can be associated with the temperature used during liquefaction. For example, Wu et al. (2006) employed a cooking temperature of 95°C during 45 minutes before α-amylase addition whereas liquefaction in this study was conducted at temperatures that never exceeded 90°C. A higher temperature enhances starch gelatinization and susceptibility to enzymatic hydrolysis and therefore is directly related to conversion efficiencies, mainly in kernels containing high amylose (Wu et al., 2006).

Glycerol, determined at the end of fermentation (Table 3.5), is a by-product of sugar metabolism. The role of this alcohol is to maintain the cytosolic redox balance by consuming NADH during its formation. According to Vriesekoop et al. (2009), an elevated glycerol is observed in fermentations under osmotic stress conditions, where it accumulates intracellularly acting as a stress protectant. Other authors indicate that glycerol differences are more related to yeast strain rather than compositional changes in the fermentation media (Attfield and Kletsas, 2000). As expected, there were no differences in glycerol concentration in fermented mashes and this is a sign of the adequate initial sugar concentration in worts for the fermenting yeast.

The calculation of ethanol yields/ton initial grain indicated that sprout-damaged kernels yielded similar amounts compared to sound kernels (381 and 376 L/ton of sorghum or maize grain -wet basis-, respectively). In the case of sorghum, this yield was higher than previously obtained for this crop without any other pretreatment but milling: 279 and 276 L/ton (Perez-Carrillo et al., 2008 and Chuck-Hernandez et al., 2009, respectively) and for maize was 15 liters less than the previously reported by the last authors (391 L/ton), highlighting again the influence of the chemical and physical composition of the tested kernels.

For insect and mold-damaged kernels, a reduction in yield was indeed observed. A decrease of 29 and 11% was observed in the case of insect and sprout-damaged maize, respectively. The use of insect-damaged sorghum reduced yield around 23%. These findings are crucial in geographic regions where *S. zeamais* is the principal infesting agent and indicates the robustness of sorghum against this particular insect. The negative impact of the use of insect or mold-damaged kernels is mainly due to dry matter losses incurred during storage and stresses the importance of first-rate storage practices. However, this research clearly demonstrates the use of already damaged kernels is feasible. These kernels can be acquired at a discount price by biorefineries and subsequently converted into bioethanol with similar efficiencies. Undoubtedly, the sprout-damage was the least detrimental because these kernels yielded similar amounts of ethanol compared to sound kernels. Comparatively, these kernels were more susceptible to amylases and thus can save hydrolysis and fermentation times.

3.5. CONCLUSIONS

This research demonstrated that there was a clear negative impact of insect, molds and intrinsic enzymes in the physical and chemical composition of maize and sorghum, being the former the most affected. Insect and mold-damaged kernels were mainly modified at the endosperm level. These kernels had reduced amount of starch and NFE and higher levels of reducing sugars and FAN. There was no evidence of germ attack because the crude fat remained at the same concentration (2.5-3.5% for maize and 1.3-2.6% for sorghum). Sprouted grains with high enzymatic activity had clear evidences of degradation of both starch and proteins. The use of sprouted kernels reduced hydrolysis time needed to achieved optimum reducing sugars concentration, because by 100 min liquefaction, the higher RdS content was reached. Conversions into bioethanol of insect, mold and sprout-damaged maize and sorghum were lower calculated in relation to the original grain weight indicating that the dry matter loss incurred during storage was the main reason for the observed detrimental effect. Insect-damaged maize was the most affected treatment, yielding 30% less ethanol followed by sorghum-infested with 24% less than their own controls. Sprouting in sorghum was the less detrimental treatment, reducing only by 8% total ethanol produced by flour unit. Despite these

losses, all already damaged kernels had similar fermentation efficiencies (76.1 to 89.9% and average of 82.6%), indicating that these feedstocks are suitable for fuel ethanol production. Furthermore the biotic damages tested within this chapter affected more to maize than sorghum in terms of reducing sugars and free amino nitrogen (as indicators of starch and protein damage respectively). Thus, after chapter 2 and 3 of this thesis, questions arose about the role of starch and protein availability (associated with ethanol production, chapter 2) in sorghum resistance to biotic detrimental factors, particularly facing insect infestation. There was a suspicion that the particular sorghum starch and protein characteristics, described previously in the frame of human and animal nutrition, could connect sorghum fermentation performance and storage resistance to biotic damages. This question was the driver for the work described into the next chapters. Hence, sorghum cultivars with different physicochemical and nutraceutical traits were selected and its susceptibility to one of the most destructive insects in stored grain: maize weevil *(Sitophilus zeamais*) was tested (Chapter 4). The same germplasm was used to evaluate the chemical changes into endosperm in order to understand the relationship among starch and protein, the main components in sorghum endosperm (Chapter 5). Fermentation trails were also performed with the selected sorghums and common traits among insect resistance and ethanol production were obtained as described in Chapter 6.

Table 3.5. Effects of insect, mold and sprout -damages of maize and sorghum on glucose (before fermentation) and ethanol, glycerol and ethanol yields and efficiencies obtained after 72 hours fermentation[1]

Samples			Glucose (mg/mL)	Ethanol (mL/L)	Glycerol[2] (mg/mL)	Fermentation efficiency (%)	Ethanol yield[3] (L/ton starch)	Ethanol yield[3] (L/ton of already damaged grain)	Ethanol yield[3] (L/ton grain)[4]
Maize	Control without damage		133.11±4.86a	92.31±8.13a	9.00±0.86a	84.24±2.44a	520.79±15.09a	376.59±10.91a	376.59±10.91a
	Damaged with:	*A. flavus*	127.63±9.95a	81.43±2.87a	10.65±0.23a	77.54±3.84a	479.40±23.71a	344.74±17.05a	331.70±16.41ab
		S. zeamais	128.50±7.68a	83.09±2.51a	10.37±0.17a	76.10±1.63a	470.47±10.10a	320.81±6.89a	264.44±5.68c
		Sprouted	133.56±6.51a	90.11±4.94a	9.73±0.08a	89.04±1.07a	550.48±6.59a	374.82±4.49a	333.67±4.00ab
Sorghum	Control without damage		140.02±2.30a	95.49±10.02a	9.89±1.03a	83.70±7.86a	517.48±48.57a	381.12±35.77a	381.12±35.77a
	Damaged with:	*A. flavus*	132.56±1.31a	91.65±8.31a	8.74±1.13a	81.83±6.65a	505.95±41.10a	353.96±28.75a	326.00±26.48ab
		S. zeamais	134.89±1.83a	87.14±1.94a	10.92±0.07a	78.72±1.28a	486.67±7.91a	341.50±5.55a	290.75±4.73abc
		Sprouted	141.2±5.95a	94.12±8.71a	10.78±0.08a	89.90±8.18a	555.78±50.55a	382.60±34.80a	349.85±31.82ab

[1] All values are expressed on dry matter basis and are the average of at least three replicas ± standard error of the mean. Means with different letter(s) within columns were statistically different ($P<0.05$). [2] The initial glycerol concentration was zero. [3] Yields calculated on 14% moisture basis. [4] Ethanol yields considering solid losses incurred during storage.

4. SUSCEPTIBILITY OF DIFFERENT TYPES OF SORGHUMS DURING STORAGE TO *SITOPHILUS ZEAMAIS* MOTSCHULSKY

4.1. ABSTRACT

Caryopses belonging to twelve sorghum cultivars (two red, one brown or high-tannin, two white heterowaxy, two white waxy, two white high-digestible protein and three white with regular endosperm) were selected to study their resistance to *S. zeamais* during storage. Five resistance parameters were evaluated: Dobie Index, Total Emerged Insects, Median Development Time, Ratio final /initial insects and Weight Loss. Biophysical characteristics (Test weight, 1000 kernel weight, endosperm texture, flotation index, true density, percentage of kernel removed with TADD, anatomical parts, color index and kernel size), chemical composition (starch, amylose, protein, free amino nitrogen and ash), and nutraceutical traits (free and bound phenolics and antioxidant capacity for the free and bound fractions) were obtained. The most resistant cultivars were both red sorghums (*RR1* and *RR2*) and a white cultivar with regular endosperm (*WR1*) whereas the most susceptible were the brown high-tannin (*Sumac*), a white waxy (*Waxy1*) and a white high digestible (*HD1*). Correlation coefficients among resistance parameters and physicochemical characteristics were calculated, yielding a clear relationship amongst different endosperm texture indicators, endosperm, ash, amylose and free amino nitrogen content, and susceptibility traits. The harder kernels (in terms of vitroussnes), higher endosperm percentage, low ash, increased amylose content and reduced free amino nitrogen concentration had more resistance to *S. zeamais.* Significant relationships among nutraceutical profiles and resistance were not detected, despite the wide range of phenolics in the array of kernels. These results indicated that endosperm structure is predominant in sorghum resistance to *S. zeamais*.

4.2. INTRODUCTION

Sorghum is the fifth cereal most produced in the world with an annual output of 55.7 million tons (FAOSTAT, 2012) and also the main source of calories and protein in some regions of Africa and Asia (Waniska and Rooney, 2000). The main producer is the United States of America with almost 9.0 million ton yearly, followed by Mexico and India (6.9 and 6.7 million ton respectively) (FAOSTAT, 2012). Sorghum is a high drought resistant crop, with a high tolerance to salinity and an outstanding performance in areas where nutrients are limited (Serna-Saldívar, 2010).

Despite the high sorghum production in the world and its high stability to abiotic and biotic factors, during the postharvest stages some qualitative and quantitative losses occur. According to Ramputh et al. (1999), cereal postharvest losses in small-farm tropical agriculture usually exceeds 30% and according to García-Lara and Bergvinson (2007), the range of worldwide postharvest losses in subsistence farming is between 10 to 40%.One of the main biotic factors associated to losses during postharvest are insect such as weevils, being *S. zeamais* the main pest in tropical and subtropical regions (García-Lara and Bergvinson, 2007). Despite the importance of the postharvest in the overall production cycle of cereals, there are few studies about resistance mechanisms of sorghum facing insect infestation, in particular to *S. zeamais*.

There are simple tests as Test Weight, Flotation Index and True Density, used as indicators of overall grain quality. These grain physical properties are related to performance during dry and wet-milling operations as well as food quality in end products (Chiremba et al., 2011; Pomeranz, 1986; Serna-Saldívar, 2010). Furthermore, these parameters are related to endosperm texture or hardness mainly affected by the ratio between vitreous and floury endosperm (Pomeranz, 1986). Moreover, according to Doggett (1982) there is a direct relationship among kernel hardness and its resistance to molds and insects. García-Lara et al. (2004) reported in maize a negative correlation (r= -0.84) between hardness and the kernel susceptibility to weevils. Thus, also in sorghum, physical parameters (simple, cheap, and quick to determine) could yield valuable information to predict the performance of a specific cultivar during storage facing *S. zeamais* infestation. Additional to mechanical resistance, in grains there is also a protective scheme associated to their chemical composition, i.e. presence of phenolic acid amides (Burt, 2003; García-Lara et al., 2004). Chandrashekar and Satyanarayana (2006) reported that phenolic

compounds such as ferulic acid and tannins are potent inhibitors of pests and pathogens. In sorghum, the effect of tannins in bird resistance and protein digestibility is already well documented (Dicko et al., 2005; Serna-Saldívar and Rooney, 1995), but there are scarce reports about the role of phenolics in the insect resistance (Ramputh et al., 1999). The function of phenolics bound to cell walls in resistance against *S. zeamais* has been demonstrated in maize (García-Lara et al., 2004), but not in sorghum.

Therefore, the objectives of this study were: 1) study the susceptibility of sorghum cultivars with different physical and chemical profiles during storage to the presence of maize weevil (*Sitophilus zeamais*) and; 2) to associate the physical, chemical and nutraceutical characteristics of sorghum with resistance/susceptibility parameters to *S. zeamais* during storage.

4.3. MATERIALS AND METHODS

4.3.1. GRAIN VARIETIES

Twelve sorghum cultivars were selected based on their color and endosperm characteristics. Genotypes used in this study were: 1) two red sorghums, commonly used as feed in the northern part of Mexico (*RR1* and *RR2*); 2) one high-tannin sorghum (*Sumac*); 3) nine white cultivars (two heterowaxy –*HTW*1, *HTW*2-, two waxy –*Waxy*1, *Waxy*2-, two described as high protein digestible –*HD*1,*HD*2- and three regular –*WR*1, *WR*2, *WR*3-). Red and high-tannin sorghum samples were commercially available material in Northern Mexico and possessed an intermediate to soft endosperm texture. White samples were kindly donated by Dr. Dirk Hays of the Texas A&M University Sorghum Breeding Program.

4.3.2. SAMPLE PREPARATION

Kernel samples, not previously treated with insecticides, were cleaned by air aspiration and sieves. The moisture was adjusted to 13% using the formula ([(100 - % Initial Moisture)/(100 – % Final Moisture)] – 1] * Sample Weight) and allowed to equilibrate for at least 7 days at 27 ± 1°C and 70 ± 5% RH, using a saturated NaCl solution. For physical, chemical, phenolics and antioxidant characterization, the sorghum kernels were milled using a coffee mill (Krups, Model GX410011, Mexico) and stored at 4°C prior to use. Whole kernels were used for susceptibility tests.

4.3.3. PHYSICAL CHARACTERIZATION

Physical characteristics were determined by triplicate using standard procedures: test weight (TW) according to Official US Grain Standard Procedures (AACC Method 55-10); thousand-kernel weight (TKW) by weighing 100 randomly selected whole kernels, and endosperm texture (ET) according to the subjective procedure previously reported by Chuck-Hernández et al. (2009). Flotation index (FI) was determined according to Salinas et al. (1992) and expressed as a percentage of floating kernels on an aqueous solution of sodium nitrate (1.25 g/cm^3 specific weight at 35°C). A pycnometer was used to obtain True Density (TD), whereas the Tangential Abrasive Dehulling Device (TADD) was employed to determine the percentage of kernel removed as an indicator for hardness. Kernel size was measured with a digital micrometer (Mitutoyo, Model MDC-1, Japan). Volume in mm^3 was calculated using the volume formula for an ellipsoid ($4/3^* \pi * R^{2*}r$). R was the half of kernel´s length and r= Thickness/2. L*, a*, b*, and other CIE color parameters of ground samples were determined using a colorimeter (Minolta CR-300, Osaka, Japan) and Color Index (CI) was estimated with the formula reported by Vignoni et al. (2006) (a*1000/L*b). Tip cap, germ, pericarp and endosperm were obtained from dissected kernels, previously soaked for 2 minutes in water according to Gutiérrez-Uribe et al. (2010).

4.3.4. CHEMICAL CHARACTERIZATION

Crude Protein (N*6.25) was determined using the micro-Kjeldahl method 46-13 (AACC, 2000) and Free Amino Nitrogen (FAN) with the ninhydrin procedure 945.30L (AOAC, 1980). Total and resistant starch and amylose were determined using enzymatic tests TStarch –AOAC 996.11-, RStarch –AOAC 2002.02- and Amylose/Amylopectin -K-AMYL- procedures respectively (Megazyme International, Ireland). Ash was assayed according to method 08-01 (AACC, 2000). Presence of pigmented testa in all sorghums was performed using the Chlorox procedure (Waniska et al., 1992) and condensed tannins were quantified with the vainillin-HCl assay (Price et al., 1978). Extracts for tannin assay were obtained using acidified methanol (1%HCl). All determinations where performed by triplicate.

4.3.5. Extraction And Determination of Free And Bound Phenolics

Free and bound phenolic compounds were extracted using the method described by Gutiérrez-Uribe et al. (2010). The extracts were used to determined total phenolics with the Folin-Ciocalteu method, briefly: 20uL of extracts with 200uL of Folin-Ciocalteau reagent (Sigma Aldrich, 2N, diluted 1:9 in distilled water) and 30uL of sodium carbonate (7.5% w/v) were maintained 90 min at 37°C in darkness and absorbance at 765 nm was obtained (Microplate Reader, Sinergy, HT Multi-Detection, BioTek, Inc., VT, USA). Gallic acid was used as standard and phenolic content was expressed as mg of Gallic Acid Equivalent (GAE) / g of flour in dry basis. All determinations where performed by triplicate.

4.3.6. Antioxidant Capacity (AOXC)

Antioxidant capacity was determined by triplicate using the Oxygen Radical Absorbance Capacity assay, using a standard of Trolox with fluorescein as a probe as described by Prior et al. (2003). Peroxyl radicals were generated by 2,2′ azobis (2-amidinopropane) dihydrochloride, and fluorescent loss was monitored in a Microplate Reader (Sinergy, HT Multi-Detection, BioTek, Inc., VT, USA). The absorbances of excitation and emission were set at 485 and 538 nm, respectively. Data was expressed as μmol of Trolox Equivalents per each gram of ground sample (dry basis).

4.3.7. Susceptibility Tests

4.3.7.1. Insect Culture

The *Sitophilus zeamais* colony was maintained in the Postharvest Laboratory at Tecnológico de Monterrey, Monterrey, Mexico. Insects were cultured in sorghum, maintained at 27 ± 1°C and 70 ± 5% RH in a 12:12 h cycle light: dark and renewed every three weeks according with methods described by Bergvinson (2001).

4.3.7.2. Confinement and Dobie Index (DI) Assays

One-hundred grams of previously equilibrated sorghum kernels (as described in subsection 4.3.2.) were infested with 80 unsexed adult maize weevils (*S. zeamais*) no older than 7 days and kept under controlled conditions (27°C, 70% RH, using a saturated NaCl solution). All trials

were performed by triplicate in glass jars with vented lids. For confinement test, samples were collected after 45 days for sorghum weight loss (WL) determination. In addition, the adult weevils were separated using sieves (mesh #10 and #20) and the ratio final /initial insects were calculated. For Dobie Index (DI) assay, adults *S. zeamais* were removed after 1 week infestation. Samples were kept under the same previous environmental conditions and adult progeny removed every third day during 45 days. DI was calculated with the formula: (log P * 100) / MDT, where P is the adult progeny and MDT the median development time (Dobie, 1974).

4.3.8. STATISTICAL ANALYSIS

Three replicates for physical and chemical traits as well as susceptibility parameters were subjected to Analysis of Variance (ANOVA) using the statistical software Statistix 9. Residual analysis was also performed in order to review the compliance of statistical assumptions (normality, independence and homoscedasticity). When means resulted different (null hypothesis rejected, *Pvalue*<0.05), differences among means were evaluated using Tukey HSD Tests with *alpha*=0.05. Homogeneous mean´s groups were indicated with the same alphabetic sub-indices within tables. Pearson correlation coefficients were obtained using the same statistical software.

4.4. RESULTS AND DISCUSSION

4.4.1. PHYSICAL AND ANATOMICAL TRAITS FOR THE EVALUATED SORGHUMS

The physical characteristics of the different sorts of caryopses before insect infestation were determined and related to storage susceptibility to *S. zeamais*. The most significant traits evaluated were kernel hardness and endosperm characteristics. Test Weight (TW), Thousand Kernel Weight (TKW), Endosperm Texture (ET), Flotation Index (FI), True Density (TD) and percentage of decortication with the Tangential Abrasive Dehulling Device (TADD), as well as kernel size, Color Index (CI) and anatomical fractions, were determined (Table 4.1 and Table 4.2). The TW values were within the typical range for sorghum (68.5 to 77.3 kg/hL) reported by Serna-Saldívar and Rooney (1995) and Serna-Saldívar (2010). The *Waxy*1 genotype was located in the lowest level of the range, different to the rest of the sorghums, which lie near to the

superior limit of the range. The same was observed for TD, where the most typical range varied from 1.20 to 1.35 g/cm^3, with cultivar *Waxy*1 ranking in the lowest range.

According to Serna-Saldívar (2010), kernels with low density values are related with a relatively high proportion of starch/protein, ratio that makes this type of material more suited to wet-milling processes. The highest TW were obtained in two white cultivars: *HTW*2 and *WR*2 a heterowaxy and regular, respectively. In the case of TKW and kernel volume (Table 4.1 and Table 4.2), these parameters are also widely evaluated in the cereal processing industry, mainly, because the size of the grain is related with milling yields. The typical range of TKW in sorghum is from 23 to 35 grams (Serna-Saldívar, 2010). All cultivars were within this range, except the *Sumac* and *Waxy*2 kernels (below and above the previously indicated range, respectively). For kernel size, the cultivars that produced the largest kernels were *Waxy*2, *HD*1, *HD*2 and *WR*3, and the lowest the high-tannin (*Sumac*) (Table 4.2). Both parameters were highly correlated (r=0.84).

Endosperm texture (ET) is mainly affected by the ratio between vitreous and floury endosperms. The lowest value corresponded to the hardest endosperm in a subjective scale from 1 to 5 (Table 4.1). Furthermore the flotation index (FI) is related with density and kernel hardness. According to Pomeranz (1986), FI is inversely correlated with vitreousness (r= -0.96) and grain hardness, measured as work required to grind the kernels (r= -0.88 to -0.92).

All the evaluated endosperms ranged from intermediate to soft (2.3 to 5.0). The hardest and softness endosperms were the *HTW*1 and *Waxy*1 kernels respectively. As expected, the lower the density, the higher the FI (Table 4.1). According to Lozano-Alejo et al. (2007), FI ranking from 0 to 37% classifies hard kernels (*RR1*, *RR2*, *Sumac*, *HTW*1, *HTW*2, *Waxy*2, *HD*2, *WR*1, *WR*2, *WR*3), from 38 to 62% intermediate endosperm samples (*HD*1) and >87% soft endosperm (*Waxy*1) kernels. These ranks are reported for maize and there are no references regarding sorghum. Both parameters, FI and ET, were highly correlated (r=0.824, $P<0.01$, Table 4.5). FI and TW also showed a good correlation coefficient (r=-0.952, $P<0.01$). TADD values for all sorghums are depicted in Table 4.1. The principle of this assay is that softer kernels will lose more material during decortication and for this reason the instrument is used as indirect measurement of grain hardness and milling performance (Chiremba et al., 2011).

Table 4.1. Physical characterization for the different sorghum cultivars [1]

Variety	Color	Test Weight (TW)	Thousand Kernel Weight (TKW)	Endosperm Texture (ET)[2]	Flotation Index (FI)	True Density (TD)[3]	Abraded Material with TADD[4]
		kg/hL	g		%	g/mL	%
RR1	Red	77.2 ± 0.7 ab	25.5 ± 0.5 e	3.5 ± 0.4 bc	5.0 ± 5.0 c	1.36	28.4 ± 0.0 bc
RR2	Red	77.4 ± 0.3 ab	26.4 ± 0.6 e	2.7 ± 0.3 cd	5.0 ± 5.0 c	1.37	28.7 ± 0.5 bc
Sumac	Brown	76.1 ± 0.2 b	15.3 ± 0.1 f	3.2 ± 0.3 bcd	25.0 ± 10.0 bc	1.35	30.9 ± 0.3 ab
HTW 1	White	76.7 ± 0.2 b	31.0 ± 0.4 d	2.3 ± 0.2 d	2.5 ± 2.5 c	1.37	14.3 ± 0.2 fg
HTW 2	White	78.5 ± 0.1 a	29.7 ± 0.1 d	2.7 ± 0.2 cd	2.5 ± 2.5 c	1.36	13.6 ± 0.1 g
*Waxy*1	White	68.7 ± 0.3 d	31.2 ± 0.3 cd	5.0 ± 0.0 a	100.0 ± 0.0 a	1.26	31.6 ± 1.6 ab
*Waxy*2	White	77.0 ± 0.2 ab	41.8 ± 0.5 a	2.5 ± 0.0 cd	17.5 ± 2.5 bc	1.37	19.2 ± 0.8 de
*HD*1	White	73.3 ± 0.4 c	30.3 ± 0.4 d	4.1 ± 0.5 ab	50.0 ± 10.0 b	1.31	26.5 ± 0.0 c
*HD*2	White	73.4 ± 0.3 c	34.3 ± 0.6 b	4.3 ± 0.3 ab	35.0 ± 0.0 bc	1.30	31.9 ± 0.2 a
*WR*1	White	77.7 ± 0.3 ab	33.3 ± 0.4 bc	2.6 ± 0.1 cd	17.5 ± 2.5 bc	1.35	15.1 ± 0.1 fg
*WR*2	White	78.7 ± 0.4 a	26.7 ± 0.4 e	2.5 ± 0.0 cd	7.5 ± 7.5 c	1.36	17.5 ± 0.4 ef
*WR*3	White	76.3 ± 0.3 b	30.4 ± 0.4 d	2.5 ± 0.0 cd	30.0 ± 10.0 bc	1.35	22.1 ± 0.3 d

[1] Means with different letter(s) within column are statistically different ($P<0.05$).[2] Endosperm Texture (ET), subjectively determined by viewing the ratio of soft to hard endosperm on dissected kernels, 1=totally vitreous or hard and 5= totally soft or chalky. [3]All means, except TD, are the average of at least three replicas ± standard error of the mean. [4]TADD: Tangential Abrasive Dehulling Device.

The TADD values ranged from 31.9% for *HD*2 to 13.6% for *HTW*2 and these values were highly correlated with other hardness indexes as TW and ET (r= -0.64 and 0.75 respectively, Table 4.5). CI (Table 4.2) is related with endosperm, testa and pericarp color, traits genetically controlled in sorghum (Serna-Saldivar and Rooney, 1995). As expected, the highest CI values were observed in the high tannin *Sumac* kernels followed by *RR1* and *RR2*. The white genotypes were located in the color range from yellow to orange according with the scale described by Vignoni et al. (2006). Based on these results, physical differences among sorghum kernels were evident. According with TKW and kernel size, the *Sumac* and *Waxy*2 kernels were the smallest and biggest, respectively. It was also evident that *Waxy*1 had the softer endosperm measured as TW, ET, FI, TD and TADD. On the other hand, the *RR1*, *RR2*, *HTW*1, *Waxy*2 and *WR*2 genotypes

produced the hardest grains. The proportion of the major anatomical parts for each sorghum genotype is depicted in Table 4.2. The relative percentage of pericarp, germ and endosperm in sorghum, varies depending on the genetic heritage and environment (Serna-Saldivar and Rooney, 1995) and these values can be useful to predict kernel performance in milling and other industrial uses. Typical values for percentage of germ, pericarp and endosperm in sorghum are 8.0 to 10.9, 4.3 to 8.7 and 81.7 to 86.5 respectively (Waniska and Rooney, 2000). According to values depicted in Table 4.2, pericarp and endosperm were within these ranges, being *Waxy*1 the only with endosperm percentage < than 81.7%.

Table 4.2 Anatomical fractions for each different sorghum cultivar (% of tip, pericarp, germ and endosperm), kernel size and Color Index (CI) [1]

Variety	Tip Cap	Pericarp	Germ	Endosperm	Kernel size [2]	Color Index (CI) [3]
	%				mm^3	
RR1	2.3 ± 0.4 a	4.5 ± 0.2 bc	6.7 ± 1.1 a	86.4 ± 0.8 ab	23.4 ± 2.5 ab	17.6 ± 1.1 b
RR2	2.5 ± 0.9 a	4.3 ± 0.6 c	7.2 ± 1.8 a	86.0 ± 1.8 ab	25.9 ± 2.2 ab	16.4 ± 1.4 b
Sumac	3.0 ± 1.1 a	7.4 ± 1.0 a	6.6 ± 0.5 a	83.1 ± 0.4 abc	14.8 ± 1.3 b	28.7 ± 2.4 a
HTW 1	2.0 ± 0.8 a	5.9 ± 0.5 abc	5.0 ± 1.1 a	87.1 ± 0.9 a	28.3 ± 0.2 ab	4.0 ± 0.5 c
HTW 2	2.5 ± 0.8 a	5.2 ± 0.1 abc	6.7 ± 0.3 a	85.6 ± 0.9 ab	28.8 ± 2.4 ab	3.6 ± 0.2 c
*Waxy*1	2.7 ± 1.0 a	7.1 ± 0.1 ab	9.9 ± 0.2 a	80.3 ± 1.0 c	27.1 ± 0.9 ab	5.1 ± 0.3 c
*Waxy*2	2.2 ± 0.9 a	5.1 ± 0.1 abc	7.6 ± 0.7 a	85.1 ± 0.8 ab	38.2±5.1 a	4.0 ± 0.4 c
*HD*1	2.4 ± 0.7 a	5.4 ± 0.7 abc	6.7 ± 1.0 a	85.6 ± 1.6 ab	35.7 ± 3.7 a	4.7 ± 0.2 c
*HD*2	3.6 ± 0.4 a	5.5 ± 0.2 abc	8.9 ± 0.6 a	82.1 ± 1.0 bc	41.8 ± 6.1 a	3.5 ± 0.1 c
*WR*1	2.9 ± 1.2 a	5.3 ± 0.5 abc	7.3 ± 1.0 a	84.6 ± 0.6 abc	29.5 ± 1.5 ab	2.7 ± 0.3 c
*WR*2	2.9 ± 0.3 a	4.9 ± 0.4 abc	7.9 ± 0.5 a	84.3 ± 0.5 abc	27.8 ± 1.4 ab	3.5 ±0.2 c
*WR*3	2.9 ± 0.4 a	6.0 ± 0.3 abc	6.8 ± 0.6 a	84.4 ± 0.6 abc	35.4 ± 2.1 a	1.6 ± 0.1 c

[1] All values are the average of at least three values ± standard error of the mean. Means with different letter(s) within columns are statistically different ($P<0.05$). [2] Kernel size was calculated with volume formula for and ellipsoid ($4/3 * \pi * R^2 * r$), using the dimensions from at least 10 kernels, (Length/ 2) as R and (thickness/2) as r. [3] Color values were estimated on ground samples. Color Index (CI)= (a*1000/L*b), where *L*, *a* and *b* are CIE Lab color space parameters.

4.4.2. Chemical and Nutraceutical Composition of Sorghum Samples

The chemical composition of all sorghum kernels is depicted in Table 4.3. Total starch in sorghum was similar to maize (average of 73.7 versus 72.4 % respectively), but regarding amylose in regular kernels is reported higher compared to maize (28.5 vs 24.0%) (Serna-Saldívar, 2010). In terms of starch content, most sorghum genotypes were below the previous described average, except the *RR2* which contained 73.55%. Nevertheless, all results are within the wide range (60.0 to 77.0%) reported by Serna-Saldivar (2010). The lowest starch contents were observed in the *Waxy*1 and *HD*1 sorghums whereas the highest in the *RR2* sorghum. Regarding amylose, the set of sorghums deployed the expected range in a set of samples that include regular, heterowaxy and waxy genotypes. Amylose concentration was 32.69% for *RR1* and 4.95% for *Waxy*1.

Crude protein was significant different among kernels. The *WR*1 and *Waxy*1 contained 14.5% protein whereas *RR2* only 9.3%. These values are considered typical because they were within the range of 7.3 to 15.6 reported by Waniska and Rooney (2000). In this context, protein is important because is the source of simpler nitrogenous compounds or FAN and at the same time, FAN could be used to describe nutrient availability and protein integrity in the endosperm matrix. Thus, this could be related to insect resistance in sorghum. The lowest and highest FAN contents were obtained in the *Sumac* and *Waxy*1 and *HD*1 kernels, respectively.

The mineral or ash contents were also within the typical range (1.1 to 2.5%) expected for whole sorghum kernels. There were significant differences ($P<0.05$) among the set of samples studied. The *Waxy*1 depicted the highest concentration (2.02%) whereas the *RR1* and *RR2* the lowest (1.3%). Ash concentration was highly correlated with endosperm percentage ($r=-0.671$, $P<0.05$) and significant to TD ($r=-0.62$, $P<0.05$). Most of the minerals are located in germ, pericarp and aleurone layer (Serna-Saldivar, 2010), thus, the ash content was expected to be correlated with the anatomical fractions of the caryopses.

Phenolic compounds were also determined as part of the nutraceutical profile. Phenolics were detected in all sorghums types and these antioxidant compounds are known to affect color, appearance and nutraceutical properties (Rooney and Serna-Saldivar, 2000). Serna-Saldivar

(2010) indicated that phenolics have an important role in protecting kernels against biotic and abiotic stresses. In maize, phenolics are related to kernel resistance through physical and toxicological mechanisms (García-Lara et al., 2004) and in sorghum, a positive relationship between phenolic content and *Sitophilus oryzae* resistance has been reported (Ramputh et al., 1999).

Significant differences in free and bound phenolics for sorghum cultivars were observed (Table 4.3). The highest concentration was detected in *Sumac* and the lowest in *HD*2, *WR*2 and *WR*3 genotypes. All samples, except *Sumac*, depicted more bound than free phenolics, around 2.0-3.0 mg GAE / g versus 0.3 to 0.8 mg GAE / g. In *Sumac* sorghum, free phenolics were two times higher compared to the bound counterparts. This result indicates that the higher content of phenolics in white varieties is related to bound compounds to cell walls (Waniska and Rooney, 2000), different, for example, to high tannin varieties (*Sumac*), where the highest phenol content is associated to the pigmented testa (Serna-Saldívar, 2010).

Phenolic content is highly variable, because of the influence of genetic and environmental factors and the extraction scheme or solvent used. Dicko et al. (2005) reported an average of 0.88 mg GAE/g of total phenols for a set of 50 cultivars where less than 20% were high-tannin genotypes. Additionally, Austin (2008) reported only for white sorghum kernels a total of 3.1 g GAE/g, close to the free and bound phenolics result obtained herein. Awika et al. (2009) reported total phenols from 7.9 to 18.2 mg GAE/g in different tannin free sorghums and almost 30 mg GAE/g in high-tannin counterparts. *RR1* and *RR2* depicted significant amounts of extractable phenols, but not condensed tannins. Moreover, the white sorghums (Table 4.3) free of condensed tannins contained lower levels of total phenols. As expected, the only type of kernel with detected condensed tannins was *Sumac* (data not shown) with 28 mg catechin equivalents/g, similar to the reported for Awika et al. (2009). These authors reported 18.1 to 23.3 mg catechin equivalent/g.

The AOXC for each type of sorghum is depicted in Table 4.3. The highest AOXC was related to the bound fraction which contained the highest phenolics. *Sumac* was the variety with the highest antioxidant activity and this result agrees with previous reports (Awika et al., 2009). Furthermore, the lowest AOXC values were detected in the *HTW*2, *WR*1 and *WR*2 genotypes.

Table 4.3 Chemical and nutraceutical composition for the different types of sorghums [1]

Variety	Total Starch (TStarch)	Amylose (AMY)	Crude Protein	Free Amino Nitrogen (FAN)	Ash	Free Phenolics (FPhenol)	Bound Phenolics (BPhenol)	Hydrophilic Antioxidant Capacity (AOXC)	
								Free Phenolics (FPhenol)	Bound Phenolics (BPhenol)
	%	%	%	mg/g	%	mg gallic acid equiv./ g	mg gallic acid equiv./ g	µM trolox equiv. / g	µM trolox equiv. / g
RR1	66.62 ± 1.89 ab	32.69 ± 1.81 a	10.90 ± 0.20 cd	0.17 ± 0.01 bc	1.34 ± 0.07 e	0.77 ± 0.02 b	2.35 ± 0.18 cd	46.67 ± 2.63 b	95.70 ± 1.23 c
RR2	73.55 ± 1.99 a	31.82 ± 1.36 a	9.30 ± 0.10 e	0.16 ± 0.02 bc	1.37 ± 0.03 e	0.87 ± 0.03 b	2.90 ± 0.17 b	57.54 ± 1.06 a	89.11 ± 1.59 d
Sumac	68.05 ± 0.58 ab	27.11±2.82ab	11.10 ± 0.20 cd	0.14 ± 0.02 c	1.78 ± 0.05 b	15.50 ± 0.16 a	7.43 ± 0.38 a	18.01 ± 0.46 g	179.39 ± 4.84 a
HTW 1	63.92 ± 1.42 ab	18.81±0.14cd	12.40 ± 0.10 bc	0.17 ± 0.01 bc	1.58 ± 0.03 cd	0.33 ± 0.01 e	2.46 ± 0.07 bcd	19.41 ± 0.42 fg	70.36 ± 1.01 f
HTW 2	65.74 ± 1.15 ab	12.32±1.74de	13.50 ± 0.20 ab	0.22 ± 0.01 bc	1.77 ± 0.02 b	0.40 ± 0.01 de	2.36 ± 0.16 cd	22.45 ± 0.49 e	70.75 ± 0.97 f
*Waxy*1	62.75 ± 0.55 b	4.95 ± 1.07 e	14.30 ± 0.00 a	0.32 ± 0.01 a	2.02 ± 0.02 a	0.43 ± 0.01 cde	2.83 ± 0.10 bc	25.80 ± 0.68 d	126.30 ± 0.82 b
*Waxy*2	69.49 ± 0.91 ab	9.62 ± 1.52 e	12.50 ± 0.30 bc	0.23 ± 0.02 b	1.86 ± 0.05 ab	0.50 ± 0.02 cd	2.72 ± 0.08 bc	37.05 ± 1.41 c	126.29 ± 2.32 b

*HD*1	59.57 ± 2.66 b	20.41±1.79bc	13.20 ± 0.30 ab	0.34 ± 0.01 a	1.82 ± 0.01 b	0.55 ± 0.03 c	2.91 ± 0.22 b	37.19 ± 0.66 c	122.34 ± 1.57 b
*HD*2	65.40 ± 2.22 ab	23.49± 0.81bc	12.40 ± 0.10 bc	0.23 ± 0.03 bc	1.82 ± 0.02 b	0.40 ± 0.00 de	2.03 ± 0.09 d	21.23 ± 0.12 ef	77.98 ± 1.20 e
*WR*1	65.99 ± 0.69 ab	25.33±1.01abc	14.50 ± 0.20 a	0.21 ± 0.02 bc	1.80 ± 0.04 b	0.34 ± 0.01 e	2.08 ± 0.18 d	21.06 ± 0.42 ef	97.00 ± 3.80 c
*WR*2	66.54 ± 2.12 ab	25.75±2.30abc	12.90 ± 0.30 bc	0.21 ± 0.01 bc	1.71 ± 0.00 bc	0.36 ± 0.01 e	2.10 ± 0.08 d	19.26 ± 0.09 fg	68.40 ± 0.78 f
*WR*3	66.82 ± 4.10 ab	26.05±0.38abc	10.30 ± 0.10 de	0.18 ± 0.01 bc	1.51 ± 0.01 de	0.35 ± 0.01 e	2.10 ± 0.07 d	17.47 ± 0.09 g	79.57 ± 1.63 e

[1] All values are expressed on dry matter basis and are the average of at least three values ± standard error of the mean. Means with different letter(s) within columns are statistically different ($P<0.05$).

For the specific case of the free fraction, the highest antioxidant activity was observed in the *RR2* kernels. Dicko et al. (2005) and Awika et al. (2009) reported AOXC values for sorghum ranging from 30 to 80 and 171 to 236 μmol of TE/g, respectively. The AOXC was related to the phenolic contents, mainly those associated to the bound fraction (r= 0.835, $P<0.01$).

4.4.3. Parameters Obtained from the Different Susceptibility Tests and their Correlation with Physical and Chemical Kernel Properties.

Results of the insect susceptibility tests made (Table 4.4) indicated that there were significant differences among genotypes, mainly in the ratio of final/initial insect number (from the confinement test) and MDT from the Dobie Index Study. The *Sumac*, *Waxy1* and *HD1* kernels were the most susceptible to *S. zeamais*, whereas counterparts *RR1*, *RR2* and *WR1* were the most resistant. The cultivar with the most severe WL was white *HD1* with 10.5% during the confinement test. The DI for this group of grains was between 14.7 for *RR1* and 18.5 for *Waxy*1. According to Dobie (1974), these damages in maize denote a highly susceptibility (DI >= 11) and agrees with the scale proposed by Teshome (1996), who established the limit between susceptible and highly susceptible materials in a DI of 13. Thus, the sorghum grains studied herein were highly susceptible to maize weevil attack.

The results in Table 4.4 describe a special group of sorghums, where despite detected differences in physical and chemical properties (Table 4.1, Table 4.2 and Table 4.3) all showed a high susceptibility to *S. zeamais* infestation. In order to set a resistant control during the susceptibility tests, a parallel assay was performed with different maize cultivars: yellow, white and a previously reported resistant landrace (P84) (García-Lara and Bergvinson, 2007). The ratio final/initial insect in the latter was practically 1.0 (0.99±0.03) and the loss of weight as low as 1.2±0.5 %.

The sorghum set evaluated in this research, showed that the harder the grain, the more resistant the genotype. The endosperm hardness is a characteristic that in this context was described as the relation between vitreous and floury endosperm, a parameter used as an index for milling quality and yield (Chiremba et al., 2011). According with results described in Table 4.5: TW, ET, FI, TD and TADD, indexes used to evaluate sorghum hardness were all well related to

susceptibility parameters (DI, TEI, insect number at 27 and 30 days, WL and MDT) and the correlation indexes were highly significant **(**Figure 4.1**)**. The physical resistance of sorghum kernel represents not only a hurdle for oviposition of *S. zeamais*, but for feeding in kernels with increased hardness.

Table 4.4. Susceptibility parameters obtained from confinement and Dobie Index tests using *S. zeamais*[1]

Variety	Dobie Index Test			Confinement Test	
	Dobie Index (DI)	Emerged Insects (TEI)	Median Development Time (MDT)	Ratio final / initial insect number	Weight loss (WL)
		#	Days		%
RR1	14.74 ± 0.39 c	160.00 ± 14.42 b	34.39 ± 0.28 a	1.53 ± 0.04 cd	4.80 ± 0.40 ab
RR2	15.27 ± 0.88 bc	200.00 ± 48.21 ab	34.32 ± 0.32 a	1.07 ± 0.53 d	5.80 ± 1.70 ab
Sumac	17.41 ± 0.47 ab	301.00 ± 35.22 ab	32.72 ± 0.27 cd	3.51 ± 1.77 a	9.40 ± 2.20 ab
HTW 1	15.63 ± 0.50 bc	210.00 ± 35.59 ab	34.05 ± 0.14 ab	2.21 ± 0.17 bcd	5.50 ± 0.70 ab
HTW 2	16.37 ± 0.06 abc	222.33 ± 14.31 ab	32.99 ± 0.32 bc	2.18 ± 0.28 bcd	4.30 ± 0.80 ab
*Waxy*1	18.47 ± 0.55 a	350.00 ± 40.63 a	31.67 ± 0.38 d	2.56 ± 0.45 bcd	7.70 ± 1.30 ab
*Waxy*2	16.86 ± 0.30 abc	286.67 ± 18.98 ab	33.54 ± 0.20 abc	2.15 ± 0.26 bcd	6.50 ± 0.70 ab
*HD*1	17.41 ± 0.51 ab	315.00 ± 47.17 ab	32.93 ± 0.26 bc	3.33 ± 1.67 b	10.50 ± 1.70 a
*HD*2	16.39 ± 0.37 abc	225.67 ± 28.26 ab	32.98 ± 0.01 bc	3.08 ± 0.19 bc	7.70 ± 1.00 ab
*WR*1	15.79 ± 0.35 bc	204.67 ± 22.84 ab	33.64 ± 0.19 abc	0.90 ± 0.48 d	3.70 ± 1.90 b
*WR*2	16.23 ± 0.15 abc	225.33 ± 6.39 ab	33.39 ± 0.19 abc	1.43 ± 0.73 bcd	4.80 ± 0.70 ab
*WR*3	16.74 ± 0.33 abc	273.67 ± 26.98 ab	33.47 ± 0.13 abc	3.39 ± 0.35 b	8.10 ± 1.00 ab

[1] All values are the average of at least three values ± standard error of the mean. Means with different letter(s) within columns are statistically different (P<0.05).

Besides the significant relationship between hardness and susceptibility parameters (Table 4.5), the percentage of anatomical parts of sorghum were also high correlated, depicting, the best, coefficients higher than 0.7. The most significant correlation was related to endosperm and pericarp percentage (r>0.72 and 0.75 respectively): cultivars with a high endosperm and low pericarp percentage showed the highest resistance (Figure 4.1). According to Serna-Saldivar (2010), kernels with a high endosperm percentage, usually are bigger and for this reason contain more starch, protein and yield during milling. Nevertheless, relationship among endosperm percentage, starch and protein was not significant, but did correlate with ET and susceptibility parameters (Insects at day 27 and 30, MDT, DI and TEI, r>|0.72|, except for the last parameter with r=-0.61). In this set of samples, the higher the endosperm percentage, the harder the kernel texture (r=0.673, P<0.05).

In other words, it was more important the access of the insects to oviposition and feeding than availability and quantity of nutrients, in this case starch and protein of the endosperm. Regarding information depicted in Table 4.3, the chemical characteristics (mainly starch, amylose and FAN) were also correlated to susceptibility parameters (Table 4.4). The ash had a high and positive correlation (r=0.8, P<0.01) with DI, as well as FAN and amylose (r >|0.6| with a P<0.05), being the latter a negative correlation. These results showed a tendency of *S. zeamais* to prefer kernels with low amylose content (waxy cultivars) and high in FAN (Figure 4.1). The latter parameter could be an indicator of the state of the proteins within the endosperm, because when the damage (or availability of protein) is high, FAN content is also high (Perez-Carrillo et al., 2012).

Although Chandrashekar and Satyanarayana (2006) described that after physical barriers, the chemical strategy is the most important for grain stability and Ramputh et al. (1999) reported a significant correlation between phenolic concentration and *S. zeamais* resistance, these mechanisms apparently did not affect insect growth (Table 4.5). The result can be related to differences in the total phenolic analysis. Ramputh et al. (1999) used in the eight analyzed cultivars the Prussian blue assay whereas in this research the Folin-Ciocalteu was selected, because of its stability and reproducibility compared to the former test (González et al., 2003).

Also, the very specific phenolic acids and composition of the total phenolic fraction in each sorghum cultivar can influence results. The DI calculated by Ramputh et al. (1999), ranged from

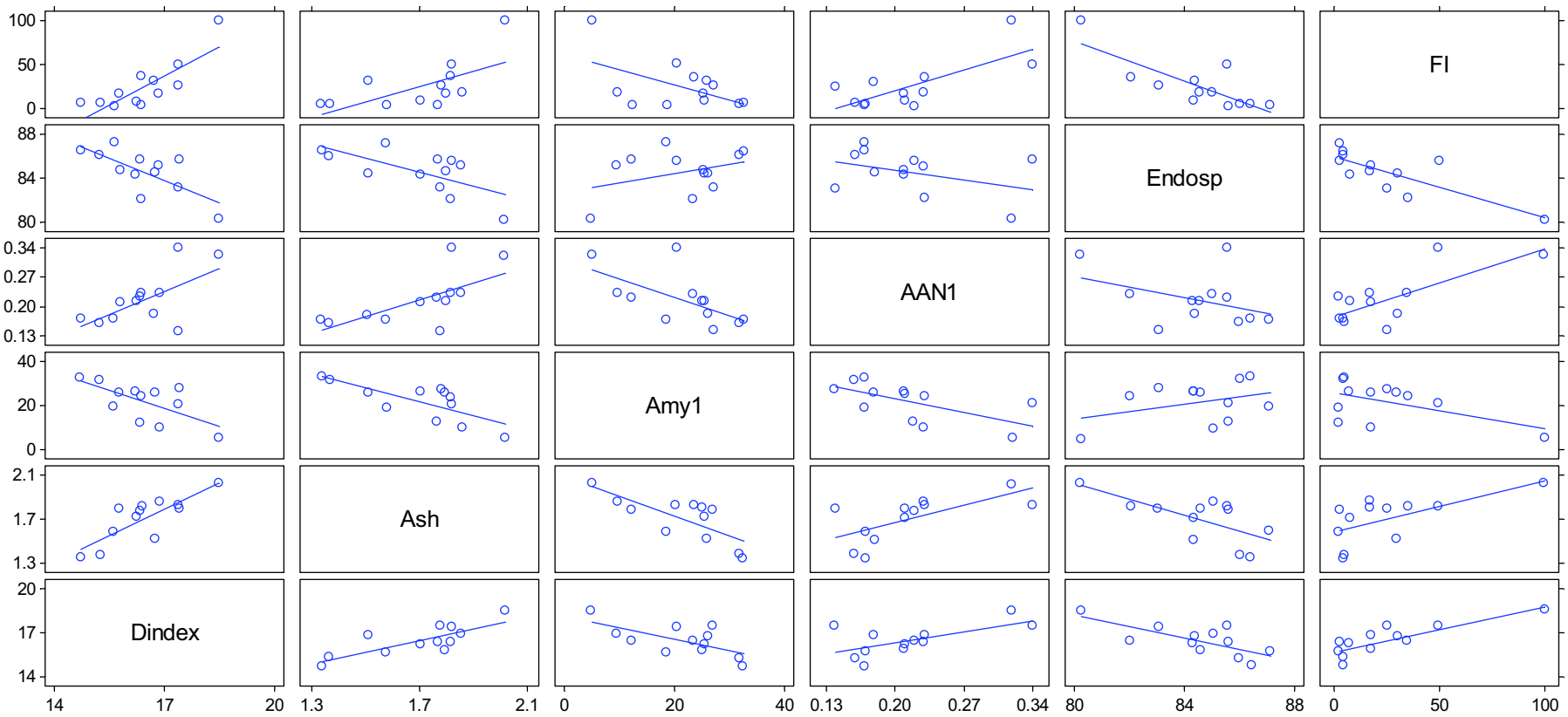


Figure 4.1. Scatter plots for Dobie Index (DI), Ash, Amylose (Amy1), Alpha Amino Nitrogen (AAN1), Endosperm percentage (Endosp) and Flotation Index (FI), indicating the correlation among these variables.

3.3 to 15.3 and had a good collection of resistance characteristics that differed from the information in Table 4.3, with Dobie indexes from 14.7 to 18.5, a range significantly higher but also narrower. At this time, hardness parameters, as FI and ET, as well as TW, showed a good potential for quick sorghum evaluations in the field to assess grain susceptibility during storage.

4.5. CONCLUSIONS

Significant differences in susceptibility to *S. zeamais* were observed amongst sorghum genotypes, indicating the wide range of evaluated germplasm. The most susceptible varieties were *Sumac*, *Waxy1* and *HD1* (Dobie Index of 17.4 to 18.4) whereas the most resistant were *RR1*, *RR2* and *WR1* (Dobie Index of 14.7 to 15.8). Susceptibility was correlated to sorghum hardness and also to anatomical fractions (mainly endosperm percentage r=-0.72). Chemical parameters associated to susceptibility were: ash, amylose and FAN contents, result that at the same time indicates a relationship among nutrient availability and sorghum response to pest infestation. The phenolic content was not correlated to *S. zeamais* susceptibility. The most resistant sorghum can be described as a material with hard and high endosperm percentage, low ash, increased amylose content and reduced free amino nitrogen concentration.

Once that the susceptibility to maize weevil was obtained for sorghum cultivars, the effect of this insect into endosperm components, namely starch and protein, was further studied and the main findings are described in chapter 5.

Table 4.5. Pearson correlations for physical, chemical and susceptibility parameters of the different sorghum genotypes[1]

	FAN	AOXC Bound	AMY	Ash	Bphenol	PDigest	DI	Emerged Insects	% Endosp	ET	FI	Ratio Final / Initial	FPhenol	% Germ	Insects at day 27	Insects at day 30	MDT	Crude Protein
AMY	-0.624*	ns																
Ash	0.671*	ns	-0.754**															
Bphenol	ns	0.835**	ns	ns														
CI	ns	0.621*	ns	ns	0.811**													
PDigest	0.594*	ns	ns	ns	-0.580*													
DI	0.633*	ns	-0.657*	0.802**	ns	ns												
Emerged Insects	0.607*	0.636*	-0.606*	0.699*	ns	ns	0.972**											
% Endosp	ns	ns	ns	-0.671*	ns	ns	-0.724**	-0.610*										
ET	0.662*	ns	ns	ns	ns	ns	ns	ns	-0.673*									
FI	0.738**	ns	ns	0.632*	ns	ns	0.821**	0.797**	-0.786**	0.824**								
Ratio Final / Initial	ns	ns	ns	ns	ns	ns	0.659*	0.665*	ns	ns	ns							
FPhenol	ns	0.762**	ns	ns	0.979**	-0.658*	ns	ns	ns	ns	ns	ns						
% Germ	ns	ns	ns	ns	ns	0.616*	ns	ns	-0.843**	0.661*	0.684*	ns	ns					
Insects at day 27	0.713**	ns	-0.722**	0.782**	ns	ns	0.869**	0.814**	-0.798**	0.763**	0.914**	ns	ns	0.689*				
Insects at day 30	0.747**	ns	-0.640*	0.766**	ns	ns	0.945**	0.904**	-0.752*	0.727**	0.914**	ns	ns	0.602*	0.950**			
WL	ns	0.600*	ns	ns	ns	ns	0.681*	0.756**	ns	ns	0.582*	0.865**	ns	ns	ns	0.617*		
MDT	-0.636*	ns	0.645*	-0.844**	ns	ns	-0.925**	-0.817**	0.835**	-0.664*	-0.823**	ns	ns	-0.612*	-0.894**	-0.936**		
% Pericarp	ns	0.607*	ns	ns	0.623*	ns	0.755**	0.713**	-0.646*	ns	0.611*	0.661*	0.611*	ns	0.650*	0.673*	-0.736**	
Crude Protein	0.656*	ns	-0.651*	0.794**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
RStarch	ns	0.658*	ns	ns	0.925**	-0.678*	ns	ns	ns	ns	ns	ns	0.970**	ns	ns	ns	ns	ns
TStarch	-0.705*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-0.652*
TADD	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.751**	ns	ns	ns	ns	ns	ns	ns	ns
TKW	ns	ns	ns	ns	-0.697*	0.649*	ns	ns	ns	ns	ns	ns	-0.733**	ns	ns	ns	ns	ns
TW	-0.690*	ns	ns	ns	ns	ns	-0.709**	-0.690*	0.716**	-0.894**	-0.952**	ns	ns	-0.621*	-0.884**	-0.833**	0.732**	ns
TD	-0.750**	ns	ns	-0.620*	ns	ns	-0.692*	-0.613*	0.796**	-0.927**	-0.932**	ns	ns	-0.726**	-0.855**	-0.831**	0.803**	ns

[1]Correlation coefficients with an asterisk (*) represent *Pvalues*<0.05 and with two (**)<0.01; *ns* stands as "non-significant".

AOXC, Antioxidant Capacity; AMY, amylose; BPhenol, Bound Phenolics; CI, Color Index; DI, Dobie Index; ET, Endosperm Texture; FAN, Free Amino Nitrogen; FI, Flotation Index; FPhenol, Free Phenolics; MDT, Median Development Time; PDigest, Protein Digestibility; RStarch, Resistant Starch; TADD, Tangential Abrasive Dehulling Device; TD, True Density; TStarch, Total Starch; TKW, Thousand Kernel Weight; TW, Test Weight; WL, Weight Loss.

5. CHANGES ELICITED BY *SITOPHILUS ZEAMAIS* IN STARCH AND PROTEIN FRACTIONS OF FLOURS OBTAINED FROM DIFFERENT SORGHUM CULTIVARS

5.1. ABSTRACT

A bioassay test of twelve different sorghums infested with *S. zeamais* was performed under confinement conditions and the changes in starch and protein characteristics evaluated. The sorghum set included two red samples with regular endosperm, one brown or high-tannin, two white waxies, two white heterowaxies, two white high-digestible genotypes and three white with regular endosperm. During storage, *S. zeamais* attacked the endosperm lowering total and resistant starch values (average 2.3%) and increased damaged starch from 1.6 to 1.8%. The waxies, one high digestible, one red and the high-tannin sorghums were the most damaged in terms of total starch losses: -7.2 to -8.4%. The higher damaged starch was correlated with percentage of abraded material with TADD and thousand kernel weight so softer and smaller grains showed the lowest damaged starch values. Amylose increased 2.8% average during infestation. The maize weevil activity also affected the protein fractions: Free Amino Nitrogen (FAN), Protein Digestibility (PD) and Crude Protein (CP) increased 50%, 44.5% and 8.3% respectively. These results describe changes in the protein structure due to the maize weevil preference for starch. *Sitophilus zeamais* affected the endosperm protein within yielding flours with more FAN and higher protein digestibility. These changes in turn allowed total and resistant starch reduction (mainly amylopectin fraction). Flours obtained from damaged grains with maize weevil were lower in starch but higher in hydrolyzed proteins. This indicates that this particular insect attacked the protein matrix in order to reach or access starch granules.

5.2. INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is a crop from the *Poaceae* family well adapted to tropical areas and dry environments and resilient to biotic stresses (Chanapamokkhot and Thongngam, 2007; Kim and Day, 2010; Yan et al., 2009). Sorghum grain and its different groups (i.e. sweet and forage sorghums) needs only one third of the fertilizer used for other crops such as sugarcane or maize (Kim and Day, 2010) and produce after a relatively short agronomic cycle (3 to 5 months). It is a highly successful cereal in Semi-Arid regions from Africa and Asia (Duodu et al., 2002) and in some North American regions. *Sorghum bicolor* is used to obtain a wide array of products as beer (malt and starch source), tortilla, bread and traditional foods (injera, tô, ogi) (Serna-Saldivar, 2010; Rooney and Serna-Saldivar, 2000). Sorghum grain has also been used for starch refining and bioethanol production (Chuck-Hernández et al., 2011; Taylor et al., 2006; Wang et al, 2008; Wu et al., 2007; Yan et al., 2011). Because of its good yield even under dry and hot environments and its high CO_2 turnover, sorghum can be described as a crop with desired characteristics under the foreseen global climatic change conditions (Anwar et al., 2012). After maize, rice, wheat and barley, sorghum is the most produced cereal worldwide with a yearly output of 55.7 million ton and a cultivated surface of 41 million hectares (FAOSTAT, 2012). The United States of America, Mexico, India and Nigeria are the main sorghum producers and altogether provide almost half of the global output.

In agricultural food production, biotic stresses as insects lower grain yields (Jackson, 1986) as well as in postharvest stages (v.gr. storage and processing). Losses can be direct, because of the insect consumption and indirect since contaminants as feces, urine, bodily parts, exoskeletons and off-odors are left in damaged cereal batches (Serna-Saldívar, 2010). From the six million existing insect species, approximately 20 to 30 are important to stored grains and five pests cause most of the commercial damage and losses: granary, rice and maize weevil, lesser grain borer and Angoumois grain moth (Serna-Saldívar, 2010). In sorghum, there are 150 insect species infesting, being *S. zeamais* the major in tropical agroecologies and one of the most important in stored products (Abebe et al., 2009; Guo et al., 2011). Insects reduce grain viability, weight and market value, affecting nutritional and functional characteristics (Abebe et al., 2009).

Global food postharvest system relies in both formal and informal markets, being the latter the most prevalent in developing countries. In informal markets, as well as small and micro

enterprises, the implementation of quality control systems is difficult and altogether with the high incidence of pest in stored grains, yields the unavoidable use of insect-damaged cereals. The influence of insects in nutritional and functional properties of cereals and its products is thus highly relevant for oriented decisions in food processing.

There are few reports about the impact of insect infestation in the functional and nutritional properties of sorghum. Park et al. (2008) studied the effect of *Rhyzopertha dominica* (F.) on physicochemical properties of sorghum kernels and flour. Abrasive hardness, milling yield and kafirin content was reduced at high levels of lesser grain borer infestation. Sorghum flour depicted an increase in the overall pasting viscosity with high insect population and damage. An infestation assay using *Trogoderma granarium* and *Rhizopertha dominica* in wheat, maize and sorghum yielded a reduction in total carbohydrates and an increase in protein and crude fiber at infestation levels of 75% (Jood et al., 1996). A reduction in starch and protein digestibility was also observed at high damages because of presence of both insects (Jood and Kapoor, 1992). All these changes could therefore affect process parameters and final product characteristics, where the quality of protein and starch are crucial. There are no reports about the effect of *S. zeamais* in sorghum during storage and altogether with the scarce information previously described about the chemical changes during infestation, were the motivations to explore changes in protein and starch of sorghum cultivars infested with maize weevil (*S. zeamais*).

5.3. MATERIALS AND METHODS

5.3.1. GRAIN VARIETIES

Twelve sorghum cultivars were selected based on their color and endosperm characteristics. Genotypes used in this study were: 1) two red sorghums, commonly used as feed in the northern part of Mexico (*RR1* and *RR2*); 2) one high-tannin (*Sumac*); 3) nine white cultivars (two heterowaxy –*HTW1, HTW2*-, two waxy –*Waxy1, Waxy2*-, two described as high protein digestible –*HD1, HD2*- and three regular –*WR1, WR2, WR3*-). The red and high-tannin sorghums were commercially available in Northern Mexico and possessed an intermediate to soft endosperm texture. The white samples were kindly donated by Dr. Dirk Hays of the Texas A&M University Sorghum Breeding Program.

5.3.2. Sample Preparation

Kernel samples, not previously treated with insecticides, were cleaned by air aspiration and sieves. Moisture was determined using gravimetric method 44-15A (AACC, 2000) and adjusted to 13% using the formula described by Serna-Saldivar (2012): ([(100 - % Initial Moisture)/(100 – % Final Moisture)] – 1] * Sample Weight). Samples were equilibrated for at least 7 days at 27 ± 1°C and 70 ± 5% RH (using a saturated NaCl solution). For chemical characterization, sorghum kernels were milled using a coffee mill (Krups, Model GX410011, Mexico) and stored at 4°C prior to use. Whole kernels were used for confinement test and all treatments were performed by triplicate.

5.3.3. Chemical Characterization

Crude Protein (N*6.25) was determined using the micro-Kjeldahl method 46-13 (AACC, 2000) and Free Amino Nitrogen (FAN) with the ninhydrin procedure 945.30L (AOAC, 1980), whereas for Protein Digestibility, the pepsin procedure described by Hamaker et al. (1987) and Axtell et al. (1981) was used with slight modifications. Briefly, ground samples (100 mg) were suspended in 17.5 ml buffer with pepsin (1.5 g pepsin –P7000, Sigma Aldrich- per liter of 0.1 M KH_2PO_4 buffer, pH 2.0) and incubated in a shaking water bath (37°C) altogether with blanks (enzyme and buffer mix). After two hours, 1 mL NaOH 2M was added, samples vortexed and centrifuged (10,000xg, 15 min, 4°C). An aliquot of the supernatant was analyzed using the micro-Kjeldahl method 46-13 (AACC, 2000) to obtain solubilized nitrogen. Protein digestibility was calculated as: (nitrogen in sample supernatant –nitrogen in blank) / total nitrogen of the sample * 100. Total, resistant and damaged starches as well as amylose were determined using enzymatic tests TStarch –AOAC 996.11-, RStarch –AOAC 2002.02-, Starch Damage –AACC 76-31- and Amylose/Amylopectin -K-AMYL- (Megazyme International, Ireland).

5.3.4. Extraction of Phenolic Compounds for Resistant Starch Evaluation

In order to evaluate the effect of phenolic and tannin compounds in resistant starch determination, extraction of phenolic compounds of sorghum samples was performed. The method reported by Chuck-Hernández et al. (2012) with modifications was used. Twenty grams of sorghum flour and 50 mL of acidified methanol (HCl 1% v/v) were mixed and kept for 12

hours at room temperature and 100 rpm before centrifugation (15 min, 4,000 rpm; Centra MP4R centrifuge, International Equipment Company, USA). The supernatant was discarded and the pellet dried in a convection oven set at 50°C during 2 hr before resistant starch assays.

5.3.5. SUSCEPTIBILITY TESTS

Insect Culture, Confinement and Dobie Index (DI) assays were performed according to methods previously described in Chapter 4.

5.3.6. STATISTICAL ANALYSIS

Three replicates for chemical and nutraceutical traits as well as susceptibility parameters were subjected to Analysis of Variance (ANOVA) using the statistical software Statistix 9. Residual analysis was performed in order to review the compliance of statistical assumptions (normality, independence and homoscedasticity). When means resulted different (null hypothesis rejected, *Pvalue<0.05*), differences among means were evaluated using Tukey HSD Tests with *alpha*=0.05. Homogeneous mean´s groups were indicated with the same alphabetic sub-indices within tables. Pearson correlation coefficients were obtained using the same statistical software.

5.4. RESULTS AND DISCUSSION

5.4.1. STARCH CHARACTERISTICS BEFORE AND AFTER CONFINEMENT

5.4.1.1. TOTAL STARCH (TS)

Starch represents two-thirds to three-fourths of the grain weight and is the main source of energy during sorghum germination (Serna-Saldivar, 2010). In dry basis, starch in sorghum, usually is reported from 60.0 to 77.0%, whereas starch in corn varies from 67.8 to 74.0%. Average starch content in both cereals could be set around 70-73% (Serna-Saldivar, 2010). Herein, total starch (TS) was 59.6 to 73.6% for *HD1* and *RR2* sorghums respectively indicating that all cultivars were within the regular reported range. After 45 days infestation, final TS was in average 2.3% less than the initial (66.2 to 63.9%) content because of the *S. zeamais* activity. *Waxy2, HD2, RR2* and *Sumac* were the most affected kernels with starch losses of 7.2 to 8.4%. In contrast, the *RR1* and *HTW1* grains were the least altered in terms of TS. This result is associated with the different susceptibilities of the sorghum kernels to *S. zeamais.*

5.4.1.2. DAMAGED STARCH (DS)

Besides TS, an interesting starch fraction is damaged starch (DS) (Table 5.1). DS increased from 1.6% before confinement to an average of 1.8% after. There are no reports about a standard range of sorghum DS, because it varies according to milling or processing characteristics, as well as genotypes. In general terms, DS is positively correlated to kernel hardness. Schober et al. (2005) for example, reported a significant correlation between starch damage and hardness (r=0.844) and also between starch damage and starch content (r=0.723). DS results (Table 5.1) correlated to TADD and TKW. Soft grains have the lowest DS (r=0.61, $P<0.05$) and counterparts with higher thousand kernel weight (TKW) values have the highest starch damage after milling and mechanical treatments (r= 0.64, $P<0.05$). The highest starch damage was detected in *HTW2* and *Waxy2* kernels (>2.0%) and the lowest for the high-tannin genotype (*Sumac*), followed by *RR1* and *RR2* with 1.2 and 1.3%, respectively. The DS average in sorghum cultivars reported by Schober et al. (2005) was 11.1 to 16.5 %, higher than the DS range values reported in Table 5.1. The TS content for the nine sorghum cultivars indicated for Schober et al. (2005) ranged from 82.1 to 77.3% in dry basis, also higher compared with the mean of 66.2% reported herein. Authors as Aboubacar and Hamaker (1999) reported for eight sorghum cultivars in couscous preparation, a range from 3.7 to 7.2% of DS, comparable to the values found by Ranhotra et al (1993) but in commercial hard and soft wheat flours (2.9–10.1%).

5.4.1.3. RESISTANT STARCH (RS)

RS can be also described as a starch susceptibility index to digestion and in this work, both RS1 and RS2 (but mainly RS1) are the main types associated to uncooked sorghum kernels. RS average before confinement was 8.1% whereas at the end decreased to 5.8%, equivalent to 16.4% compared to the initial RS value (Table 5.1). Among the different starch fractions, the RS showed the highest change during storage. The W*axy1* cultivar contained the lowest RS before and after the confinement test (1.9 and 1.0% respectively). The *WR3, HTW1* and *HD1* cultivars increased RS (+50, 18 and 13.8% respectively), whereas a decrease was observed in the rest of sorghum types (Table 5.1). The highest drop was for *Sumac*, *Waxy1* and *Waxy2* (>40%) and this result is related to the susceptibility to α-amylase hydrolysis. Comparatively, these cultivars possessed the softest endosperm texture. The *Sumac* cultivar (sorghum 3), had the highest RS values before and after confinement (almost 34% and 16% respectively). Reported RS in sorghum varies from

5.1 to 6.5% (Niba and Hoffman, 2003; Platel and Shurpalekar, 1994). These values were close to values summarized in Table 5.1 (except for the 33.9% value of *Sumac*). In a storage test using *Trogoderma granarium* Everts and *Rhizopertha dominica* Fabricius as insect models in wheat, maize and sorghum, a significant reduction in starch digestibility was reported in kernels with grain infestations of 25, 50 and 75% (Jood and Kapoor, 1992). As previously described, not all sorghum cultivars depicted an increase in resistant starch and therefore a reduction in starch digestibility as previously described by Jood and Kapoor (1992). The difference could be associated with the insect feeding preferences, where *S. zeamais* affects more the endosperm starch granules and protein matrix.

5.4.1.4. Effect of Phenolic Extraction in RS.

In Table 5.1, the high RS content in *Sumac* cultivar is noteworthy. Resistant starch is a trait that could be positively linked to endosperm texture, but this characteristic was not substantially different in *Sumac* regarding other evaluated sorghum, in fact *Sumac* had one of the softest endosperm of all genotypes (Table 4.1). Moreover, the Flotation Index (FI) related to endosperm texture, was not statistically different in comparison to the other types of sorghum kernels (Table 4.1). The only remarkable difference was indeed the phenolic content, because *Sumac* was the only high-tannin cultivar. Tannins have been widely reported as antinutritional factors because of their ability to bind food proteins and carbohydrates, yielding insoluble complexes that cannot be hydrolyzed by digestive enzymes. Tannins have therefore capacity to direct binding of digestive enzymes including amylases, trypsin, chymotrypsin and lipases; thus, inhibiting their activity (Awika and Rooney, 2004; Dicko et al., 2006a; Dykes et al., 2005). RS of *Sumac* after phenolic extraction was 2.4±0.1%, 14 times less compared to the initial RS, clearly indicating the effect of tannins in sorghum digestibility.

5.4.1.5. Amylose

Within the evaluated sorghum set (Table 5.1), there were two cultivars that can be considered as waxy (*Waxy1* and *Waxy2),* because they contained lower amylose contents (5.0 and 9.6%) compared to regular sorghums which usually contain 20 to 30% amylose (Serna-Saldivar and Rooney, 1995; Serna-Saldivar, 2010). This trait varies according to the cultivar itself. Starch granule structure can be divided in crystalline and amorphous areas, being the former mainly composed from amylopectin and the latter for amylose, being also less dense than crystalline

areas. Some starch hydrolases, as α amylase, starts its attack in the amorphous regions, while hydrolysis of crystalline areas occurs more slowly (Rooney and Pfulgfelder, 1986). For this reason, and because of the enzymatic machinery of insects infesting the grain, it was expected to observe a decrease in amylose after confinement. Nevertheless and increase was observed (21.5% versus 22.7%). This result altogether with the decreased in TS previously described indicates that *S. zeamais* had preference for amylopectin instead of amylose. The *HTW2, HD1* and *WR2* cultivars yielded the highest deltas in amylose, with -30.1, +33.8 and +39.1% respectively.

5.4.1.6. Differences in Starch During Storage and Insect Infestation.

Based on results depicted in Table 5.1, *Sitophilus zeamais* had a significant TS consumption throughout the 45 days of storage. Its activity within sorghum kernels was different in each starch fraction: TS and RS values reduced whereas DS values increases. The increase in damaged starch, under the same milling conditions, could be indeed considered as a sign of insect activity within the endosperm. Resistant starch was reduced, with some exceptions and amylose percentage was maintained almost without changes. This latter result could be interpreted that the insect had preference for the amylopectin fraction affecting starch structure and integrity.

5.4.2. Protein Characteristics Before and After Confinement (and Fractions of Protein)

Starch availability and susceptibility to hydrolysis and deterioration is affected by protein structure and composition. The Crude Protein (CP), Free Amino Nitrogen (FAN) and Protein Digestibility (PD) for the different sorghum grains were obtained (Table 5.2).

5.4.2.1. Crude Protein (CP)

Typically, sorghum crude protein ranges from 7.3 to 15.6%. The sorghums analyzed herein (Table 5.2) varied from 9.3 to 14.5% and therefore lie within that range. The *RR2* and *WR1* kernels contained the lowest and highest CP values, respectively. Table 5.2 also depicts differences of protein fractions as affected by infestation with *S. zeamais*. In almost all cultivars there was an increase in CP related to TS reduction previously described in subsection 5.4.1.1. (average +8.3% compared to the initial average content).

Table 5.1. Starch characteristics of sorghum cultivars before and after *S. zeamais* damage: total, damage and resistant starch and amylose percentage for all evaluated sorghum and maize samples[1]

Sorghum cultivar	Kernel color	Starch, %	Damaged Starch, %	Resistant Starch, %	Amylose, %	Starch, %	Damaged Starch, %	Resistant Starch, %	Amylose, %
		Before insect damage				After confinement test			
RR1	Red	66.6 ± 1.9 ab	1.2 ± 0.0 f	6.9 ± 0.4 bcd	32.7 ± 1.8 a	66.0 ± 0.7 a	1.7 ± 0.0 def	6.4 ± 0.6 b	31.1 ± 2.2 a
RR2	Red	73.6 ± 2.0 a	1.3 ± 0.0 ef	8.7 ± 0.2 b	31.8 ± 1.4 a	67.5 ± 1.8 a	1.7 ± 0.0 de	6.1 ± 0.1 b	33.9 ± 1.8 a
Sumac	Brown	68.1 ± 0.6 ab	1.1 ± 0.0 g	33.9 ± 2.7 a	27.1 ± 2.8 ab	62.4 ± 0.4 ab	1.3 ± 0.0 g	16.0 ± 1.8 a	23.2 ± 1.3 abc
HTW 1	White	63.9 ± 1.4 ab	1.6 ± 0.0 c	5.0 ± 0.4 bcde	18.8 ± 0.1 cd	64.5 ± 1.0 ab	2.0 ± 0.0 b	5.9 ± 0.6 b	20.7 ± 1.1 abc
HTW 2	White	65.7 ± 1.2 ab	2.1 ± 0.0 a	6.3 ± 0.3 bcd	12.3 ± 1.7 de	64.5 ± 1.6 ab	2.0 ± 0.0 b	5.0 ± 0.2 bc	8.6 ± 0.2 bc
Waxy1	White	62.8 ± 0.6 b	1.5 ± 0.0 cd	1.9 ± 0.0 e	5.0 ± 1.1 e	60.7 ± 0.1 b	1.5 ± 0.0 f	1.0 ± 0.0 c	4.3 ± 0.0 c
Waxy2	White	69.5 ± 0.9 ab	2.1 ± 0.0 a	6.2 ± 0.2 bcde	9.6 ± 1.5 e	64.5 ± 0.5 ab	2.3 ± 0.0 a	3.7 ± 0.3 bc	8.7 ± 0.8 bc
HD1	White	59.6 ± 2.7 b	1.6 ± 0.0 cd	2.9 ± 0.2 de	20.4 ± 1.8 bc	60.0 ± 0.9 b	1.6 ± 0.0 ef	3.3 ± 0.1 bc	27.3 ± 4.2 ab
HD2	White	65.4 ± 2.2 ab	1.5 ± 0.0 d	5.6 ± 0.4 bcde	23.5 ± 0.8 bc	60.7 ± 1.3 b	1.6 ± 0.0 ef	4.1 ± 0.3 bc	25.3 ± 2.0 ab
WR1	White	66.0 ± 0.7 ab	1.3 ± 0.0 e	8.1 ± 0.7 bc	25.3 ± 1.0 abc	62.4 ± 0.9 ab	1.8 ± 0.1 cd	5.7 ± 0.1 b	26.0 ± 0.4 ab
WR2	White	66.5 ± 2.1 ab	1.7 ± 0.0 b	8.2 ± 0.2 b	25.8 ± 2.3 abc	66.4 ± 0.4 a	1.9 ± 0.0 c	6.2 ± 0.9 b	35.9 ± 8.5 a
WR3	White	66.8 ± 4.1 ab	1.8 ± 0.0 b	3.8 ± 0.3 cde	26.1 ± 0.4 abc	67.4 ± 1.3 a	1.9 ± 0.0 c	5.7 ± 0.3 b	27.7 ± 0.5 ab

[1] All values are expressed on dry matter basis and are the average of at least three replicas ± standard error of the mean. Means with different letter(s) within column are statistically different ($P<0.05$).

The *WR2* was the only genotype without change in CP, and was also the less affected in terms of TS. These results could be related to the feeding strategy of insects. *Sitophilus oryzae*, for example, in its adult stage feeds mainly on the endosperm reducing thus carbohydrate content, but larvae feeds preferentially in the germ, the grain structure where most of the fat, minerals and vitamins are located, as well as the portion where 15% of protein is stored. These proteins are mainly constituted by albumins and globulins, the water and salt-soluble fractions respectively (Dal Bello et al., 2001). In wheat, *Cryptolestes ferrugineus* (rusty grain beetle) feeds exclusively on germ, whereas *Rhyzopertha dominica* (lesser grain borer) and *Sitophilus granarius* (granary weevil) feed both in germ and endosperm during larval and adult feeding (Campbell and Sinha, 1976). The *S. granarius* is considered the most damaging pest in terms of weight loss in a single kernel experiment (60% versus 17 and 4% for *R. dominica* and *C. ferrugineus* respectively). Thus, the feeding characteristics of the pest will affect the nutrimental composition of the grain and in the specific case of germ-feeding also will be detrimental to kernel´s viability. According to the results shown in Table 5.1 and Table 5.2 is clear that *S. zeamais* preferred starch above the protein located in protein bodies and endosperm matrix.

5.4.2.2. FREE AMINO NITROGEN (FAN)

In this context, FAN can be defined as amino acids or peptides already present in the analyzed sorghum flour or derived from the hydrolysis of its proteins during storage and insect infestation. FAN is determined through a colorimetric method using ninhydrin as reagent that yields a purple complex with peak absorption at 570 nm.

Original FAN content in cereals depends on the cultivar and agronomic conditions among others factors. Table 5.2 summarizes FAN values for the twelve evaluated sorghums that ranged from 0.34 to 0.14 mg/g (42.4 to 17.8 mg/L). These values are lower compared to results obtained by Anibaba and Osagie (1997) in two malting sorghums (60 mg/L). *Sumac* was the variety with the lowest FAN content (0.14 mg/g) whereas the *Waxy1* and *HD1* contained the highest concentrations (0.32 and 0.34 mg/g respectively). At the end of confinement, an increase in FAN (average of +50% regarding initial FAN content) was observed (Table 5.2), indicating the physical and chemical damage of proteins. The *S. zeamais* activity over starch affected the state of the proteins, because of the reduction of TS and the increase of FAN which indicates the degree of protein hydrolysis.

Table 5.2. Crude protein, alpha amino nitrogen and digestibility of protein (before and after *S. zeamais* damage) for all evaluated cultivars[1]

Sorghum cultivar	Crude protein, %	Alpha amino nitrogen (mg/g)	Protein digestibility, %	Crude protein, %	Alpha amino nitrogen (mg/g)	Protein digestibility, %	Crude protein	Alpha amino nitrogen	Protein digestibility
	Before insect damage			After confinement test			Diferences, %		
RR1	10.9 ± 0.2 cd	0.2 ± 0.0 bc	21.2 ± 0.9 ab	12.0 ± 0.3 ef	0.3 ± 0.0 de	41.8 ± 0.6 cd	10.1	47.1	97.2
RR2	9.3 ± 0.1 e	0.2 ± 0.0 bc	44.4 ± 14.0 a	10.5 ± 0.0 g	0.2 ± 0.0 de	48.0 ± 2.1 abcd	12.9	50.0	8.1
Sumac	11.1 ± 0.2 cd	0.1 ± 0.0 c	11.1 ± 1.8 b	12.1 ± 0.2 ef	0.2 ± 0.0 e	20.5 ± 1.2 e	9.0	50.0	84.7
HTW 1	12.4 ± 0.1 bc	0.2 ± 0.0 bc	27.6 ± 4.7 ab	13.4 ± 0.1 cd	0.3 ± 0.0 cde	49.6 ± 3.2 abcd	8.1	70.6	79.7
HTW 2	13.5 ± 0.2 ab	0.2 ± 0.0 bc	27.7 ± 5.0 ab	14.2 ± 0.0 bc	0.3 ± 0.0 cde	46.7 ± 3.4 abcd	5.2	31.8	68.6
Waxy1	14.3 ± 0.0 a	0.3 ± 0.0 a	44.4 ± 5.4 a	15.6 ± 0.4 a	0.5 ± 0.0 ab	61.7 ± 2.0 a	9.1	43.8	39.0
Waxy2	12.5 ± 0.3 bc	0.2 ± 0.0 b	41.3 ± 2.8 a	13.0 ± 0.2 de	0.4 ± 0.0 bc	46.6 ± 1.7 abcd	4.0	60.9	12.8
HD1	13.2 ± 0.3 ab	0.3 ± 0.0 a	41.0 ± 6.4 a	14.9 ± 0.0 ab	0.5 ± 0.0 a	58.4 ± 1.6 ab	12.9	47.1	42.4
HD2	12.4 ± 0.1 bc	0.2 ± 0.0 bc	47.0 ± 4.9 a	14.1 ± 0.0 bc	0.4 ± 0.0 c	52.7 ± 1.2 abc	13.7	52.2	12.1
WR1	14.5 ± 0.2 a	0.2 ± 0.0 bc	30.3 ± 1.4 ab	14.9 ± 0.3 ab	0.3 ± 0.0 cd	46.5 ± 2.4 bcd	2.8	47.6	53.5
WR2	12.9 ± 0.3 bc	0.2 ± 0.0 bc	37.7 ± 1.4 ab	12.9 ± 0.1 de	0.3 ± 0.0 de	34.4 ± 10.5 d	0.0	19.0	-8.8
WR3	10.3 ± 0.1 de	0.2 ± 0.0 bc	31.9 ± 5.8 ab	11.5 ± 0.0 fg	0.3 ± 0.0 cd	46.3 ± 3.4 bcd	11.7	72.2	45.1

[1] All values are expressed on dry matter basis and are the average of at least three replicas ± standard error of the mean. Means with different letter(s) within column are statistically different ($P<0.05$).

5.4.2.3. PROTEIN DIGESTIBILITY (PD)

PD is the sorghum susceptibility to proteolysis using pepsin at low pH. Table 5.2 summarizes PD results of the set of sorghum samples. The genotype with the lowest initial PD was *Sumac* with 11.1% and the highest *RR2* with 44.4%. The low value obtained for *Sumac,* could be associated to the interference mechanism of tannins in enzymatic activity, as described for RS (subsections 5.4.1.3. and 5.4.1.4.) . Moreover, the other values are lower compared to previous results for sorghum (without cooking) reported in the literature. Aboubacar et al. (2001) evaluated 48 sorghum lines and obtained PD of 56.1 to 91.0% of samples containing original crude protein content values between 7.8 and 15.7%. Weaver et al. (1998), described for normal and high lysine sorghum lines, ranges of PD between 65.8 to 80.2% and 79.8 to 80.7% respectively. These higher results compared with the depicted in Table 5.2 could be related to the intrinsic differences among sorghum cultivars. Maclean et al. (1981) for example, reported for sorghum gruels produced from tannin-free specimens, a PD of 46%, while rice, maize and wheat gave values of 66, 73 and 81% respectively. This value is closer to the depicted in Table 5.2 and denotes the lower PD of sorghum regarding other cereals. Millet, a small grain from the same botanical family, is also similar in terms of PD to sorghum. Pushparaj and Urooj (2011) reported in raw pearl millet flour PD values between 45.5 – 49.3 %. This digestibility was not increased by wet heat treatments as boiling and pressure cooking, but roasting and germination improved the *in vitro* PD values (Dicko et al., 2006b).

After confinement (Table 5.2), protein digestibility increased in more than 40% in all cultivars. This result is related to the breakage of the protein matrix and bodies surrounding the starch granules in sorghum and differs from studies with other pest as *Tribolium castaneum.* Pant and Susheela (1977) studied sorghum infestation with red flour beetle and concluded that protein quality was not affected with infestation level under 30%, but it was damaged at higher levels. Jood and Kapoor (1992), using *Trogoderma granarium* Everts and *Rhizopertha dominica* Fabricius in separated and mixed populations on wheat, maize and sorghum concluded that protein digestibility was reduced in grain infestation levels of 25, 50 and 75% (an insect content higher compared to the evaluated herein with *S. zeamais*). *T. granarium,* a germ feeder, reduced protein digestibility more than an endosperm feeder as *Rhizopertha dominica*. According to the authors, the reduction in digestibility was "dependent on the distribution of proteins in seed components

as well as feeding preferences of insects". Being the sorghum germ a portion rich in high digestible proteins would be expected (with a germ-feeder insect) the overall reduction of protein digestibility because of the proportional increase of the less digestible protein associated to the endosperm. The opposite for an endosperm-feeder as *S. zeamais*, because in order to access starch must affect the less digestible protein that glues starch granules, yielding small peptides (FAN) increasing thus pepsin digestibility values.

5.4.3. Correlations Among Starch and Protein Fractions Remained in Samples After Confinement

In Table 5.3 correlations among final starch and protein fractions are depicted. Coefficients indicate that the highest the protein content the lowest the starch (Figure 5.1). Furthermore, the protein damage within sorghum cultivars (FAN) yielded an increase in Protein Digestibility and reduced Resistant Starch. These results describe the intimate relationship of protein and starch within the sorghum endosperm. Furthermore, RS was not correlated with Total Starch at the end of confinement, but negatively to Protein Digestibility and FAN (Figure 5.1) indicating clearly that resistance mechanism in sorghum starch is linked to the integrity of proteins within the endosperm.

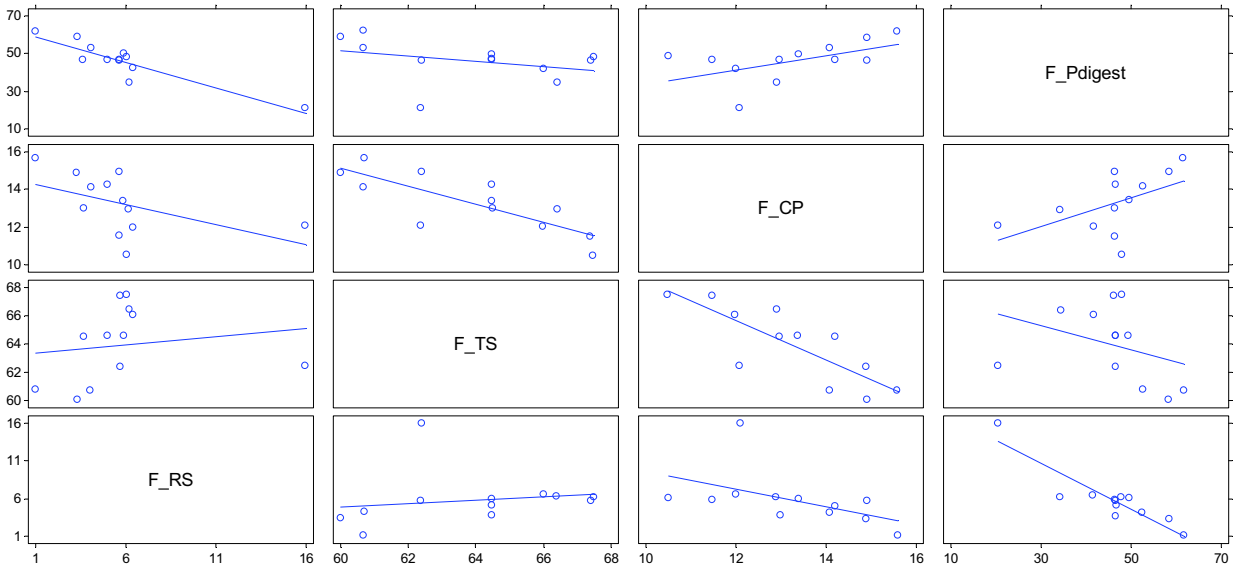


Figure 5.1. Scatter plots for Resistant Starch (F_RS), Total Starch (F_TS), Crude Protein (F_CP) and Protein Digestibility (F_Pdigest), depicting the correlation among these variables.

All final parameters were positively and significant correlated ($P<0.01$ and $P<0.05$ for TS) to its initial content in sorghum caryopsis, thus the higher the protein and starch contents at the beginning of confinement test the higher the final contents. Moreover, final damaged starch was correlated to endosperm texture and percentage of kernel abraded in TADD, as well as to TKW. The softer endosperm kernels loss more material during TADD and with low TKW (small) showed the lowest DS at the end of confinement. This result is associated to the previous commented correlation among DS and kernel hardness (subsection 5.4.1.2.).

5.5. CONCLUSIONS

Starch and protein characteristics of sorghum were evaluated facing a 45-day storage period with *S. zeamais*. Maize weevil in sorghum depicted endosperm-feeding characteristics, lowering TS, reducing RS and increasing DS. Average total starch loss during confinement was 2.3%, being *Waxy2, HD2, RR2* and *Sumac* the most damaged cultivars, with a starch reduction between 7.2 to 8.4%. Damaged starch increased during confinement (1.6 to 1.8%) and was correlated with TADD and TKW. Thus, softer and smaller grains had the lowest damaged starch. Resistant starch dropped 2.3% whereas amylose increased 2.8% in average, a signal of maize weevil preference for amylopectin. RS quantification was affected by the tannin content present in samples (*Sumac*). The activity of *S. zeamais* on the starch affected also the protein portion. FAN and PD increased (50% and 44.5% average regarding initial content), depicting changes in the protein structure. CP also increased (average of 8.3%), highlighting again that *S. zeamais* had preference for the starch. Protein modifications enhanced total and resistant starch consumption (mainly amylopectin fraction) during confinement. These results showed the close relationship among protein and starch into sorghum endosperm and the influence of *S. zeamais* into these components. Once susceptibility indicators for sorghum cultivars were obtained and the effect of *S. zeamais* within the endosperm was evaluated, the ethanol production performance for the same sorghum varieties was also tested. Results are described in the next and last thesis chapter. Emphasis was placed on common physical, chemical and nutraceutical traits among sorghum fermentation efficiency and susceptibility to maize weevil.

Table 5.3. Correlations among content of starch and protein fractions after confinement with *S. zeamais* and original physicochemical properties of the evaluated sorghum cultivars.

	F _ AAN	F _ PD	F_R Starch	F_ T Starch	Ph _ AAN	Ph_ Amy	Ph _ Ash	Ph_BP	Ph_ CI	Ph_ PD	Ph _ ET	Ph _ FI	Ph _ FP	Ph_CP	Ph _ RStarch	Ph_ T Starch	Ph _ TADD	Ph _ TKW	Ph _ TW	Ph_TD	Ph_Vol
F_Amy	ns	ns	ns	ns	ns	0.92°	-0.65°	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
F_CP	0.74°	ns	ns	-0.81°	0.78°	-0.66*	0.80°	ns	ns	ns	ns	ns	ns	0.95°	ns	-0.78°	ns	ns	ns	-0.65*	ns
F_DStarch	ns	ns	ns	ns	ns	ns	ns	ns	-0.59*	ns	-0.66*	ns	ns	ns	ns	ns	-0.77°	0.68*	ns	ns	ns
F_AAN		0.75°	-0.74°	-0.66*	0.94°	-0.65*	0.63*	ns	ns	0.58*	0.68*	0.74°	ns	0.60*	-0.59*	-0.72°	ns	ns	-0.74°	-0.75°	0.59*
F_ PD			-0.91°	ns	0.72°	ns	ns	-0.65*	-0.65*	0.73°	ns	ns	-0.75°	ns	-0.86°	ns	ns	0.66*	-0.58*	ns	0.63*
F_RStarch				ns	-0.70*	ns	ns	0.83°	0.79°	-0.79°	ns	ns	0.90°	ns	0.95°	ns	ns	-0.78°	ns	ns	-0.69*
F_TStarch					-0.67*	ns	-0.78°	ns	ns	ns	-0.72°	-0.65*	ns	-0.65*	ns	0.68*	ns	ns	0.69*	0.75°	ns

[1]Correlation coefficients with an asterisk (*) represent *Pvalues* <0.05 and with (°) *Pvalues*<0.01. (F_) stands for final results (after 45 days of confinement test using *S. zeamais* as infestation insect); (Ph_) stands for a physicochemical trait of sorghums evaluated at the beginning of confinement test. AAN, Alpha Amino Nitrogen or Free Amino Nitrogen; Amy, Amylose; BP, Bound Phenolics; CI, Color Index; CP, Crude Protein; DSTarch, Damaged Starch; ET, EndospermTexture; FI, Flotation Index; FP, Free Phenolics; PD, Protein Digestibility using pepsin; RStarch, Resistant Starch; TADD, Tangential Abrasive Dehulling Device; TD, True Density; TKW, Test Kernel Weight; TSTarch, Total Starch; Vol, Volume.

6. COMMON CHARACTERISTICS OF SORGHUM GRAIN INVOLVED IN FUEL ETHANOL PRODUCTION AND ITS RESISTANCE TO MAIZE WEEVIL (*SITOPHILUS ZEAMAIS*).

6.1. ABSTRACT

Sorghum grain has good potential as feedstock for fuel ethanol, but some of the best cultivars for yeast fermentation also show low resistance to insects during storage. Therefore, the study of the characteristics affecting both ethanol productivity and pest susceptibility is desired. Twelve cultivars were selected for ethanol production using dry-grind procedure (two red sorghums, one high-tannin, two white heterowaxy, two white waxy, two white high digestible and three white with regular endosperm). During liquefaction all cultivars reached 41.7 to 53.7% of theoretical reducing sugars, except the high- tannin (38.7%) genotype. Both red sorghums were the highest producers of reducing sugars. The best fermentation efficiency was observed for the red sorghum (RR1) and a high digestible white cultivar (89%) and the lowest for the high-tannin (79%). This trait was highly correlated to performance and productivity during liquefaction or hydrolysis with thermoresistant α-amylase. Reducing sugars were positively correlated to the amount of endosperm and negatively to protein content. A significant correlation between susceptibility to *S. zeamais* and liquefaction was observed, being the highest sugar-producing sorghums the most resistant to maize weevils. The amount of reducing sugars obtained after liquefaction was a good and quick indicator of the fermentation efficiency. The evaluation of a wider sample set of sorghums is recommended. This set should include sorghums with large endosperm and soft texture.

6.2. INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is a crop from *Poaceae* family widely adapted to dry environments, with a good yield in areas where water and nutrients are scarce (Chanapamokkhot and Thongngam, 2007; Kim and Day, 2010). Sorghum grain is a naked caryopsis, spherical and slightly flat in one side, with general dimensions of 3.0 to 5.0 mm length, 2.5 mm wide and 1.2 mm thickness (Gélinas and McKinnon, 2000). One half to three quarters of its weight is composed mainly by starch and the rest of protein, structural carbohydrates and fat. This cereal ranks fifth in the most produced worldwide (55.7 million tons), being the United States of America the main harvester followed by Mexico and India (9.0, 6.9 and 6.7 million ton respectively) (FAOSTAT, 2012). Most of this production is employed for human and feed consumption, but also as construction material (its stems) and for general industrial purposes, as starch production. In Mexico, 99% of sorghum is used for feeding and the remaining 1% as seedlings (FR, 2011). One special use of sorghum in the United States is fuel ethanol production (around 3 to 5% of total volume is obtained from this cereal). In that country, ethanol as a transportation fuel has an increasing tendency driven mainly by energy regulation (Renewable Fuel Standard) that urges substitution of fossil fuel from renewable sources. The goal of the US government is to produce 36,000 million gallons of ethanol from renewable sources by 2022. In Mexico, the *Ley de Promoción y Desarrollo de Bioenergéticos*, determine the substitution of MTBE (Methyl Terbutyl Ether) as gasoline oxygenate starting 2011. In order to meet this law, at least 74.6 million gallons of ethanol are required. In Mexico, where the use of maize for fuel industrial production is restricted and in practical terms prohibited, sorghum represents a good option, primarily because allows the use of a mature well known technology, instead of the implementation of immature and unavailable processes to convert lignocellulose feedstocks into second-generation ethanol. After reviewing the factors that restricts the use of sorghum for ethanol production in the United States, is clear that some special features of its protein (hydrophobicity and cross-linking tendency), as well as phenolics (mainly tannin content) associated to the pericarp and testa of some cultivars, amylose content and association between amylose and lipids, differentiate the sorghum from maize hydrolysis and fermentation. Previous work has already been done using sorghum as source for ethanol (Chuck-Hernández et al., 2009; Chuck-Hernández et al., 2012a; Chuck-Hernández et al., 2012b; Perez-Carrillo et al.,

2008; Wang et al., 2008; Wu et al., 2007; Yan et al., 2009; Yan et al., 2010; Zhang and Hamaker, 1998; Zhao et al., 2008; Zhao et al., 2009) and some characteristics within sorghum cultivars as well as process recommendations has been described to upgrade and optimize production: no-tannin cultivars, low amylose, high protein digestibility, mechanical decortication, steam flaking, germination and use of proteases and last generation amylases. Nevertheless some of these characteristics, mainly tannin content and protein digestibility, have been empirically associated to a higher susceptibility to insects during storage (Rooney, 2003).

According to Ramputh et al. (1999), cereal postharvest losses in small-farm tropical agriculture usually exceeds 30% and according to García-Lara and Bergvinson (2007), the range of worldwide postharvest losses in subsistence farming is between 10 to 40%. One of the main biotic factors associated to losses during postharvest are insect such as weevils (Teetes and Pendleton, 2000). *S. zeamais* is the main pest in tropical and subtropical regions and also the most harmful (Abebe et al., 2009; García-Lara and Bergvinson, 2007). The use of postharvest damaged sorghum and maize for ethanol production has been already evaluated (Chuck-Hernández et al., 2012a), yielding no differences in fermentation efficiency but in total ethanol production derived from starch losses due to mold and insect infestation. The use of damaged sorghum is then feasible, but not ideal, so the best material for sorghum producers and processors should be a kernel with good fermentation performance, but also with good resistance during storage.

Therefore, the objectives of this study were to evaluate fermentation performance of different sorghum cultivars following a dry-grind process and to obtain the common traits underlying ethanol productivity and sorghum resistance during infestation with *Sitophilus zeamais.*

6.3. MATERIALS AND METHODS

6.3.1. GRAIN VARIETIES.

Twelve sorghum cultivars were selected based on their color and endosperm characteristics. Genotypes used in this investigation were: 1) two red sorghums, commonly used as feed in the northern part of Mexico (*RR1* and *RR2*); 2) one high-tannin sorghum (*Sumac*); 3) nine white cultivars (two heterowaxy –*HTW1, HTW2*-, two waxy –*Waxy1, Waxy2*-, two described as high protein digestible –*HD1, HD2*- and three regular –*WR1, WR2, WR3*-). Red and high-tannin sorghum samples were commercially available material in Northern Mexico and possessed an

intermediate to soft endosperm texture. White samples were kindly donated by Dr. Dirk Hays of the Texas A&M University Sorghum Breeding Program.

6.3.2. Sample Preparation.

Kernel samples, not previously treated with insecticides, were cleaned by air aspiration and sieves. The moisture was adjusted to 13% using the formula described by Serna-Saldívar (2012): ([(100 - % Initial Moisture)/(100 – % Final Moisture)] – 1] * Sample Weight) and allowed to equilibrate for at least 7 days at 27 ± 1°C and 70 ± 5% RH using a saturated NaCl solution. For ethanol production as well as physical, chemical, phenolics and antioxidant characterization, the sorghum kernels were milled using a coffee mill (Krups, Model GX410011, Mexico) and stored at 4°C prior to use. Whole kernels were used for susceptibility tests.

6.3.3. Susceptibility Tests.

Insect Culture, Confinement and Dobie Index (DI) assays were performed according previously described in Chapter 4.

6.3.4. Ethanol Production.

6.3.4.1. Liquefaction.

Ground meals (15 g dry basis) were mixed with distilled water to obtain mashes with 30% (wt/vol) solids. pH was initially adjusted to 5.6 with 0.1N HCl and temperature was increased to 95°C in a water bath (Boekel Grant VSP, Pennsylvania, USA). When slurries reached 90°C, 50µL of Liquozyme (240 KNU-S/g, Novozymes, Bagsvaerd, Denmark) was added. After 25 min a second Liquozyme dose (50µL) was applied. Mashes were mixed carefully with a metallic spatula and maintained at 95°C during 200 min. In order to determine the progressive extent of starch hydrolysis, aliquots were taken before first enzyme addition, and after 100 and 200 min hydrolysis.

6.3.4.2. Simultaneous Saccharification and Fermentation (SSF).

Mashes were cool down from 80°C to 30°C, adjusted to 15°Plato and pasteurized (65°C for 30 min) in a water bath (Boekel Grant VSP, Pennsylvania, USA). A commercial mixture (Dextrozyme DX 2X, Novozymes) of glucoamylase (EC 3.2.1.3) and pullulanase (EC 3.2.1.14) produced from genetically modified strains of *Bacillus subtilis* and *Aspergillus niger* was added

altogether with dry yeast (*Saccharomyces cerevisiae*) (Nevada, Safmex, Toluca, Mexico). Dextrozyme was added at a rate of 1 mL/100mL of liquefied slurry whereas the yeast was previously suspended in sterile water and pitched (1.5 x 10^7cells/mL) according to Chuck-Hernández et al. (2009). Reaction vessels were sealed and maintained for 72 h in an incubator-shaker (VWR International, Model RF1575) set at 30°C with agitation (100 rpm) during the first 24 h.

6.3.5. Sugars and Ethanol Determination.

Total reducing sugars (RdS) during liquefaction were determined with the Dinitrosalicylic Acid Method (DNS) of Miller (1959). Maltotriose, maltose, glucose, fructose, ethanol and glycerol were quantified throughout 72 h SSF by HPLC-IR (Waters 2114, Milford, MA) furnished with an ion-exchange column (Aminex HPX-87H, Bio-Rad®, Hercules, CA) at 60°C using 5 mM sulfuric acid at a rate of 0.6 mL/min as mobile phase (Chuck-Hernández et al., 2009).

6.3.6. Physical and Chemical Characteristics.

Physical characteristics, as previously described in chapter 4, were determined using standard procedures. Briefly: test weight (TW) according to Official US Grain Standard Procedures (AACC Method 55-10); thousand-kernel weight (TKW) by weighing 100 randomly selected whole kernels, and endosperm texture (ET) according to the subjective procedure previously reported by Chuck-Hernández et al. (2009). Flotation index (FI) was determined according to Salinas et al. (1992) and Munck (1995) and expressed as a percentage of floating kernels on an aqueous solution of sodium nitrate (1.25 g/cm^3 specific weight at 35°C). A pycnometer was used to obtain True Density (TD), whereas the Tangential Abrasive Dehulling Device (TADD) was employed to determine the percentage of kernel removed as an indicator for hardness. Kernel size was measured with a digital micrometer (Mitutoyo, Model MDC-1, Japan). Volume in mm^3 was calculated using the volume formula for an ellipsoid ($4/3^* \pi * R^2*r$). R was the half of kernel´s length and r= Thickness/2. L*, a*, b*, and other CIE color parameters of ground samples were determined using a colorimeter (Minolta CR-300, Osaka, Japan) and Color Index (CI) was estimated with the formula reported by Vignoni et al. (2006) (a*1000/L*b). Tip cap, germ, pericarp and endosperm were obtained from dissected kernels, previously soaked for 2 minutes in water according to Gutiérrez-Uribe et al. (2010).

Regarding chemical characteristics: Crude Protein (N*6.25) was determined using the micro-Kjeldahl method 46-13 (AACC, 2000) and Free Amino Nitrogen (FAN) with the ninhydrin procedure 945.30L (AOAC, 1980). Total and resistant starch and amylose were determined using enzymatic tests TStarch –AOAC 996.11-, RStarch –AOAC 2002.02- and Amylose/Amylopectin -K-AMYL- procedures respectively (Megazyme International, Ireland). Ash was assayed according to method 08-01 (AACC, 2000) and protein digestibility using the pepsin procedure described by Hamaker et al. (1987) and Axtell et al. (1981) with slightly modifications as briefly described: ground samples (100 mg) were suspended in 17.5 ml of a buffer containing pepsin (1.5 g pepsin –P7000, Sigma Aldrich- per liter of 0.1 M KH_2PO_4 buffer, pH 2.0). Samples were incubated in a shaking water bath (37°C) altogether with blanks (enzyme and buffer mix). After two hours, 1 mL NaOH 2M was added, samples vortexed and centrifuged (10,000xg, 15 min, 4°C). Supernatant was analyzed using the micro-Kjeldahl method 46-13 (AACC, 2000) to obtain solubilized nitrogen. Protein digestibility was calculated as (nitrogen in sample supernatant – nitrogen in blank) / total nitrogen of the sample * 100.

6.3.7. Extraction and Determination of Free and Bound Phenolics and Antioxidant Capacity (AOXC).

Free and bound phenolics as well as antioxidant capacity was performed as previously described in Chapter 4.

6.3.8. Statistical Analysis

All determinations were performed in triplicated and data analyzed with Analysis of Variance (ANOVA) using the statistical software Statistix 9. Residual analysis was performed in order to review the compliance of statistical assumptions (normality, independence and homoscedasticity). When means resulted different (null hypothesis rejected, $Pvalue<0.05$), differences among means were evaluated using Tukey HSD Tests with $alpha$=0.05. Homogeneous mean´s groups were indicated with the same alphabetic sub-indices within tables. A table with Pearson correlation coefficients and Principal Component Analysis (PCA) were obtained using the same statistical software.

6.4. RESULTS AND DISCUSSION

6.4.1. PHYSICAL AND CHEMICAL ANALYSIS

The main physical and chemical characteristics of the twelve sorghum genotypes used in this research are depicted in Table 6.1. These parameters are related to ethanol production (v.gr. total starch, TS), but also with endosperm components and its association, for example: amylose (AMY), crude protein (CP) and endosperm texture (ET). In sorghum, these traits are close related to each other and all have an impact in ethanol production (Wu et al., 2007). ET indicates the ratio among floury/corneous endosperm portions, where the highest value (5) describes a totally floury or chalky endosperm and the lowest value (1) a totally vitreous (Table 6.1). Both floury and vitreous areas are composed by starch granules, cell walls, protein matrix and protein bodies. Starch granules are the principal components within the endosperm cell and are attached to the protein matrix (Serna-Saldivar, 2010). Thus, differences amid sorghum cultivars can be linked to the arrangement of each of the previous listed endosperm components: the vitreous section has a close and tight union between starch granules and the protein matrix (without air spaces), being a region with angular starch granules, while the floury section (usually the kernel central part) contains larger starch granules and less angular in shape because of the weaker association with the protein matrix. The chalky appearance is related to the smaller air spaces or voids present in the protein matrix (Doudu et al., 2003). The lowest and highest value for ET was obtained in cultivars *HTW1* and *Waxy1,* respectively (Table 6.1). These cultivars in turn can be described as intermediate and totally floury. ET is usually correlated to the grain density and protein content (Wu et al., 2008), the higher the protein, the more vitreous the endosperm. Besides, starch and protein in sorghum are typically inversely related and both influences ethanol productivity (Lacerenza et al., 2008; Zhan et al., 2003). Thus, bio-refineries prefer sorghums with soft endosperm (in this case with high ET) and low protein, because these kernels usually contain a higher proportion of starch, but also because of the smaller particle size after milling (compared with hard grains).

Following the ET analysis, Crude Protein (CP) and Total Starch (TS) were directly quantified and are depicted in Table 6.1. CP stands for the total nitrogen in sample converted to protein, assuming 16% of nitrogen weight in protein (FAO, 2003). Protein results were in the range of 9.3 to 14.5% for *RR2* and *WR1* respectively, in agreement to previous reports for sorghum used for

ethanol production (7.56 to 16.35 %, Wu et al., 2008). However, compared to other cereals as maize (8.1 to 11.5%), the CP range was wider to the regularly reported (Serna-Saldivar, 2010). According to Taylor and Belton (2002), protein content is one of the most variable chemical traits in sorghum and is linked to genotype and agronomical characteristics. Total Starch (TS), in sorghum is usually observed within 64 to 74% (Wu et al., 2007) and, as previously stated, is inversely related to CP (Zhan et al., 2003). In this case, both parameters (TS and CP), had indeed a negative and significant correlation (Table 6.4). The highest the protein content, the lowest starch percentage, indicating *a priori*, that the best cultivars for ethanol production would be the highest in starch and the lower in protein. The highest TS percentage was assayed in the *RR2* genotype with 73.5% and the lowest in the *HD1* (59.5%) counterpart, followed by the *Waxy1* with 62.7%. *HD1* TS is lower that the reported by Wu et al. (2008 and 2010), whereas the higher percentage is within the sorghum typical values (74.8% and 73.5%) reported by other authors. Is then expected that *HD1* (in general terms) would have a low ethanol yield compared with the rest of the genotypes, because of the low starch content.

Table 6.1 also depicts amylose contents. Starch is a polymer formed by glucose linked by glycosidic bonds and is composed of two kinds of molecules: amylose and amylopectin, being the former a linear chain linked mainly by *alpha*-1,4 bonds and the latter a branched molecule with *alpha*-1,6 linkages (Wu et al., 2006). Regular endosperm cereals contain from 23 to 30% amylose, waxy up to 5% whereas heterowaxy around 12%. Heterowaxy genotypes have a waxy and a non-waxy parent, yielding thus a heterozygous endosperm. In order to obtain a completely waxy sorghum, the triploid endosperm must be homozygous recessive (Serna-Saldívar and Rooney, 1995); these genotypes contain starch granules more susceptible to enzymatic hydrolysis compared with the non-waxy counterparts (Rooney and Pflugfelder, 1986). Amylose content in sorghum cultivars evaluated herein ranged from 4.9 to 32.7% for *Waxy1* and *RR1* respectively. According to results (Table 6.1), all cultivars can be grouped as: waxy (*Waxy1* and *Waxy2*), heterowaxy (*HTW1* and *HTW2*) and regular (the rest of the set). Thus, some differences in ethanol production can be expected, mainly because of the differences in substrate content (TS), type of starch (AMY), protein content and endosperm texture.

Table 6.1. Main physical and chemical characteristics of sorghum used for ethanol production [1]

Variety	Color	ET[2]	CP[3]	TS[3]	AMY[3]
			%	%	%
RR1	Red	3.5 ± 0.4 bc	10.90 ± 0.20 cd	66.62 ± 1.89 ab	32.69 ± 1.81 a
RR2	Red	2.7 ± 0.3 cd	9.30 ± 0.10 e	73.55 ± 1.99 a	31.82 ± 1.36 a
Sumac	Brown	3.2 ± 0.3 bcd	11.10 ± 0.20 cd	68.05 ± 0.58 ab	27.11 ± 2.82 ab
HTW1	White	2.3 ± 0.2 d	12.40 ± 0.10 bc	63.92 ± 1.42 ab	18.81 ± 0.14 cd
HTW2	White	2.7 ± 0.2 cd	13.50 ± 0.20 ab	65.74 ± 1.15 ab	12.32 ± 1.74 de
Waxy1	White	5.0 ± 0.0 a	14.30 ± 0.00 a	62.75 ± 0.55 b	4.95 ± 1.07 e
Waxy2	White	2.5 ± 0.0 cd	12.50 ± 0.30 bc	69.49 ± 0.91 ab	9.62 ± 1.52 e
HD1	White	4.1 ± 0.5 ab	13.20 ± 0.30 ab	59.57 ± 2.66 b	20.41 ± 1.79 bc
HD2	White	4.3 ± 0.3 ab	12.40 ± 0.10 bc	65.40 ± 2.22 ab	23.49 ± 0.81 bc
WR1	White	2.6 ± 0.1 cd	14.50 ± 0.20 a	65.99 ± 0.69 ab	25.33 ± 1.01 abc
WR2	White	2.5 ± 0.0 cd	12.90 ± 0.30 bc	66.54 ± 2.12 ab	25.75 ± 2.30 abc
WR3	White	2.5 ± 0.0 cd	10.30 ± 0.10 de	66.82 ± 4.10 ab	26.05 ± 0.38 abc

[1]All results are the average of at least three replicas ± standard error of the mean. Values with different letter within columns were statistically different (P<0.05). [2] Endosperm Texture (ET), subjectively determined by viewing the ratio of soft to hard endosperm on dissected kernels, 1=totally vitreous or hard and 5= totally soft or chalky. [3] Crude Protein (CP), Total Starch (TS) and Amylose (AMY) are calculated in dry basis.

6.4.2. Reducing Sugars (RdS) During Liquefaction

Results for RdS during the liquefaction are depicted in Table 6.2. RdS are carbohydrates with a free carbonyl group that can act as a mild reducing agent (Wrolstad, 2012). Examples of RdS are glucose and common monosaccharides as well as disaccharides (maltose), fermentable by *S. cerevisiae*. Initial RdS (Table 6.2), was statistically different in varieties and according with these results, three main groups can be separated within sorghum set: 1) waxy, heterowaxy and high digestible cultivars (RdS around 13g/L or 4.3 g per 100g initial flour); 2) white sorghums (10 to

11 g/L or 3.3 to 3.7 g per 100 g initial flour) and 3) red and high tannin or brown sorghums: *RR1, RR2* and *Sumac* (6 to 7 g/L or 2.0 to 2.3 g RdS per 100 g initial flour). These results describe original differences among sorghum kernels.

Table 6.2. Reducing sugars [RdS] (g/L) at 0, 100 and 200 minutes of liquefaction and ratio between final RdS and total theoretical glucose based on initial starch of sample used[1]

Variety	Hydrolysis time (min) RdS (g/L)			*k* for RdS production ($g\ L^{-1}\ min^{-1}$)	RdS at the end of liquefaction / theoretical glucose (%)
	0	100	200		
RR1	6.9 ± 0.6 bc	150.8 ± 13.1 a	148.8 ± 5.5 a	1.44	53.7 ± 1.2 a
RR2	6.1 ± 1.8 c	155.8 ± 10.3 a	150.4 ± 4.3 a	1.50	49.1 ± 0.8 ab
Sumac	6.3 ± 0.5 c	110.1 ± 18.2 b	109.7 ± 5.3 b	1.04	38.7 ± 1.1 b
HTW1	12.6 ± 2.2 a	139.5 ± 11.0 ab	138.0 ± 6.5 ab	1.27	51.9 ± 1.4 ab
HTW2	13.9 ± 2.1 a	130.7 ± 12.1 ab	125.0 ± 20.1 ab	1.17	45.7 ± 4.3 ab
Waxy1	14.5 ± 1.6 a	109.7 ± 20.0 b	110.5 ± 19.8 b	0.95	42.3 ± 4.4 ab
Waxy2	14.9 ± 2.1 a	123.9 ± 16.4 ab	120.6 ± 11.1 ab	1.09	41.7 ± 2.2 ab
HD1	12.6 ± 2.2 a	135.1 ± 20.6 ab	133.7 ± 14.0 ab	1.23	53.9 ± 3.3 a
HD2	11.8 ± 1.5 ab	122.0 ± 1.8 ab	127.1 ± 10.0 ab	1.10	46.7 ± 2.1 ab
WR1	11.5 ± 1 abc	124.1 ± 2.1 ab	124.7 ± 10.6 ab	1.13	45.4 ± 2.2 ab
WR2	10.6 ± 2.6 abc	123.6 ± 6.3 ab	124.7 ± 12.4 ab	1.13	45.0 ± 2.6 ab
WR3	11.2 ± 2.4 abc	129.0 ± 10.0 ab	140.2 ± 18.3 ab	1.18	50.4 ± 3.8 ab

[1]All values are the average of at least three replicas ± standard error of the mean. Means with different letter(s) within columns were statistically different ($P<0.05$).

The simplest carbohydrates detected in cereals are: fructose, glucose and sucrose in a concentration around 2%. Sorghum´s soluble sugars in particular, ranges between 2.2 to 3.8%, 0.9-2.5% free reducing sugars and 1.3 to 1.4% non-reducing sugars, most of them are located in the germ (Serna-Saldivar, 2010; Serna-Saldivar and Rooney, 1995). According to Anglani (1998) the primary sugars in sorghum grain are fructose, glucose, maltose, sucrose and raffinose and all, except fructose and glucose, are below 1.0% (Watson and Hirata, 1960). Regarding sugar content of sorghum samples before liquefaction (Table 6.2), this concentration is clearly linked to variety and also could be related to maturity or growth stage of the grain and intrinsic enzymatic activity. Some cultivars, such as high lysine (high digestible) and sugary, contain more soluble sugars than regular endosperm sorghums, and this concentration decreases during grain maturation (Anglani, 1998; Serna-Saldivar and Rooney, 1995). For example, a sorghum cultivar reported by Murty et al. (1985), 22 days before anthesis contained 5.2% soluble sugars, whereas at physiological maturity only 2.3%.

After 100 min of liquefaction (Table 6.2), a different arrangement within samples could be observed. Genotypes with the highest RdS concentration were *RR1* and *RR2* (150 to 155 g/L or 50 g RdS per 100g flour) and the lowest *Sumac* and *Waxy1* (110 g/L around 37 g RdS per 100g flour). These results seems to be linked to TS content but mainly to the amount of endosperm ($r=0.81$, $P<0.01$, Table 6.4), indicating the importance of this anatomical parameter in hydrolysis efficiency as well as in other industrial process, like wet and dry milling operations (Lee et al, 2002; Serna-Saldivar, 2010). Usually sorghum cultivars with high endosperm also contained the highest starch content (Munck, 1995; Serna-Saldivar, 2010), but herein a significant correlation among both factors was not observed (Table 6.4).

During liquefaction, RdS in almost all samples was enough to reach around 50% of theoretical from initial starch (Table 6.2). Dextrose Equivalents (DE) obtained during liquefaction is determined by process conditions, as well as enzyme efficiency and dosage (Novozymes, without date). According to Olsen (2002), a DE of about 40 could be obtained at the end of a regular liquefaction. DE is inversely related to the degree of polymerization, thus is an indicator of the hydrolysis extent: glucose has a DE value of 100 and intact starch, an effective DE of zero (Sun et al., 2010). Almost all results at the end of liquefaction (Table 6.2) were higher than the 40% described by Olsen (2002), except for *Sumac* (39%). RdS result depicted by the high tannin

cultivar was statistically different from the most efficient treatments: *RR1* and *HD1* (54%). This low RdS shows the detrimental effect of tannin in *alpha* amylase activity, as previously described by Wang et al. (2008) and Yan et al. (2011). Sorghum tannins retard liquefaction, yielding a mash with high-viscosity (Wu et al., 2007). According to Yan et al. (2011), tannins could enhance sorghum protein cross-linking during heating, retarding starch hydrolysis or enzymatic degradation, nevertheless because of the ability of tannins to bind proteins (around 12 times its own weight), the formation of indigestible protein-tannin complexes is the most accepted explanation for the reduced *alpha* amylase activity (Duodu et al., 2003; Wang et al., 2008; Wu et al., 2007), being thus a direct detrimental effect in enzymes rather than non-accessible starch.

A rough velocity hydrolysis parameter is depicted in Table 6.2 (velocity constant *k,* as the slope among time 0 and 100, assuming a straight line). The lowest values were observed for *Waxy1* and *Sumac*, intermediate rates for all white cultivars (a rate of change of 1.10) and the highest for the *RR1* and *RR2*genotypes (>1.40). From liquefaction time 100 to 200 min, RdS concentration remained practically the same. *RR1* and *RR2* were the highest in terms of RdS while *Waxy1* and *Sumac* the lowest, as in t100. Thus the rate previously described was not useful to evaluate differences in starch susceptibility, given that at time 100 practically all the possible starch hydrolysis was reached.

6.4.3. Ethanol Production (TEthanol), Fermentation Efficiency (FF) and Yield

Table 6.3 summarizes results for fermentation and ethanol production. There were no significant differences among treatments in terms of Total Ethanol (TEthanol), glycerol and residual sugars. Glycerol is a major by-product in alcohol fermentation, playing an important role in redox balance, osmotic regulation and thermal protection of *S. cerevisiae*, the most important glycerol producing yeast (Bideaux et al., 2006; Brumm and Hebeda, 1988). Elevated glycerol concentrations are detected in fermentations under an osmotic stress conditions and some authors indicate that besides compositional changes in fermentation media, the yeast strain is the major factor influencing glycerol production (Attfield and Kletsas, 2000; Vriesekroop et al., 2009). In bio-refineries is important to maintain glycerol production at the lowest possible level, in order to increase final ethanol yield. Besides, the presence of glycerol in the stillage stream increases the moisture of the byproduct and therefore, the required drying energy (Brumm and

Hebeda, 1988). From total available carbon in wort, 4 to 10% is used by *S. cerevisiae* to produce glycerol, in other words, glycerol represents 7 to 10% of total ethanol, depending on yeast strain, medium and process conditions (Scanes et al., 1998).

Table 6.3. Ethanol production (TEthanol), fermentation efficiency (FF) and final yield of the evaluated sorghum cultivars after 72 hours fermentation[1]

Variety	Ethanol, TEthanol (mL/L)	Glycerol (g/L)[2]	Residual Sugars (g/L)	Fermentation Efficiency, FF (%)	Ethanol Yield[3]	
					(L/ton starch)	(L/ton grain)
RR1	86.4 ± 2.6 a	6.5 ± 0.2 a	0.6 ± 0.0 a	89.1 ± 0.8 a	640.4 ± 5.8 a	400.0 ± 3.6 abc
RR2	86.2 ± 3.3 a	6.4 ± 0.2 a	0.6 ± 0.0 a	82.2 ± 2.0 abcd	590.6 ± 14.2 abcd	407.3 ± 9.8 a
Sumac	90.5 ± 4.2 a	7.1 ± 0.2 a	1.8 ± 0.9 a	79.6 ± 0.2 d	571.9 ± 1.5 d	364.9 ± 1.0 efg
HTW1	85.0 ± 2.8 a	6.5 ± 0.2 a	1.0 ± 0.4 a	87.9 ± 1.0 ab	631.9 ± 7.4 ab	378.7 ± 4.4 bcdef
HTW2	85.5 ± 3.4 a	6.7 ± 0.3 a	0.7 ± 0.0 a	83.0 ± 1.5 abcd	596.5 ± 10.5 abcd	375.5 ± 4.1 cdef
Waxy1	77.0 ± 4.4 a	6.4 ± 0.3 a	0.6 ± 0.0 a	82.1 ± 0.9 abcd	590.3 ± 6.1 abcd	347.3 ± 3.6 g
Waxy2	86.0 ± 3.5 a	6.5 ± 0.2 a	0.8 ± 0.0 a	82.5 ± 1.5 abcd	593.3 ± 10.7 abcd	394.8 ± 3.2 abcd
HD1	83.7 ± 2.3 a	6.7 ± 0.2 a	0.7 ± 0.1 a	88.4 ± 0.9 a	635.8 ± 6.6 a	355.1 ± 3.7 fg
HD2	84.1 ± 2.2 a	6.5 ± 0.2 a	0.7 ± 0.0 a	81.1 ± 1.1 bcd	583.2 ± 7.7 bcd	373.3 ± 4.2 def
WR1	84.0 ± 2.7 a	6.6 ± 0.2 a	0.7 ± 0.1 a	80.1 ± 1.4 cd	576.1 ± 9.8 cd	372.0 ± 3.4 def
WR2	87.6 ± 3.4 a	6.7 ± 0.2 a	1.0 ± 0.0 a	83.1 ± 1.7 abcd	597.5 ± 12.0 abcd	389.9± 0.9 abcde
WR3	87.1 ± 3.0 a	6.4 ± 0.2 a	1.0 ± 0.1 a	87.3 ± 2.7 abc	628.2 ± 19.1 abc	401.9 ± 8.2 ab

[1]All results are the average of at least three replicas ± standard error of the mean. Values with different letter within columns were statistically different (P<0.05). [2] Depicted glycerol is at the end of fermentation. At the beginning glycerol content was zero. [3] Yields calculated in wet basis (14% moisture).

With data depicted in Table 6.3 is clear that glycerol concentration, regarding total ethanol, was in the lowest level reported by Scanes et al. (1998). These results imply an adequate *S. cerevisiae* fermentation.

Considering TS, fermentation efficiency was calculated (Table 6.3). *Sumac* with 79% showed the lowest value, whereas *RR1* and *HD1* the highest with 89% efficiency. These efficiencies were similar to data reported for Wu et al. (2010), using a set of white sorghums with different endosperm textures (87.1 to 91.8%). Wang et al. (2008) obtained efficiencies from 86 to 93%, Chuck-Hernández et al. (2012a) reported a range from 78 to 90% in sorghums with different sorts of damage (mold, insect and sprouted), whereas Wu et al. (2008) for nine genotypes grown in different locations and irrigation regimes, found efficiencies between 87.5 to 93.9%. In general terms, fermentation efficiency and ethanol yields are affected mainly by genotype (Wu et al., 2008) and process parameters, as *alpha* amylase dosage during liquefaction or temperatures through all production stages (Wu et al., 2006; Zhao et al., 2008).

Regarding ethanol yield (Table 6.3), the highest content per ton of initial starch was for the *RR1* and *HD1* genotypes and per ton of grain for *RR2*. The lowest yield was obtained in *Sumac* and *Waxy1* (from starch and grain respectively). Ethanol per ton of starch and grain ranged from 571 to 640 L and 347 to 407 L respectively. Chuck-Hernández et al. (2012a and 2012b) reported yields from 490 to 555 and 470 to 610 liter per ton starch, and 290 to 380 L/ton flour. In some of these results, despite similar TS content in raw materials, yields were statistically different, implying that not all starch contributed equally to ethanol production. Wang et al. (2008) previously reported that ethanol from sorghums with comparable starch contents varied as much as 7.4%.

The lowest fermentation efficiency and yield was for the *Sumac* type, the only sorghum with condensed tannins within the evaluated set. Wu et al. (2007) reported for high tannin cultivars a fermentation efficiency average of 85.2%, higher than the 79.6% observed in this research work (Table 6.3). High tannin cultivars converted by Wu et al. (2007) had similar chemical composition compared to the *Sumac* (68% starch, 11 % protein, Table 6.1) employed herein, but a lower tannin content (1.6 versus 2.8%, data not shown). Therefore, this difference in tannin content had a negative impact in sugars generated during liquefaction. The lower starch conversion contributed to the poor yeast fermentation efficiency. Furthermore, protein digestibility was also obtained for this *Sumac* cultivar (data not shown) and interestingly was less than a half the average value reported by Wu et al., (2007) (11.1% vs 28.3%), reinforcing the influence of these compounds on digestibility (Elmaki et al., 1999; Serna-Saldivar and Rooney, 1995) and its interference with starch hydrolysis (Taylor and Belton, 2002; Rooney and

Pflugfelder, 1986; Wang et al., 2008). The lower starch hydrolysis observed in the Sumac possibly increased final mash viscosity which is known to negatively affect ethanol production and fermentation efficiency (Duodu et al., 2003; Mullins and NeSmith, 1986; Salunke et al., 1982; Wu et al., 2007).

Furthermore the low observed yield for *Waxy1* was probably associated only to the low initial TS content (Table 6.1) because the FF was similar to *RR2*, the highest ethanol yielding cultivar. Waxy cultivars are preferred in ethanol production because amylose has a significant and detrimental effect on conversion efficiency. In fact, waxy cultivars always perform better during ethanol fermentation (Wu et al., 2007; Wu et al., 2010; Yan et al., 2011; Zhao et al., 2008). This phenomenon is linked to the positive correlation of amylose and cooking temperature. Waxy sorghum starch has a lower cooking temperature, low heating stability, high water-binding capacity (Serna-Saldivar and Rooney, 1995) and more resistance to gel formation and retrogradation compared to regular sorghum starch (Waniska and Rooney, 2000). Therefore, waxy cultivars are more prone to gelatinization and susceptibility to hydrolytic enzymes, resulting in better conversion to glucose (Wang et al., 2008). Despite these differences, fermentation efficiency (as previously stated) was not statistically higher for waxy and heterowaxy genotypes compared to non-waxy kernels (Table 6.3). Pasting temperature for regular and waxy sorghum flour is 82.1°C and 72.7°C respectively and during liquefaction 90°C was set as minimum. Also, according to Wu et al. (2006), when amylose content is lower than 30% its adverse effect on ethanol fermentation efficiency is not significant. The same authors stated that amylose affects conversion efficiency specially when is higher than 35% and herein practically all evaluated genotypes portrayed less than 30% amylose.

6.4.4. Correlations Among Hydrolysis and Fermentation Performance with Sorghum Physicochemical Characteristics and Susceptibility to *S. zeamais*

Correlations among hydrolysis and fermentation indicators with physicochemical and resistance characteristics of sorghum are summarized in Table 6.4. The most significant correlations are depicted in scatter plots (Figure 6.1). DNS at initial time of the process had a significant correlation with FAN, ash, protein, TKW and grain volume. Thus, DNS at minute zero of liquefaction was associated to parameters of the own sorghum cultivar and not associated to the

liquefaction or fermentation process. The largest kernels, with more protein, a high proportion of peptides and amino acids (FAN), low amylose and resistant starch were the higher in RdS.

The profile of RdS at time zero changed for the next two liquefaction times (minutes 100 and 200), where the highest positive correlations were linked to the percentage of endosperm and the most negative to ash, pericarp and protein (Figure 6.1). The last factor have a role on its own, because ash and pericarp (%) are usually negatively correlated to the amount of endosperm: cereals with a high endosperm have a smaller proportion of pericarp and thus, less ash. In fact, ash and pericarp are significant (and negative) correlated to endosperm (r= -0.67 and -0.65 respectively, Table 6.4), but not to protein. Thus, the highly significant (and negative) relationship with RdS previously described, can be due to the same nature of sorghum proteins (high hydrophobicity, disulfide and non-disulfide crosslinking during cooking processes) (Duodu et al., 2003; Hamaker et al., 1987). An interesting observation is that Total Starch (TS) was not correlated to liquefaction, contrary to crude protein, where calculated correlation coefficients for DNS minute 100 and 200 were -0.54 and -0.61 respectively (Figure 6.1). This result indicates that level of RdS obtained with α amylase depends on the endosperm and also on crude protein that altogether could be described as: *sorghum endosperm characteristics*. Endosperm characteristic has been previously described as one of the main variables influencing fermentable sugars and ethanol yield (Chuck-Hernández et al., 2012b), but for the twelve sorghum genotypes analyzed herein, endosperm texture *per se* was not correlated to RdS during liquefaction. This is understandable given that this parameter (ET) is an evaluation of the starch/protein through a subjective assessment of the vitreous and floury endosperm parts. In fact, one striking significant correlation (Table 6.4) was detected between DNS (t100) and Flotation Index (FI) (r=-0.56): the less floating kernels, or the most vitreous were also the higher in reducing sugars. This result could be associated to the very special characteristic of the evaluated sorghum. Other non-expected correlation was the obtained between Endosperm Texture (ET) and Endosperm percentage, as well as ET with Germ percentage (r= -0.67 and 0.66 respectively).

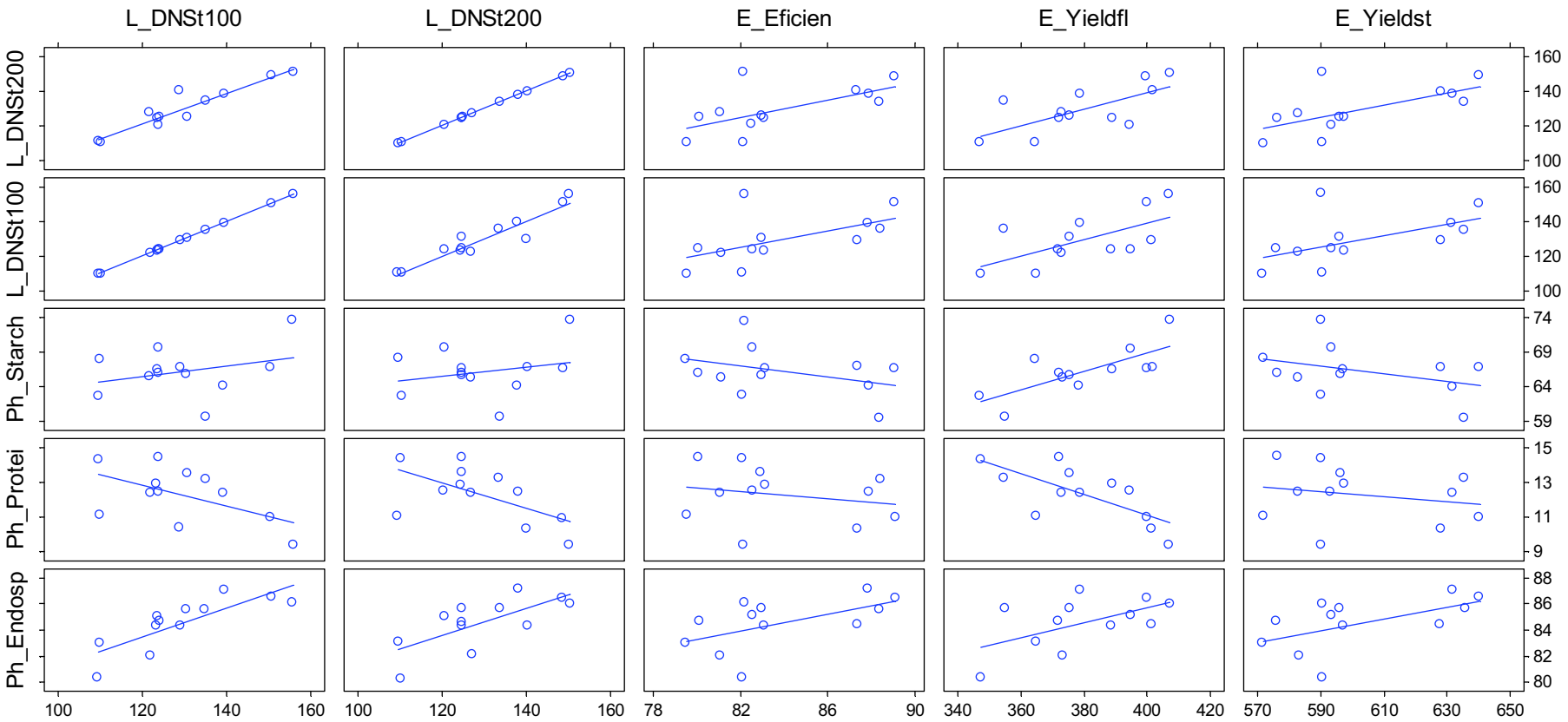


Figure 6.1. Scatter plots for Endosperm Percentage (Ph_Endosp), Crude Protein (Ph_Protei), Total Starch (Ph_Starch), Reducing Sugars during liquefaction at 100 and 200 minutes of reaction (L_DNSt100 and L_DNSt200 respectively), Fermentation Efficiency (E_Eficien), Ethanol yield from Flour and from Starch (E_Yieldfl and E_Yieldst respectively), depicting the correlation among these variables.

These results indicate that softer kernels have the lowest endosperm percentage and therefore were the smallest, indeed a non-desired bias in the sorghum set. Some of the softer kernels, as *Waxy1* had also one of the lowest starch content, affecting thus RdS during liquefaction. Hence, it would be desirable to include cultivars with a soft texture, but high endosperm proportion and high TKW.

Regarding liquefaction and resistance to insects, data of Table 6.4 clearly shows the inverse relationship between RdS and kernel susceptibility to *S. zeamais:* the highest sugar-producing sorghum cultivars were also the most resistant to maize weevil.

Table 6.4 also depicts the coefficients for ethanol production (fermentation efficiency, total ethanol and yield from flour as well as from total starch). All these factors could be directly linked to the sorghum performance (in terms of RdS) during liquefaction (Figure 6.1). It is clear that α amylase hydrolysis was the limiting step for fermentation. TS was non-significant related to ethanol producing variables, except for yield from flour (Table 6.4 and Figure 6.1). According to authors as Wang et al. (2008), TS is a good ethanol yield indicator in sorghum, but Wu et al., (2007) reported that only 78% of ethanol yield in sorghum could be explained by total starch content. According to the same authors, a better correlation can be obtained using protein as predictive factor. This result is indeed different when maize is used instead of sorghum.

Based on the results (Table 6.4), RdS generated during liquefaction described better the subsequent fermentation process (Fermentation Efficiency and yield from starch and flour). Similarly, Zhao et al. (2009), working with a small scale mashing procedure, reported that the complete hydrolyzed starch (glucose) was a better indicator for predicting ethanol yield than TS. This can be linked to total available starch for reducing sugar production. Then, the higher the RdS at the end of liquefaction, the higher ethanol yield and fermentation efficiency. There was not a significant correlation between liquefaction or fermentation with phenolics (total, bound or free), in agreement with previous reports (Chuck-Hernández et al., 2012b). On the other hand, condensed tannins (as a special phenolic group) had a detrimental effect on fermentation efficiency and liquefaction as previously described in subsections 6.4.2. and 6.4.3.

Table 6.4. Pearson correlations for physicochemical, susceptibility to *S. zeamais* and fermentation parameters of different sorghum genotypes[1]

	E_EficiencyFerment	E_EthanolTotal	E_Glycerol	E_YieldFlour	E_YieldStarch	L_DNS0	L_DNS100	L_DNS200	L_RatioTeor/Real	Ph_AAN	Ph_AOXCB	Ph_AOXCF	Ph_Amylose	Ph_Ash	Ph_BoundPhenolics	Ph_CI	Ph_DigestibProtein	Ph_Endosperm%	Ph_EndospermText	Ph_FI	Ph_FreePhenolics	Ph_Germ	Ph_Pericarp	Ph_Protein	Ph_ResistantStarch	Ph_StarchTotal	Ph_TADD	Ph_TKW	Ph_TW	Ph_TrueDensity	R_DobieIndex	R_EmergedInsects	R_FinalvsInitInsect	R_InsectsDay27	R_InsectsDay30
E_EthanolTotal	ns																																	
E_Glycerol	ns	ns																																
E_YieldFlour	ns	0.55^	ns																															
E_YieldStarch	1.00**	ns	ns	ns																														
L_DNS0	ns	-0.59*	ns	ns	ns																													
L_DNS100	0.57^	ns	ns	0.63*	0.57^	ns																												
L_DNS200	0.64*	ns	ns	0.69*	0.65*	ns	0.94**																											
L_RatioTeor/Real	0.86**	ns	ns	ns	0.86**	ns	0.78**	0.86**																										
Ph_AAN	ns	-0.75**	ns	-0.66*	ns	0.66*	ns	ns	ns																									
Ph_AOXCB	ns	ns	0.58*	ns	ns	ns	ns	-0.55^	ns	ns																								
Ph_AOXCF	ns	ns	ns	ns	ns	ns	0.72**	0.58*	ns	ns	ns																							
Ph_Amylose	ns	0.63*	ns	ns	ns	-0.87**	ns	0.60*	ns	-0.62*	ns	ns																						
Ph_Ash	ns	ns	ns	-0.79**	ns	0.68*	-0.84**	-0.89**	-0.62*	0.67*	ns	ns	-0.75**																					
Ph_BoundPhenolics	ns	ns	0.80**	ns	ns	ns	ns	ns	ns	ns	0.84**	ns	ns	ns																				
Ph_CI	ns	ns	0.54^	ns	ns	-0.82**	ns	ns	ns	ns	0.62*	ns	ns	ns	0.81**																			
Ph_DigestibProtein	ns	-0.58*	-0.66*	ns	ns	ns	ns	ns	ns	0.59*	ns	ns	ns	ns	-0.58*	-0.58*																		
Ph_Endosperm%	0.57^	ns	ns	0.55^	0.57^	ns	0.80**	0.71**	0.62*	ns	ns	ns	ns	-0.67*	ns	ns	ns																	
Ph_EndospermText	ns	-0.66*	ns	-0.65*	ns	ns	ns	ns	ns	0.66*	ns	ns	ns	ns	ns	ns	ns	-0.67*																
Ph_FI	ns	-0.75**	ns	-0.69*	ns	ns	-0.56^	ns	ns	0.74**	ns	ns	-0.53^	0.63*	ns	ns	ns	-0.79**	0.82**															
Ph_FreePhenolics	ns	ns	0.83**	ns	ns	ns	ns	ns	ns	ns	0.76**	ns	ns	ns	0.98**	0.8**	-0.66*	ns	ns	ns														
Ph_Germ	ns	-0.59*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.62*	-0.84**	0.66*	0.68*	ns													
Ph_Pericarp	ns	ns	ns	-0.66*	ns	ns	-0.77**	-0.71**	ns	ns	0.61*	-0.61*	ns	0.54^	0.62*	ns	ns	-0.65*	ns	0.61*	0.61*	ns												
Ph_Protein	ns	-0.60*	ns	-0.72**	ns	0.73**	-0.54^	-0.61*	ns	0.66*	ns	ns	-0.65*	0.79**	ns	-0.55^	ns	ns	ns	ns	ns	ns	ns										
Ph_ResistantStarch	ns	0.63*	0.83**	ns	ns	-0.60*	ns	ns	-0.54^	ns	0.66*	ns	ns	ns	0.93**	0.81**	-0.68*	ns	ns	ns	0.97**	ns	ns	ns										
Ph_StarchTotal	ns	ns	ns	0.75**	ns	ns	ns	ns	ns	-0.70**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-0.65*	ns								
Ph_TADD	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.75**	ns	ns	ns	ns	ns	ns								
Ph_TKW	ns	ns	-0.63*	ns	ns	0.77**	ns	ns	ns	ns	ns	ns	ns	ns	-0.70*	-0.79**	0.65*	ns	ns	ns	-0.73**	ns	ns	ns	-0.73**	ns	ns							
Ph_TW	ns	0.77**	ns	0.68*	ns	ns	ns	ns	ns	-0.69*	ns	ns	ns	ns	ns	ns	ns	0.72**	-0.89**	-0.95**	ns	-0.62*	-0.57^	ns	ns	ns	-0.64*	ns						
Ph_TrueDensity	ns	0.77**	ns	0.74**	ns	ns	ns	ns	ns	-0.75**	ns	ns	ns	-0.62*	ns	ns	ns	0.80**	-0.93**	-0.93**	ns	-0.73**	ns	ns	ns	0.60*	-0.58*	ns	0.94**					
Ph_Volume	ns	ns	ns	ns	ns	0.63*	ns	ns	ns	ns	ns	ns	ns	ns	-0.66*	-0.78**	0.71**	ns	ns	ns	-0.66*	ns	ns	ns	-0.69*	ns	ns	0.84**	ns	ns				
R_DobieIndex	ns	ns	ns	-0.71**	ns	ns	-0.79**	-0.76**	ns	0.63*	0.57^	ns	-0.66*	0.80**	ns	ns	ns	-0.72**	ns	0.82**	ns	ns	0.76**	ns	ns	ns	ns	ns	-0.71**	-0.69*				
R_EmergedInsects	ns	ns	ns	-0.62*	ns	ns	-0.68*	-0.65*	ns	0.61*	0.64*	ns	-0.61*	0.70*	ns	ns	ns	-0.61*	ns	0.80**	ns	ns	0.71**	ns	ns	ns	ns	ns	-0.69*	-0.61*	0.97**			
R_FinalvsInitInsect	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.66*	ns	ns	ns	ns	ns	ns	0.66*	0.66*		
R_InsectsDay27	ns	-0.70**	ns	-0.75**	ns	ns	-0.68*	-0.69*	ns	0.71**	ns	ns	-0.72**	0.78**	ns	ns	ns	-0.80**	0.76**	0.91**	ns	0.69*	0.65**	ns	ns	ns	ns	ns	-0.88**	-0.85**	0.87**	0.81**	ns	
R_InsectsDay30	ns	-0.59*	ns	-0.78**	ns	ns	-0.68*	-0.69*	ns	0.75**	ns	ns	-0.64*	0.77**	ns	ns	ns	-0.75**	0.73**	0.91**	ns	0.60*	0.67*	ns	ns	ns	-0.53 ^	ns	ns	-0.83**	-0.83**	0.95*	0.90**	ns	0.95**
R_LossWeight	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.60*	ns	ns	ns	ns	ns	ns	ns	ns	0.58*	ns	ns	0.55^	ns	ns	ns	0.62*	ns	-0.61*	ns	0.68*	0.76**	0.87**	ns	0.62*
R_MDT	ns	ns	ns	0.81**	ns	ns	0.82**	0.81**	0.55^	-0.64*	ns	ns	0.65*	-0.84**	ns	ns	ns	0.83**	-0.66*	-0.82**	ns	-0.61*	-0.74**	-0.55^	ns	ns	ns	ns	0.73**	0.80**	-0.93**	-0.82**	-0.56^	-0.89**	-0.94**

[1]Correlation coefficients with a circumflex accent (^) represent *Pvalues* <0.07, with an asterisk (*), *Pvalues* <0.05 and with two (**), *Pvalues*<0.01

Name of variables initiated with E_ means obtained from ethanol process, L_ from Liquefaction, Ph_ from physicochemical analysis and R_ stands for an insect resistance related factor. AAN, Alpha Amino Nitrogen; AOXCB, Antioxidant Capacity Bound Fraction; AOXCF, Antioxidant Activity Free Fraction; CI, Color Index; DNS, Reducing Sugars; FI, Flotation Index; MDT, Median Development Time; TADD, Tangential Abrasive Dehulling Device; TKW, Thousand Kernel Weight; TW, Test Weight.

Ethanol most producing sorghum cultivars were the more resistant to *S. zeamais* infestation, indicating the possibility to use the same physicochemical traits to describe the best cultivars in terms of storage and ability to fermentation. Digestibility of sorghum proteins was significant related to total ethanol.

6.4.5. PRINCIPAL COMPONENT ANALYSIS (PCA)

A Principal Component Analysis (PCA) was performed using the 38 variables obtained for the 12 sorghum cultivars (22 physicochemical, 7 resistance /susceptibility to *S. zeamais*, 4 related to liquefaction and 5 to ethanol production). According to Smith et al. (2002), the objective of PCA is to reduce the dimensionality of the data or to reduce the number of variables without (or with the less possible) loss of information. PCA allows an observed structure or pattern of variables and this analysis is usually performed before regression (Gurrea, 2005).

Table 6.5. Eigenvalues, percent of variance and cumulative percent for each principal component (PC)

PC	Eigenvalue	% of variance	Cumulative % of variance
1	15.6555	41.2	41.2
2	9.23262	24.3	65.5
3	4.44577	11.7	77.2
4	3.32299	8.7	85.9
5	1.7864	4.7	90.6
6	1.32863	3.5	94.1
7	0.92789	2.4	96.6
8	0.50376	1.3	97.9
9	0.36459	1.0	98.9
10	0.31698	0.8	99.7

In Table 6.5 are depicted the results of the PC factor analysis through orthogonal transformation of the correlation matrix. Five factors explained 90.6% of the total variation being further described in Table 6.6. Column 1 in this last table includes a simpler description of the PC according to the most significant correlation with the factors. The highest correlations with the PC1 are depicted by variables

associated to *liquefaction* (DNS or RdS content) and *resistance* to *S. zeamais*. In this first factor, ash percentage, as well as FI showed a positive correlation, the same that susceptibility (DI and Insects emerged at different times of storage). In other words, the highest the FI the highest the susceptibility, an observation already described in the previous chapter. All these variables were in the opposite side of liquefaction descriptors (RdS at time 100 and 200 of liquefaction), implying that the susceptibility of sorghum to weevil and its performance in liquefaction were negatively correlated (described in subsection 6.4.4.). Endosperm percentage and TD can also be associated, as previously described, with the high RdS yield during liquefaction (Table 6.6).

The second principal component was associated to the *nutraceutical* characteristics of the sorghum cultivars. Bound and Free Phenolics altogether with Resistant Starch were positively correlated to PC2, whereas DNS at initial time, protein digestibility, volume and TKW were in the other side of the vector. PC3 was related to the percentage of abraded material in TADD as well as loss of weight during confinement tests with *S. zeamais* and positively to Crude Protein and Test Weight, depicting again the important role of protein in the quality characteristics of sorghum (described as Test Weight) and *hardness* (measured as TADD), but also with the resistance to insects (*S. zeamais*).

PC4 was the *ethanol* production component, where the starch and germ percentage as well as protein digestibility were in the opposite side of ethanol yield and efficiency. This relationship is very interesting, considering that starch and protein digestibility (within the evaluated sorghum cultivars) are both positive and in the opposite side of ethanol production variables. In wet milling of 24 grain sorghum hybrids, Buffo et al. (1998) reported a negative correlation among total starch and starch recovery, but they didn't evaluate the protein digestibility, a factor that could also be related to this low starch yield. The last factor described in Table 6.6 (PC5) was related to resistance and protein content. This last variable is opposite to number of final insects and loss of grain weight during storage with *S. zeamais* and to volume of caryopsis.

Table 6.6. Variables with the most significant correlations within each of the principal components (|r|>0.2 or >0.3 when indicated*)

Principal component (PC)	Factors with the most significant correlation within each principal components	
	negative(-)	**positive (+)**
1. Resistance and liquefaction	R_MDT Ph_TD Ph_EndospermPercentage E_Yieldflour Ph_TW L_DNSt100 L_DNSt200	R_Total Emerged Insects Ph_Ash Ph_FI R_DI R_InsectsatDay27 R_InsectsatDay30
2. Phenolics	Ph_TKW Ph_Volume Ph_ProteinDigestibility L_DNSt0	Ph_BoundPhenolics* Ph_FreePhenolics* Ph_RS*
3. Hardness	Ph_TADD* R_LossWeight	Ph_TW Ph_CProtein
4. Ethanol production	E_Yieldstarch* E_Efficiency*	Ph_ProteinDigestibility Ph_Starch* Ph_Germ*
5. Protein and Resistance	Ph_Volume* R_FinalvsInitialInsects* R_LossWeight*	Ph_CProtein*

6.5. CONCLUSIONS

Twelve sorghum cultivars were physical and chemical characterized. Total starch, crude protein, amylose and endosperm texture differed among cultivars. After liquefaction with α amylase, all sorghum cultivars ended with similar RdS (efficiencies from 41.7 to 53.7%), except for the high tannin *Sumac* (38.7%). The tannins inhibited α amylase and thus had a detrimental effect during the key step of liquefaction. The *Sumac* and the *Waxy1* genotypes yielded the lowest amounts of RdS during liquefaction (109.7 and 110.5 g/L respectively). The low initial starch content present in the *Waxy1* (62.8% versus total average of 66.5% for all treatments) was the driver for the poor performance of this specific genotype. The red cultivars *RR1* and *RR2* were the best in terms of RdS produced, reinforcing the previous result about the null influence of non-tannin phenolics in amylase activity. In terms of ethanol production and efficiency of fermentation the different sorghum genotypes were statistically different. The lower efficiency was for *Sumac* (79%) and the highest for the *HD1* and *RR1* kernels (89%). RdS in both 100 and 200 minutes, was highly correlated to endosperm percentage (r=+0.80 and +0.71, respectively) and negatively to protein content (r=-0.54 and -0.61, respectively). Ethanol production values were in turn, correlated to liquefaction performance, indicating the feasible use of RdS as indicator for sorghum fermentation potential. A significant correlation between susceptibility and liquefaction was also observed: the highest sugar-producing sorghums were the most resistant to maize weevil (r=0.76 to 0.79). In PCA, five principal components explained 90% of data variance and were associated to: 1) liquefaction and insect resistance; 2) nutraceutical traits; 3) hardness; 4) ethanol production and 5) protein and resistance. The best sorghum cultivars in terms of ethanol production and resistance to maize weevil were non-tannin and hard genotypes, with high endosperm percentage. Red cultivars are one of the most promissory because of its good resistance to insects and high ethanol production. Protein digestibility and resistant starch, under the evaluated processes conditions have a non-significant impact in the reducing sugars production. A quick evaluation to predict fermentability of sorghum cultivars is indeed liquefaction at bench scale. Based on these results and, as further described next in general conclusions, the hypothesis established at the beginning of this work must be rejected. Sorghum cultivars highly efficient in ethanol production

were not the most susceptible to insect damage during storage. In fact were the most resistant. Nevertheless kernel physical and chemical properties mainly endosperm type and texture, influenced both fermentation and infestation results. Phenolic sorghum content had a detrimental effect only in bioethanol production and didn't have influence in insect activity during storage.

7. PRINCIPAL CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

- Phenolics and bran from red sorghum cultivars were not detrimental to liquefaction, saccharification and fermentation with *S. cerevisiae*.
- Tannins in sorghum flour affected negatively activity of amylase in ethanol production, yielding lower fermentation efficiencies compared to non-tannin counterparts.
- Damaged sorghum with molds, insects or sprouted were successfully bio-converted into fuel ethanol production without detrimental effects on α-amylase, amyloglucosidase and yeast (*S. cerevisiae*) performance during fermentation.
- The use of sprouted kernels reduced liquefaction (hydrolysis) time, reaching an optimum level of fermentable sugars faster than the control treatment.
- Ethanol production from sorghum was highly dependent of the performance during liquefaction with α-amylase.
- Liquefaction was positively correlated to sorghum endosperm percentage, starch and negatively to protein content and tannins.
- *Sitophilus zeamais* in sorghum fed and damaged endosperm affecting proteins and reducing resistant and total starch.
- The most resistant sorghum cultivars to *S. zeamais* were the harder kernels with high endosperm percentage and that contained high amylose and reduced free amino nitrogen concentration.
- In the evaluated sorghum kernels, the endosperm characteristics affected oppositely bioethanol production and insect resistance.
- The more resistant cultivars during storage with *S. zeamais* were also the highest sugar producers after treatment with α -amylase.
- Endosperm type and texture was thus related to the efficiency in fuel ethanol production and at the same time affected resistance to *S. zeamais* infestation.
- The starch and protein ratio affecting liquefaction efficiency for bioethanol and also resistance to maize weevil can be measured through relatively fast tests as subjective endosperm texture, flotation index as well as starch and crude protein contents.

- Protein digestibility *per se* was not related to the efficiency in fuel ethanol production and at the same time did not affect its resistance during *S. zeamais* infestation.
- Phenolic content and antioxidant activity were not correlated to sorghum susceptibility to *S. zeamais* during storage, implying a different mechanism of resistance compared with other grains like maize.
- Phenolic profile, specifically condensed tannin content, was related with efficiency in fuel ethanol production but not with sorghum resistance to *S. zeamais* infestation.
- Regarding initial hypothesis, sorghum cultivars highly efficient in ethanol production were not the most susceptible to insect damage during storage. In fact, those genotypes were the most resistant to *S. zeamais* deleterious effect.

RECOMMENDATIONS

- To increase the set of sorghum cultivars during confinement tests with *S. zeamais* and fermentation trials with *S. cerevisiae*. Specifically the use of germplasm with high endosperm percentage and soft texture as well as a second high tannin variety will be desired.
- To study the influence of specific phenolic compounds in fermentation efficiency and insect resistance, mainly of flavan-4-ols.
- To study the role of pest related protein, as ribosome inactivating protein, in sorghum cultivars facing *S. zeamais* infestation.
- To obtain a mathematical correlation among sorghum physicochemical characteristics and ethanol production altogether with its specific pest resistance.
- To study the effect of *S. zeamais* in sorghum protein molecular weight compared with other cereals as maize.
- To analyze the microscopic changes in sorghum endosperm facing insect infestation and *alpha* amylase hydrolysis with special emphasis in starch granules and protein matrix (and bodies) characteristics.

- To screen several sorghum cultivars looking for a natural resistance germplasm to insect damage and to perform an evaluation to describe the differences among a resistant and susceptible genotype.
- To study the changes in functional properties of sorghum after *S. zeamais* infestation, using the viscoamylographic properties of the damaged flour.
- To study differences among *S. zeamais* activity in sorghum cultivars compared with other insect as *S. oryzae* and *P. truncatus.*

There are few studies about pest resistance in sorghum. Its defense mechanisms against insects have not been described as in other cereals as maize. Furthermore, the impact of chemical, physical and nutraceutical sorghum properties on pest resistance and their relationship with industrial processes as bioethanol production have not been reported until now. Information obtained herein is useful as an initial assessment of insect resistance mechanisms in sorghum influencing its productivity during fermentation.

8. STATEMENT OF ADDITIONAL CONTRIBUTIONS

Hereby the main collaborations and contributions are detailed. Published and submitted articles, as well as material to be submitted derived from this dissertation are included. Reviews, book chapters, oral and poster presentations in conferences within the period of the PhD program are also detailed. To all collaborators thanks a lot for your help and support to achieve these contributions.

8.1. ORIGINAL ARTICLES IN JOURNALS

F.J. Dávila-Gómez, C. Chuck-Hernández, E. Pérez-Carrillo, W.L. Rooney, S.O. Serna-Saldivar. 2011. Evaluation of bioethanol production from five different varieties of sweet and forage sorghums (*Sorghum bicolor* (L) Moench). Industrial Crops and Products 33, 611-616.

Chuck-Hernández, C., Gutiérrez-Uribe, J., Serna-Saldivar, S.O. 2011. Effect of thermoplastic extrusion and cell wall degrading enzymes on the phenolic and flavonoid compounds extracted from whole and dehulled black beans (*Phaseolus vulgaris* L.). Insights in Biotechnology, 1(4): 15-18.

Chuck-Hernández, C., Peralta-Contreras, M., Bando-Carranza, G., Vera-García, M., Gaxiola-Cuevas, N., Tamayo-Limón, R., Cárdenas-Torres, F., Pérez-Carrillo, E., Serna-Saldívar, S.O. 2012. Bioconversion into ethanol of decorticated red sorghum (*Sorghum bicolor* L. Moench) supplemented with its phenolic extract or spent bran. Biotechnology Letters 34, 97–102.

Chuck-Hernández, C., García-Lara, S., Serna-Saldívar, S.O. 2012. Conversion into bioethanol of insect (*Sitophilus zeamais* Motschulsky), mold (*Aspergillus flavus* Link) and sprout-damaged maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench). Journal of Cereal Science 55, 285-292.

Perez-Carrillo, E., Serna-Saldivar, S.O., Chuck-Hernandez, C., Cortes-Callejas, M.L. 2012. Addition of protease during starch liquefaction affects free amino nitrogen, fusel alcohols and ethanol production of fermented maize and whole and decorticated sorghum mashes. Biochemical Engineering Journal 67, 1-9.

Chuck-Hernández, C., González-Ruiz, J., Luna-Salazar, R., Serna-Saldívar, S.O. 2012. Pilot-Scale performance of sweet sorghum juice for fuel-ethanol production: efficiency and production rates using commercial yeast. Insights in Biotechnology, 2(5): 5-9.

Peralta-Contreras, M., Chuck-Hernandez, C., Perez-Carrillo, E., Bando-Carranza, G., Vera-Garcia, M., Gaxiola-Cuevas, N., Tamayo-Limon, R., Cardenas-Torres, F., Serna-Saldivar, S.O. 2012 Fate of free amino nitrogen during liquefaction and yeast fermentation of maize and sorghums differing in endosperm texture. Food and Bioproducts Processing. In press: 31 August 2012.

8.2. ARTICLES SUBMITTED

Chuck-Hernández, C., Serna-Saldívar, S.O., García-Lara, S.O. Susceptibility of different types of sorghums during storage to *Sithophilus zeamais* Motschulsky. Journal of Cereal Science. Date of submission: October 9th, 2012.

8.3. ARTICLES TO BE SUBMITTED

Chuck-Hernández, C., Serna-Saldívar, S.O., García-Lara, S.O. Resistance characteristics of sorghum grain to maize weevil (*Sitophilus zeamais*) and its digestibility properties correlated with ethanol production using *Saccharomyces cerevisiae* as fermentation microorganism.

Chuck-Hernández, C., Serna-Saldívar, S.O., García-Lara, S.O. Influence of *Sitophilus zeamais* in the starch and protein fractions of flour obtained from different stored sorghum cultivars.

8.4. REVIEWS

Chuck-Hernández, C., Pérez-Carrillo, E., Heredia-Olea, E., Serna-Saldívar, S.O. 2011. Sorgo como un cultivo multifacético para la producción de bioetanol en México: Tecnologías, avances y áreas de oportunidad. Sorghum as a multifunctional crop for bioethanol production in Mexico: Technologies, advances and improvement opportunities. Revista Mexicana de Ingeniería Química. 10(3): 529-549.

8.5. BOOK CHAPTERS

Serna-Saldívar, S.O., Chuck-Hernández, C., Pérez-Carrillo, E., Heredia-Olea, E. 2012. Sorghum as a Multifunctional Crop for the Production of Fuel Ethanol: Current Status and Future Trends. Chapter 3 in Pinheiro-Lima, M.A., Pardo Policastro-Natalense, A.P. (Eds.). Bioethanol. In Tech. ISBN 978-953-51-0008-9, pp. 51-74.

8.6. ORAL PRESENTATIONS IN NATIONAL AND INTERNATIONAL MEETINGS

Chuck-Hernández, C., Pérez-Carrillo, E. y Serna-Saldívar, S.O. 2010. Efecto del rolado al vapor en la producción de alcoholes de fusel durante fermentación con *Sacharomyces cerevisiae*. En: 40° Congreso de Investigación y Desarrollo. Tecnológico de Monterrey, Campus Monterrey. 20-22 de enero 2010.

Chuck-Hernández, C., Peralta-Contreras, M., Bando-Carranza, G., Vera-García, M., Rivas-Briones, A., Gaxiola-Cuevas, N., Tamayo-Limón, R., Cárdenas-Torres, F., Pérez-Carrillo, E., Serna-Saldívar, S.O. 2010. Efecto de fibra y compuestos fenólicos en la eficiencia de licuefacción de sorgo rojo (*Sorghum bicolor* L. Moench) como etapa inicial del proceso de producción de bioetanol. En: 40° Congreso de Investigación y Desarrollo. Tecnológico de Monterrey, Campus Monterrey. 20-22 de enero 2010.

Chuck-Hernández, C., Peralta-Contreras, M., Bando-Carranza, G., Vera-García, M., Rivas-Briones, A., Gaxiola-Cuevas, N., Tamayo-Limón, R., Cárdenas-Torres, F., Pérez-Carrillo, E., Serna-Saldívar, S.O. 2010. Efecto de fibra y compuestos fenólicos en el perfil de generación de fructosa, glucosa, maltosa y maltotriosa durante la sacarificación de sorgo rojo (*Sorghum bicolor* L. Moench). En: 40° Congreso de Investigación y Desarrollo. Tecnológico de Monterrey, Campus Monterrey. 20-22 de enero 2010.

Chuck-Hernández, C., Peralta-Contreras, M., Bando-Carranza, G., Vera-García, M., Rivas-Briones, A., Gaxiola-Cuevas, N., Tamayo-Limón, R., Cárdenas-Torres, F., Pérez-Carrillo, E., Serna-Saldívar, S.O. 2010. Efecto de fibra y compuestos fenólicos en el perfil de generación de etanol y alcoholes superiores durante la fermentación de sorgo rojo

(*Sorghum bicolor* L. Moench). En: 40° Congreso de Investigación y Desarrollo. Tecnológico de Monterrey, Campus Monterrey. 20-22 de enero 2010.

Dávila-Gómez, F.J., Chuck-Hernandez, C., Perez-Carrillo, E., Rooney, W., Serna-Saldivar, S.O. 2010. Producción de bioetanol a partir de cinco diferentes variedades de sorgos dulces y forrajeros (*Sorghum bicolor* (L) Moench). En: VII Encuentro Latinoamericano y del Caribe sobre Biotecnología Agropecuaria. Del 1 al 5 de noviembre del 2010. Guadalajara Jalisco México.

Chuck-Hernández, C., Lozano-Martínez, E., Serna-Saldivar, S.O. 2011. Análisis económico marginal de diferentes tecnologías disponibles en el mundo para la producción de etanol a partir de sorgo. En: 41 Congreso de Investigación y Desarrollo. Ciencia para el Desarrollo. Del 19 al 21 de enero del 2011. Monterrey Nuevo León México.

Chuck-Hernández, C., Serna-Saldivar, S.O. 2011. Factibilidad económica de una biorefinería para la producción de 160 millones de litros anuales de bioetanol en el noreste de México. En: 41 Congreso de Investigación y Desarrollo. Ciencia para el Desarrollo. Del 19 al 21 de enero del 2011. Monterrey Nuevo León México.

Chuck-Hernández, C., Pérez-Carrillo, E., Serna-Saldívar, S.O. 2011. Tecnologías viables de producción de bioetanol a partir de sorgo en México. En: 41 Congreso de Investigación y Desarrollo. Ciencia para el Desarrollo. Del 19 al 21 de enero del 2011. Monterrey Nuevo León México.

Luna Salazar, R., González Ruíz, J., Díaz González, E.E., Guardado Virgen, E., Martín, E., Aguilar Zamarripa, E., Pérez Carrillo, E., Chuck-Hernández, C., Serna Saldívar, S.O. 2011. Desempeño de una cepa comercial de levadura en la producción de bioetanol a partir de jarabe glucosado y jugo de sorgo dulce en fermentaciones por lotes de 45 litros. En: 41 Congreso de Investigación y Desarrollo. Ciencia para el Desarrollo. Del 19 al 21 de enero del 2011. Monterrey Nuevo León México.

González Ruíz, J., Luna Salazar, R., Díaz González, E.E., Guardado Virgen, E., Martín, E., Aguilar Zamarripa, E., Pérez Carrillo, E., Chuck-Hernández, C., Serna Saldívar, S.O. 2011. Constantes cinéticas y tasa de producción de bioetanol a partir de sorgo dulce y jarabe glucosado en fermentaciones por lotes de 45 litros usando una cepa comercial de

Saccharomyces cerevisiae. En: 41 Congreso de Investigación y Desarrollo. Ciencia para el Desarrollo. Del 19 al 21 de enero del 2011. Monterrey Nuevo León México.

Chuck-Hernández,C., Pérez-Carrillo, E., Heredia-Olea, E., Serna-Saldívar. S.O. 2012. Sorghum as a Multifunctional Crop for the Production of Fuel Ethanol: Current Status and Future Trends. En: 42 Congreso de Investigación y Desarrollo. Del 18 al 20 de enero del 2012. ITESM Campus Monterrey. Monterrey, N.L. México.

Chuck-Hernández, C., García-Lara, S., Serna-Saldívar, S.O. 2012. Conversion into bioethanol of insect (*Sitophilus zeamais* Motschulsky), mold (*Aspergillus flavus* Link) and sprout-damaged maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench). En: 42 Congreso de Investigación y Desarrollo. Del 18 al 20 de enero del 2012. ITESM Campus Monterrey. Monterrey, N.L. México.

García-Lara, S., Chuck-Hernandez, C., Serna-Saldivar, S.O. 2012. Impacts in biophysical, nutrimental and nutraceutical properties of tortillas made using maize kernels damaged by larger grain borer (*Prostephanus truncatus* Horn.). En: 4 Congreso Internacional de Nixtamalización. 16 al 20 de Octubre del 2012. Juriquilla, Querétaro, México.

Chuck-Hernández, C., García-Lara, S., Serna-Saldívar, S.O. 2012. Características físicas, químicas y de resistencia a *Sitophilus zeamais* de doce diferentes variedades de sorgo usadas en el sur de Texas y norte de México. En: XXIV Congreso Nacional Y IV Internacional De Fitogenética. Del 24 al 28 de Septiembre de 2012. Monterrey, Nuevo León, México.

8.7. POSTER PRESENTATIONS IN NATIONAL AND INTERNATIONAL MEETINGS

Chuck-Hernandez, C., Perez-Carrillo, E., Serna-Saldivar, S.O. 2009. "Effect of steam flaking in higher alcohol production during fermentation with *Saccharomyces cerevisiae*" en: AACC International Annual Meeting. 13 al 16 de septiembre 2009. Baltimore, MD, USA.

Chuck-Hernandez, C., Perez-Carrillo, E., Serna-Saldivar, S.O. 2010. "Production of Bioethanol from steam-flaked sorghum and maize" en: 11a. Feria de Posgrados. Llevado a cabo en: Distrito Federal, Pachuca y Campeche. 17 al 22 de mayo del 2010. Consejo Nacional de Ciencia y Tecnología (CONACyT).

Chuck-Hernández, C., Peralta-Contreras, M., Gaxiola-Cuevas, N.., Tamayo-Limón, R., Cárdenas-Torres, F., Perez-Carrillo, E., Serna-Saldívar, S.O. 2010. "Effect of Red Sorghum Bran and Its Phenolics on the Conversion of Fermentable Sugars and Free Amino Nitrogen during Yeast Fermentation" en: AACC International Annual Meeting. 24 al 27 de octubre del 2010. Savannah, Georgia, USA.

Pérez-Carrillo, E., Serna-Saldívar, S.O., Alvarez, M., Cortés-Callejas, M.L., Chuck-Hernandez, C. 2010. "Effects of Sorghum Decortication and Protease Addition during Liquefaction on Bioethanol, Free Amino Nitrogen and Fusel Alcohol Production Throughout Yeast Fermentation" en: AACC International Annual Meeting. 24 al 27 de octubre del 2010. Savannah, Georgia, USA.

González Ruíz, J., Luna Salazar, R., Díaz González, E.E., Guardado Virgen, E., Martín, E., Aguilar Zamarripa, E., Pérez Carrillo, E., Serna Saldívar, S.O. 2010. Constantes cineticas, eficiencias y tasa de producción de bioetanol a partir de sorgo dulce y jarabe glucosado en fermentaciones por lotes de 45 litros usando una cepa comercial de *Saccharomyces cerevisiae*. En: V Congreso Regional de Biotecnología y Bioingeniería del Sureste. Del 27 al 29 de octubre del 2010. Mérida Yucatán México.

Chuck-Hernández, C., Lozano-Martínez, E., Serna-Saldivar, S.O. 2010. Análisis económico marginal de diferentes tecnologías disponibles en el mundo para la producción de etanol a partir de sorgo. En: VII Encuentro Latinoamericano y del Caribe sobre Biotecnología Agropecuaria. Del 1 al 5 de noviembre del 2010. Guadalajara Jalisco México.

Chuck-Hernández, C., Serna-Saldivar, S.O. 2010. Factibilidad económica de una biorefinería para la producción de 160 millones de litros anuales de bioetanol en el noreste de México. En: VII Encuentro Latinoamericano y del Caribe sobre Biotecnología Agropecuaria. Del 1 al 5 de noviembre del 2010. Guadalajara Jalisco México.

Chuck-Hernández, C., Peralta-Contreras, M., Bando-Carranza, G., Vera-García, M., Rivas-Briones, A., Gaxiola-Cuevas, N., Tamayo-Limón, R., Cárdenas-Torres, F., Pérez-Carrillo, E., Serna-Saldívar, S.O. 2010. Efecto del Salvado y Compuestos Fenólicos en la Eficiencia de Producción de Bioetanol durante la Fermentación de Sorgo Rojo (*Sorghum bicolor* (L) Moench). En: VII Encuentro Latinoamericano y del Caribe sobre Biotecnología Agropecuaria. Del 1 al 5 de noviembre del 2010. Guadalajara Jalisco México.

Chuck-Hernandez, C., García-Lara, S., Serna-Saldivar, S.O. 2011. Bioconversion of insect (*Sitophilus zeamais*), mold (*Aspergillus flavus*) and sprouted damaged maize (*Z. mays*) and sorghum (*S. bicolor* (L) Moench) into bioethanol en: AACC International Annual Meeting. 16 al 19 de Octubre del 2011. Palm Springs, CA, USA.

Chuck-Hernandez, C., García-Lara, S., Serna-Saldivar, S.O. 2012. Impacto de diferente grado de daño con *Prostephanus truncatus* Horn. en las propiedades viscoamilográficas de harina y tortilla obtenidas con maíz susceptible y resistente. En: 4 Congreso Internacional de Nixtamalización. 16 al 20 de Octubre del 2012. Juriquilla, Querétaro, México.

Chuck-Hernández, C., Serna-Saldívar, S.O., García-Lara, S. 2012. Estudio de la susceptibilidad de sorgo a *Sitophilus zeamais* Motschulsky durante almacenamiento. En: VI Congreso de Biotecnología y Bioingeniería del Sureste. Del 24 al 26 de octubre de 2012. Mérida Yucatán, México.

9. APPENDIX

Biotechnol Lett (2012) 34:97–102
DOI 10.1007/s10529-011-0752-z

ORIGINAL RESEARCH PAPER

Bioconversion into ethanol of decorticated red sorghum (*Sorghum bicolor* L. Moench) supplemented with its phenolic extract or spent bran

Cristina Chuck-Hernandez · Mayeli Peralta-Contreras · Gabriela Bando-Carranza · Mariano Vera-Garcia · Nallely Gaxiola-Cuevas · Ranses Tamayo-Limon · Feliznando Cardenas-Torres · Esther Perez-Carrillo · Sergio O. Serna-Saldivar

Received: 8 August 2011 / Accepted: 20 September 2011 / Published online: 6 October 2011

Abstract The effect of extracted phenolics or spent bran added to decorticated red sorghum kernels during fuel ethanol production was studied and compared to maize and whole red and white sorghums. After liquefaction, free amino nitrogen ranged from 65 to 101 mg/l and at the end of saccharification all mashes had approx. 80 g glucose and 2–5 g maltose/100 g meal (dry basis). Saccharified worts were fermented giving 50–90 ml ethanol/l. The lowest fermentation efficiency (76%) was obtained in the white sorghum. Ethanol yields indicate that sorghum bran or its associated phenolics did not significantly affect the efficiency of the sequential steps involved in ethanol production. Red sorghum is a good alternative to maize to produce ethanol and the difference regarding white sorghum and maize was mainly due to endosperm protein structure and composition.

Keywords Bioethanol · Phenolic extract · Sorghum · Sorghum bran · Sorghum decortication

Electronic supplementary material The online version of this article (doi:10.1007/s10529-011-0752-z) contains supplementary material, which is available to authorized users.

C. Chuck-Hernandez · M. Peralta-Contreras · G. Bando-Carranza · M. Vera-Garcia · E. Perez-Carrillo · S. O. Serna-Saldivar (✉)
Departamento de Biotecnología e Ingeniería de Alimentos, Centro de Biotecnología, Tecnológico de Monterrey, Av. Eugenio Garza Sada 2501 Sur, CP 64849 Monterrey, N.L., Mexico
e-mail: sserna@itesm.mx

N. Gaxiola-Cuevas · R. Tamayo-Limon · F. Cardenas-Torres
Ciudad Universitaria Facultad de Ciencias Químicas Biológicas, Universidad Autónoma de Sinaloa, Av. de las Américas y Josefa Ortíz de Dominguez S/N, CP 80010 Culiacan, Sinaloa, Mexico

Introduction

Sorghum is an excellent alternative to maize for fuel ethanol production because it is cheaper and contains almost the same amount of starch. It can be grown in drier and harsher lands where maize could not be planted. A drawback of the use of sorghum in biorefineries is the lower yield compared to maize and its comparatively higher starch gelatinization temperature as well as the reduced protein and starch digestibility (Duodu et al. 2003). Several investigations have studied the factors that affect sorghum starch and protein digestibility: the phenolic content and disulfide cross-linking among endosperm storage proteins being the two most relevant (Awika and Rooney 2004; Duodu et al. 2003). To overcome these disadvantages and improve grain sorghum ethanol yield, physical and chemical treatments have been devised, including particle size reduction, decortication, steam-flaking and use of proteases (Chuck-Hernandez et al. 2009; Corredor et al. 2006; Perez-Carrillo et al. 2008). In case of decorticated red sorghum, the improvement in fermentation yield apparently is related to reduction in

phenolic compounds found in the bran and to the increase of starch concentration resulting from the mechanical removal of the kernel's outer layers (Corredor et al. 2006). Abrasive decortication reduces the levels of fiber, fat and phenolics and can lead to an increase as much as 10% of starch and improve protein digestibility. The effect of bran fraction and phenolic compounds found in the pericarp fraction has not yet been investigated individually. This information could be useful in order to avoid or reduce the use of specific anatomical parts of the grain in ethanol production from red sorghum considered as the most widely used sorghum class. Thus, this research was performed to evaluate the effects of addition of red sorghum bran or its extracted phenolics to decorticated red sorghum kernels on the sequential steps of ethanol production in terms of fermentable sugars and free amino nitrogen (FAN) production, ethanol yield and fermentation efficiency. This information was contrasted with results obtained for maize and white sorghum kernels as controls grains.

Materials and methods

Grain sources and meal production

Grains used were commercial regular soft-endosperm yellow dent maize, a commercial red sorghum with a relatively soft endosperm texture and an experimental white sorghum (ATX631 TX436) with intermediate endosperm texture (Texas A&M University, Sorghum Breeding Program). Decortication, milling and particle size distribution were performed according to Perez-Carrillo et al. (2008). Physical and chemical characterization methods are detailed in supplementary material.

Extraction of phenolic compounds and spent-bran production

For extraction, 200 g red sorghum abraded bran was mixed with 500 ml 1% HCl/80% (v/v) methanol and maintained at 100 rpm for 12 h at room temperature. The extract was centrifuged (15 min, $\sim 4{,}000 \times g$) and then decanted. The resulting extract (260 ml) was concentrated in a rotary evaporator at 60°C. The spent-bran was dried at 50°C during 2 h.

Liquefaction and saccharification

Ground meal (150 g dry wt) was mixed with water to obtain mashes with 30% (w/v) solids. pH was adjusted to 6.5 with 0.1 M HCl. The slurry was held at 60°C and 225 µl of Liquozyme SC DS (Novozymes) was added. The temperature was gradually increased to 90°C and maintained for 195 min. Mashes were cooled down to 60°C and 1 ml Dextrozyme DX (Novozymes) per 200 ml liquefied slurry was added. Reaction vessels were maintained for 16 h at 60°C and 100 rpm. Mashes were then filtrated using a plastic sieve (1 mm) to remove spent grains and residues were washed with 200 ml distilled water.

Fermentation

Saccharified worts were adjusted to 13° Plato and enriched with yeast extract (150 mg/l of –FAN-, Yeastex 1433 Probamex, Naucalpan, Mexico). Total volume was recorded and worts (125 ml) were pasteurized at 70°C for 30 min and cooled to 35°C. Yeast was added at 1.3×10^7 cells/ml (*Saccharomyces cerevisiae,* Nevada-Safmex, Toluca, Mexico). The yeast suspension was prepared 20 min before pitching (1 g dry yeast in 100 ml distilled sterile water). The sealed flasks were incubated for 72 h at 30°C and 125 rpm.

Analytical methods

Reducing sugars were determined according to Miller (1959) and FAN by the Official Method 945.30 (AOAC 1980). Fructose, glucose, maltose and maltotriose were analyzed in a HPLC-IR chromatograph according to Chuck-Hernandez et al. (2009). Ethanol was determined by GC using an HP-Innowax (30 m × 0.53 mm × 1 µm) column.

Treatments and statistical analysis

Six treatments were evaluated: (1) whole red sorghum, (2) decorticated red sorghum with spent-bran free of phenolics, (3) decorticated red sorghum supplemented with phenolic extract, (4) decorticated red sorghum, (5) whole yellow maize and (6) whole white sorghum. In treatment 2, solids were composed of 90% decorticated sorghum meal and 10% ground spent-bran whereas in treatment 3 the phenolic extract was added to reach the concentration originally found in the red

whole sorghum. Data of at least three replicates for all determinations were analyzed using analysis of variance with a confidence level of 95% and Fisher's least significant difference test was used to compare treatment means (Minitab 14, Minitab Inc.).

Results and discussion

Sugars and FAN during enzymatic treatments

At the end of liquefaction a lower total reducing sugars in the red whole sorghum hydrolyzate compared to the other five treatments was obtained. However, the difference between the whole red and white sorghums was not significant (0.54 ± 0.08 vs. 0.57 ± 0.09 g/g flour–data not shown), despite the notorious difference in original starch content. Per se phenolic compounds present in red sorghum and fiber components did not have effect on α-amylase performance. Thus, the harder endosperm texture of the white sorghum kernels (Supplementary results and Supplementary Table 1) and the comparatively coarser meal granulation must be the factors that influenced the rate of hydrolysis. Naidu et al. (2007) concluded that the coarser granulation of maize meals negatively influenced fermentation and ethanol yields but they did not examine the effect of this factor during the stages of liquefaction and saccharification. In relation to the endosperm texture, starchy cells located in the floury and vitreous regions of the endosperm have similar components (cell walls, starch granules, matrix and protein bodies) and chemical composition, but starch granules present in the vitreous endosperm are tightly bound to the protein matrix and therefore are more difficult to hydrolyze.

The FAN content for all treatments ranged from 65 to 101 mg/l at the end of liquefaction (data not shown) and there were not significant differences among treatments. The maize hydrolyzates contained the highest FAN (101 mg/l), similar to values reported by Perez-Carrillo et al. (2008). The variation between sorghum and maize is related to the susceptibility of proteins to denaturation especially during high temperature cooking and use of proteases. Cooking reduces protein digestibility of sorghum and correlated with the formation of disulfide-bonded oligomeric proteins (Wu et al. 2007). Despite the high homology between zeins and kafirins, the sorghum prolamins have more susceptibility to crosslinking during cooking due to the higher content of hydrophobic sequences (Duodu et al. 2003). Previous studies have reported improvements in fermentation performance in decorticated sorghum samples (Corredor et al. 2006) and decorticated sorghum treated with proteases during liquefaction (Perez-Carrillo et al. 2008). Figure 1 depicts changes in glucose, maltose, maltotriose and fructose throughout saccharification with glucoamylase/pullulanase. There were no significant differences among treatments in terms of glucose (Fig. 1a). The curve showed a biphasic kinetic behavior consistent with product inhibition and enzymatic inactivation reported by several authors (Perez-Carrillo et al. 2008).

Maltose concentrations for the different hydrolyzates were slightly higher compared to maltotriose at the start of saccharification (Fig. 1b, c). At the end of the programmed hydrolysis, maltose was 10–20 mg/ml (2–5 g/100 g meal) higher than maltotriose. The decorticated red sorghum, maize and white sorghum hydrolyzates contained the highest maltose concentration. The residual maltose at the end of process could be due to glucoamylase inhibition by product. Maltotriose showed the highest rate of hydrolysis during the first 30 min followed by a slight increase in concentration. The highest concentration (approx. 6 g/100 g meal) was observed in the decorticated red sorghum plus spent bran. Profiles for this carbohydrate were similar among treatments ($P > 0.05$). The increase of maltotriose before 100 min could be linked to the gradual hydrolysis of existing dextrins. The hydrolysis of maltotriose in the first stage of saccharification had an effect in glucose and maltose concentrations (Fig. 1a, b). The lowest amount of maltotriose was reached at 5 h and this trisaccharide disappeared by the end of saccharification. Thus, glucoamylase hydrolyzed maltotriose with a good efficiency even though it has a higher affinity for longer chain oligosaccharides and preference for α-1,4 rather than α-1,6 bonds.

In some fermentation processes, such as beer production, maltotriose, dextrins and other oligosaccharides account up to 38% of total sugars present in wort (Serna-Saldivar 2010). Nevertheless, in bioethanol production a higher level of monosaccharides is desired because regular yeast cannot metabolize linear and branched dextrins. Maize and white sorghum hydrolyzates contained the highest fructose concentration during saccharification (Fig. 1d). Fructose in all treatments was less than 1/100 g original meal and can be related to the amount present in original grains or to the

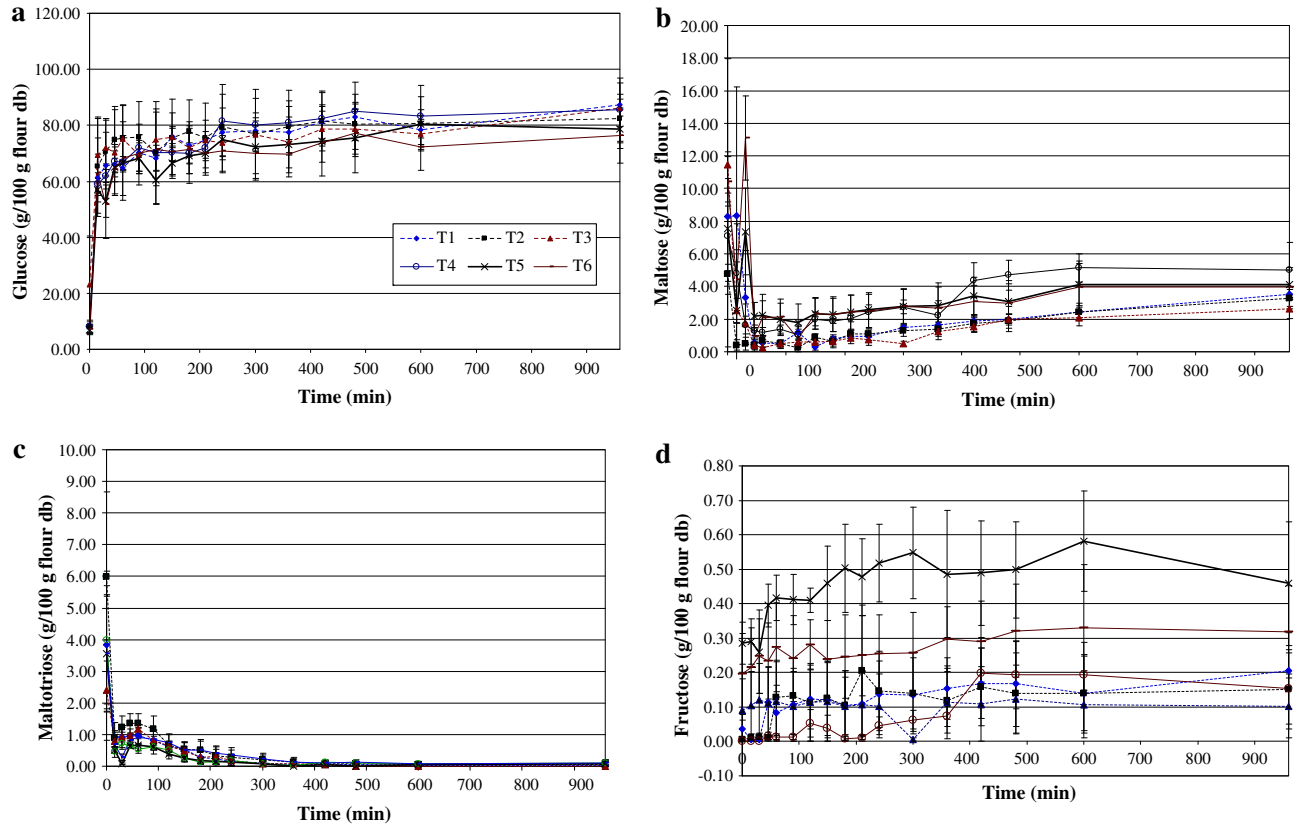


Fig. 1 **a** Glucose, **b** maltose, **c** maltotriose and **d** fructose (g/100 g flour) during saccharification of *T1* whole red sorghum, *T2* decorticated red sorghum + spent-bran, *T3* decorticated red sorghum + phenolic extract, *T4* decorticated red sorghum, *T5* whole maize and *T6* whole white sorghum

hydrolysis or inversion of the small quantity of sucrose in cereal grains. Mature kernels contain small amounts of mono and disaccharides and most of these soluble sugars are associated to the germ (Serna-Saldivar 2010). According to the same author, sorghum and maize have roughly the same level of soluble sugars (1.5–1.9%), being the average of sorghum slightly lower compared to maize.

At the start of saccharification the percentage of fermentable carbohydrates, calculated as glucose, fructose, maltose and maltotriose, was between 22–46% of the total reducing sugars. The decorticated red sorghum + phenolic extract hydrolyzate produced the highest amounts of fermentable species. At the end of saccharification, glucose was the main sugar reaching 96% in relative abundance. The maltose content of hydrolyzates prepared from decorticated red sorghum, maize and white sorghum were slightly higher compared to the other treatments, although these dissimilarities were not statistically different ($P > 0.05$). In short, there were no significant differences in soluble carbohydrate profile after saccharification for the evaluated treatments.

Sugars and ethanol profile during fermentation

Fructose consumption during fermentation is shown in Fig. 2a. The maize wort contained the highest initial fructose concentration whereas all sorghum treatments had similar amounts. After 24 h fermentation, fructose was completely consumed in all mashes. Consumption of fructose and glucose (Fig. 3) showed similar patterns indicating the consumption preferences of *S. cerevisiae*. Maltose and maltotriose contents were relatively low (Fig. 2b, c). The former sugar was around 6–8 g/l for all treatments and the latter less of 1 g/l. In the case of maltose, treatment 4 (decorticated red sorghum) reached the lowest concentration after 72 h and the higher level between 30–50 h when maltotriose was present at the lowest concentration (Fig. 2c). At the end of fermentation, residual maltose could be related to glucoamylase inhibition by product

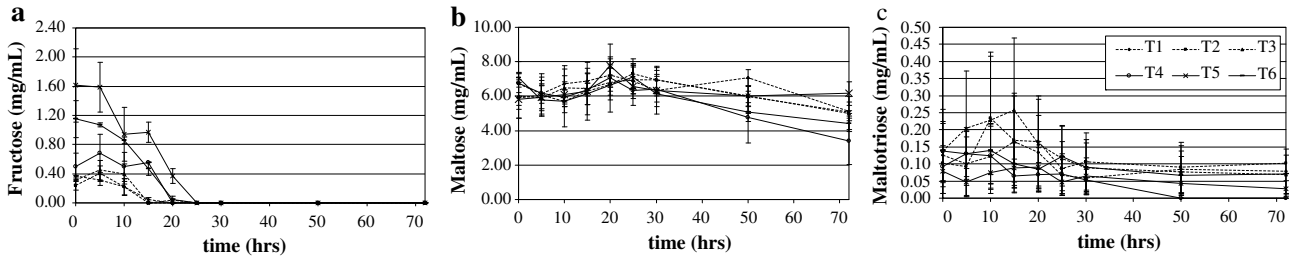


Fig. 2 **a** Fructose, **b** maltose and **c** maltotriose profile during fermentation for all evaluated treatments (*T1* whole red sorghum, *T2* decorticated red sorghum + spent-bran, *T3* decorticated red sorghum + phenolic extract, *T4* decorticated red sorghum, *T5* whole maize and *T6* whole white sorghum)

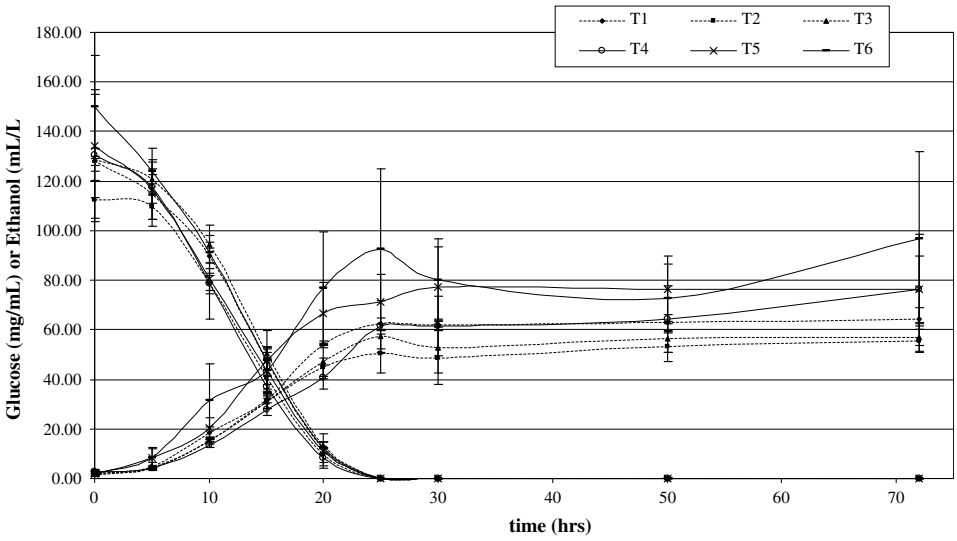


Fig. 3 Glucose consumption and ethanol production profile during fermentation for all evaluated treatments (*T1* whole red sorghum, *T2* decorticated red sorghum + spent-bran, *T3* decorticated red sorghum + phenolic extract, *T4* decorticated red sorghum, *T5* whole maize and *T6* whole white sorghum)

or to glucose inhibition of maltose transport in yeast, a dominant factor for the control of maltose catabolism to ethanol during fermentation. As stated before, the principal substrate present in worts was glucose (Fig. 3) which was totally consumed through the first 25 h of fermentation when the highest amount of ethanol was also produced. The consumption and production profile observed was similar to previous reports (Chuck-Hernandez et al. (2009); Perez-Carrillo et al. 2008). Glucose was also the substrate with the highest rate of consumption mainly compared to maltose and this result is related to transport mechanisms of *S. cerevisiae* mentioned above.

Ethanol yields and fermentation efficiencies for the six treatments are shown in Table 1. Yields for maize and red sorghum were 0.36 and 0.35 ml/g, respectively whereas for the decorticated red sorghum 0.37 ml/g. In terms of ethanol yield expressed per unit of starch, the decorticated with spent bran treatment was the highest followed by red sorghum (0.6 versus 0.58 ml/g, respectively). The higher fermentation efficiency of decorticated with spent bran treatment was probably due to the presence of starch in the bran after phenolic extraction. On the other hand, the lowest yield and efficiency was observed in white sorghum treatment due to its harder endosperm texture (Supplementary results and Supplementary Table 1) which also negatively affected performance during liquefaction. Despite the observed differences in yield and fermentation efficiencies, the statistical analysis showed no significant differences among treatments ($P > 0.05$).

In summary, the effect of sorghum bran and phenolic compounds was studied in sorghum meals subjected to liquefaction with thermostable alpha-amylase, saccharification with glucoamylase/pullulanase and fermentation with *S. cerevisiae*. Red sorghum contained the lowest starch content whereas the white counterpart and maize the hardest endosperm textures. No significant differences were found in FAN and reducing sugars among treatments, nevertheless, maize was slightly higher compared to all

Table 1 Effect of grain type and addition of phenolic extract or spent red sorghum bran to decorticated kernels on ethanol yield and fermentation efficiency

Treatment	ml Ethanol/g flour (mb)	ml Ethanol/g starch (mb)	Fermentation efficiency (%)
Whole red sorghum	0.35 ± 0.04	0.58 ± 0.06	94 ± 9.45
Decorticated red sorghum + spent bran	0.38 ± 0.04	0.61 ± 0.06	98 ± 9.6
Decorticated red sorghum + phenolic extract	0.35 ± 0.08	0.51 ± 0.12	84 ± 19.7
Decorticated red sorghum	0.37 ± 0.04	0.55 ± 0.06	88 ± 9.5
Maize	0.36 ± 0.05	0.53 ± 0.07	86 ± 11.9
Whole white sorghum	0.31 ± 0.04	0.47 ± 0.06	76 ± 10.2

sorghum treatments. No significant differences were found in maltotriose and glucose production profiles during saccharification of liquefied starch. All treatments had residual maltose after saccharification and fermentation. At the end of process, *S. cerevisiae* produced the equivalent of 313–384 l ethanol/ton meal, being the white sorghum treatment the one which had the lowest fermentation efficiency. The harder endosperm texture and coarser particle size distribution of ground meals observed in white sorghum negatively affected performance. Treatments supplemented with spent red sorghum bran (without phenolics) and with added phenolic were not significantly different compared to the decorticated red sorghum in terms of glucose production after liquefaction and saccharification. Thus, results clearly demonstrate that the red sorghum bran and its phenolics did not affect performance of *alpha*-amylase during conventional liquefaction, glucoamylase/pullulanase during saccharification or *S. cerevisiae* during fermentation.

Acknowledgments This research was supported by grants from Fondos Mixtos N.L.-CONACyT NL-2006-C09-33363 "*Creación del centro de investigación y desarrollo de biocombustibles del Estado de Nuevo León: producción de bioetanol y biodiesel a partir de recursos renovables*" and Research Chair Fund CAT-005 from Tecnológico de Monterrey-Campus Monterrey.

References

AOAC (1980) Official methods of the association of official analytical chemists. AOAC, Washington, DC

Awika JM, Rooney LW (2004) Sorghum phytochemicals and their potential impact on human health. Phytochem 65: 1199–1221

Chuck-Hernandez C, Perez-Carrillo E, Serna-Saldivar SO (2009) Production of bioethanol from steam-flaked sorghum and maize. J Cereal Sci 50:131–137

Corredor DY, Bean SR, Schober T, Wang D (2006) Effect of decorticating sorghum on ethanol production and composition of DDGS. Cereal Chem 83:17–21

Duodu KG, Taylor JRN, Belton PS, Hamaker BR (2003) Factors affecting sorghum protein digestibility. J Cereal Sci 38: 117–131

Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428

Naidu K, Singh V, Johnston DB, Rausch KD, Tumbleson ME (2007) Effects of ground corn particle size on ethanol yield and thin stillage soluble solids. Cereal Chem 84:6–9

Perez-Carrillo E, Serna-Saldivar SO, Alvarez MM, Cortes-Callejas ML (2008) Effect of sorghum decortication and use of protease before liquefaction with thermoresistant *alpha*-amylase on efficiency of bioethanol production. Cereal Chem 85:792–798

Serna-Saldivar SO (2010) Cereal grains: properties, processing and nutritional attributes. CRC (Taylor & Francis), Boca Raton

Wu X, Zhao R, Bean SR, Seib PA, McLaren JS, Madl RL, Tuinstra M, Lenz MC, Wang D (2007) Factors impacting ethanol production from grain sorghum in the dry-grind process. Cereal Chem 84:130–136

Journal of Cereal Science 55 (2012) 285–292

Contents lists available at SciVerse ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Conversion into bioethanol of insect (*Sitophilus zeamais* Motschulsky), mold (*Aspergillus flavus* Link) and sprout-damaged maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench)

C. Chuck-Hernández, S. García-Lara, S.O. Serna-Saldívar*

Departamento de Biotecnología e Ingeniería de Alimentos, Centro de Biotecnología, Tecnológico de Monterrey, Av. Eugenio Garza Sada 2501 Sur, CP 64849 Monterrey, N.L., Mexico

ARTICLE INFO

Article history:
Received 26 June 2011
Received in revised form
26 November 2011
Accepted 14 December 2011

Keywords:
Sorghum
Maize
Bioethanol
Postharvest losses

ABSTRACT

The bioconversion into ethanol of insect (*Sitophilus zeamais*), mold (*Aspergillus flavus*) and sprout-damaged maize and sorghum was investigated. Kernel test weight losses due to insect damage in maize were almost twice compared to sorghum (18.6 *vs.* 10.7%). All damaged kernels lost some of the starch and increased soluble sugars, ash and crude fiber. The mold-damaged sorghum contained approximately five times more FAN compared to the control. The sprout-damaged kernels contained the highest amounts of reducing sugars prior (11 g/L) to and at the end (146.5 g/L) of liquefaction with α-amylase. Ethanol yields based on the already damaged grain indicated that sprout-damaged kernels yielded similar amounts compared to sound kernels (381.1 vs. 382.6 L/ton and 376.6 vs. 374.8 L/ton of sorghum or maize respectively). The insect-damaged maize and sorghum have reduced ethanol yields compared with the controls (29 and 23% respectively), and this negative result was mainly due to dry matter losses during the inadequate storage. Despite differences in ethanol yield, all treatments have similar conversion efficiencies (76.1–89.9%) indicating the robustness of yeast facing biotic-damaged feedstocks. This research demonstrates that the use of already damaged insect, mold or sprouted kernels is feasible and a good alternative for biorefineries.

1. Introduction

Worldwide ethanol production is growing at a remarkable pace derived from the rising concern of fuel sustainability and due to environmental problems associated with fossil energy. In 2010, bioethanol production in the USA reached 13 billion gallons, 30% more than the previous year (RFA, 2011), whereas in Brazil 9.7 billion gallons are expected to be distilled in 2012 (Walker, 2011). This unprecedented ethanol production has been supported mainly with maize and sugar cane, respectively.

In Latin America and some areas of Africa, maize is the main source of caloric intake and for this reason its use for biofuels is not socially feasible. In the specific case of Mexico, maize for bioethanol is limited by a federal law, where it is established that only surplus domestic production can be channeled to biorefineries (CD, 2008). However there are other options to produce biofuels such as agricultural wastes and insect, fungi and sprout-damaged kernels which are not fit for human consumption or industrial processing.

In the postharvest system of cereals, some qualitative and quantitative losses occur. Overall postharvest losses in maize can be around 29%, mainly during drying in the field and storage, and can predominantly be attributed to biotic factors such as insects, molds, rodents and sprouting. According to FAO (1993), the range of worldwide postharvest losses is between 10 and 37%. Considering these values, the USA losses are between 33 to 123 and 1 to 3 million tons of maize and sorghum, representing economic losses of $5.5 to 20.1 billion dollars.

One of the main biotic factor associated with these losses are insects, being *Sitophilus zeamais* the main pest in tropical agro-ecologies and also the most harmful, causing 20–40% of losses in stored grain (García-Lara and Bergvinson, 2007). Besides insect damage, fungal infections are also a major postharvest problem causing undesirable effects such as discoloration, off-odors, loss of germination capacity and contamination with harmful mycotoxins. According to Bandyopadhyay et al. (2000), the estimated annual losses due to molds in semi-arid tropical areas of Asia and Africa reached US$130 million. Fungi affecting cereal grains are diverse,

Abbreviations: ANOVA, analysis of variance; DNS, dinitrosalicylic acid method; FAN, free amino nitrogen; FI, flotation index; FID, flame ionization detector; ITESM, Instituto Tecnológico y de Estudios Superiores de Monterrey; RdS, reducing sugars; RH, relative humidity; SSF, Simultaneous Saccharification and Fermentation.

* Corresponding author. Tel.: +52 81 83284322; fax: +52 81 83284262.
E-mail address: sserna@itesm.mx (S.O. Serna-Saldívar).

0733-5210/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jcs.2011.12.007

but the genus *Aspergillus* and *Fusarium* are the most widespread in storage (Serna-Saldívar, 2010). In tropical areas, field and storage sprouting also causes direct and indirect losses due to the activation of degrading intrinsic enzymes that break down proteins, carbohydrates and lipids into simpler forms.

The use of insect, fungi and sprout-damaged grain for human consumption is not always possible and its utilization in other industrial processes could reduce, at least to some extent, the producer losses. Regarding the use of damaged kernels in ethanol production, Yan et al. (2010) tested field-sprouted-sorghum and concluded that the use of these kernels significantly reduced fermentation time and yielded higher ethanol. The detrimental effect of aflatoxins associated with maize mashes was studied by Murthy et al. (2005). These authors concluded that maize containing 775 ppb or less aflatoxins did not affect ethanol yields.

The present study was undertaken to compare the bioethanol conversion efficiency of insect (*S. zeamais*), mold (*Aspergillus flavus*) and sprout-damaged lots of maize and sorghum. These comparisons were made to investigate the behavior of damaged kernels with different chemical compositions in terms of susceptibility to enzyme hydrolyses and efficiency of ethanol production.

2. Materials and methods

2.1. Grain sources and evaluated treatments

Grains used were commercial yellow dent maize and type II red sorghum, obtained from a local market (Monterrey, N.L. Mexico). The undamaged maize and sorghum kernels had soft and intermediate endosperm textures rated as 1.5 and 2.5, respectively (in a subjective scale where 1 is a totally soft and 5 is totally hard endosperm). These grains were not treated with insecticides or fungicides. Four subsamples with three replicas were taken from each type of cereal grain. One was kept as control (sound kernels) whereas the others were purposely damaged with *S. zeamais*, *A. flavus* and sprouting.

2.2. Sample preparation

Original grains were cleaned by air-aspiration and sieves, tempered to 14% moisture and stored in closed polyethylene terephthalate containers at 27 °C and 75% relative humidity (RH).

2.2.1. Insect infestation

For insect infestation, kernels were equilibrated for 30 days at 27 ± 1 °C and 70 ± 5% RH. Then, grains were infested with 200 unsexed adult weevils (*S. zeamais*) no older than 7-days and then stored under controlled conditions (27 °C, 70% RH) with a photoperiod of 12:12 h (dark/light). The infestation experiments were run during 7 weeks or until the level of damage reached 17.6 and 14.8% of dry matter losses for maize and sorghum (53 and 18% of damaged kernels, respectively). Mesh sieves (No. 10 and 16) were used to sort grain and adult weevils. Grain weight loss was calculated by subtracting the final from the initial dry grain weight. Damaged kernels were separated and counted based on visible tunneling or emergence holes. These losses and extent of damage are typically observed in grain elevators in northern Mexico.

2.2.2. Mold inoculation

A. flavus isolate from the experimental field station at Agua Fria, Puebla, Mexico (19° North latitude, 60 m above sea level) was used to inoculate kernels. The fungus was grown on V8 juice agar plates (5% juice – vol/vol – pH 5.2 and 2% agar – wt/vol) at 30 °C in the dark. Conidia from 7-day old cultures suspended in deionized water were used as inoculums. Then, the infection experiment was run during 7 weeks at 27 °C, 75% RH and a dark/light period of 12:12 h. The level of infection was visually evaluated and when the fungi mycelia reached 75% of the grain-container, simulation was finished and grain used for further tests. Both insect and mold infestations were performed at the Entomology and Phytopathology Laboratory facilities at CIMMYT (Texcoco, Estado de Mexico, Mexico) and samples sent to the Biotechnology Center at ITESM (Monterrey, N.L., Mexico) for further analysis and processing.

2.2.3. Grain sprouting

Kernels were steeped in distilled water (1:4) with 0.01% (vol/vol) of formaldehyde during 36 h at 22 °C with constant aeration. In order to avoid mold growth, a 30 ppm sodium hypochlorite water wash was applied. Then, the soaked kernels were spread onto trays covered with wet paper towel and placed in a germination cabinet (Seedburo Equipment Co., Chicago IL, USA) set at 27 °C and >75% RH. Maize and sorghum kernels were sprouted for 36 and 12 h, until they reached 40 and 60% of visually germinated kernels, respectively. Then, the sprouted kernels were dehydrated at 50 °C during 12 h and stored at 4 °C.

2.2.4. Milling

Control and induced-damaged kernels were ground in a Wiley mill equipped with 2 mm round orifices screen. The particle-size distribution of resulting meals was determined by the Rotap sieving method reported by Chuck-Hernández et al. (2009).

2.3. Physical and chemical characterization

Grain physical characteristics were determined using standard procedures: test weight according to Official US Grain Standard Procedures (AACC Method 55-10); thousand-kernel weight by weighing 100 randomly selected kernels and endosperm texture according to the subjective procedure reported by Chuck-Hernández et al. (2009). Flotation index (FI) was determined according to Gutiérrez-Uribe et al. (2010) and expressed as a percentage of floating kernels on an aqueous solution of sodium nitrate (1.25 g/cm^3 specific weight at 35 °C). L^*, a^*, b^*, and other CIE color parameters of ground samples were determined using a colorimeter (Minolta CR-300, Osaka, Japan). Moisture content was assayed using a gravimetric method AACC (2000) 44-15A. Total starch was calculated using a commercial kit (Method 76-13, AACC, 2000, Megazyme International, Wicklow, Ireland). Protein (N*6.25) was determined using the micro-Kjeldhal method 46-13 whereas crude fiber, fat, and ash were assayed according to methods 32-10, 30-20 and 08-01, respectively (AACC, 2000).

2.4. Liquefaction

Ground meals (15 g dry basis) with 0.02% of calcium hydroxide were mixed with distilled water to obtain mashes with 30% (wt/vol) solids. pH was initially adjusted to 5.6 with 0.1N HCl and temperature was increased to 85 °C in a shaking water bath (BellCo Glass, Vineland, NJ). When slurries reached 80 °C, 25 μL of Liquozyme (240 KNU-S/g, Novozymes, Bagsvaerd, Denmark) was added. Mashes were maintained at 90 °C during 200 min. In order to determine the progressive extent of starch hydrolysis, aliquots were taken before enzyme addition, and after 100 and 200 min hydrolysis.

2.5. Simultaneous Saccharification and Fermentation (SSF)

Mashes were cooled down to 30 °C, adjusted to 15°Plato and pasteurized (65 °C for 30 min). A commercial mixture (Dextrozyme DX, Novozymes) of glucoamylase (EC 3.2.1.3) and pullulanase (EC

3.2.1.14) produced from genetically modified strains of *Aspergillus* and *Bacillus* was added altogether with dry yeast (*Saccharomyces cerevisiae*) (Nevada, Safmex, Toluca, Mexico). Dextrozyme was added at a rate of 1 mL/100 mL of liquefied slurry whereas the yeast was previously suspended in sterile water and pitched at a cell concentration of 1.5×10^7 cells/mL according to Chuck-Hernández et al. (2009). Reaction vessels were sealed and maintained for 72 h in an incubator-shaker (VWR International, Model RF1575) set at 30 °C with agitation (100 rpm) during the first 24 h.

2.6. Analysis of hydrolyzates and fermented mashes

Free amino nitrogen (FAN) during liquefaction and SSF was determined using the ninhydrin method 945.30 L (AOAC, 1980). Total reducing sugars during liquefaction were determined with the dinitrosalicylic acid method (DNS) of Miller (1959). Ethanol concentration after fermentation was determined by gas chromatography (GC Agilent 6850) with Flame Ionization Detector (FID) at 220 °C. The GC was furnished with an HP Innowax column (30 m × 0.53 mm × 1.0 μm) and helium at 4.0 mL/min was used as carrier. Injection volume was 0.2 μL at a split rate of 1:50. Maltotriose, maltose, glucose, fructose and glycerol were quantified throughout 72 h SSF by HPLC-IR (Waters 2114, Milford, MA) furnished with an ion-exchange column (Aminex HPX-87H, Bio-Rad®, Hercules, CA) at 60 °C using 5 mM sulfuric acid at a rate of 0.6 mL/min as mobile phase (Chuck-Hernández et al., 2009).

2.7. Statistical analysis

All determinations were performed in triplicate and data analyzed with ANOVA (Minitab 14, 2004). The variables studied were type of grain and damage. Additionally, the generation of FAN throughout liquefaction was statistically analyzed. Means were compared with Tukey's test ($alpha = 0.05$).

3. Results and discussion

3.1. Physical and chemical analysis

The initial test weights of maize and sorghum were within ranges specified by Serna-Saldívar (2010), indicating that both types of kernels were initially sound and healthy. As expected, all sorts of damaged kernels had a lower bulk density and average kernel weight (Table 1). Test weight losses due to insect damage in maize were higher (18.6%) compared to sorghum (10.7%). Insect damage and sprouting were more detrimental in maize compared to sorghum. The observed difference could be associated with the softer endosperm texture of maize and with the higher resistance of the sorghum pericarp which contains more phenolics. These compounds are related to kernel resistance through two main mechanisms: physical (hydroxycinnamic acids bound to cell wall) and toxicological (due to the effect of phenolic acid amides in the invasive organism) (Bergvinson and García-Lara, 2011; García-Lara et al., 2004). According to Dicko et al. (2006), fifty different sorghum varieties contained from 0.5 to 3% (w/w) of total phenolics and these compounds were mainly associated with pericarp cell walls. Phenolics have an important role in protecting kernels against biotic and abiotic stresses (Serna-Saldívar, 2010). Ramputh et al. (1999) reported a positive relationship between phenolic content of sorghum and *Sitophilus oryzae* resistance.

The FI was also greatly affected by the different sorts of damages. This parameter estimates the relative density of the grain and therefore is an indicator inversely related to grain hardness (Roselena et al., 2009). In ethanol production, an intermediate or soft endosperm texture is desired because these kernels usually have a higher proportion of starch (Serna-Saldívar, 2010). In all treatments, an increase of FI was observed because of the reduction of density due to endosperm damage or starch consumption or degradation (Tables 1 and 2). According to Lozano-Alejo et al. (2007), soft and intermediate-textured maize and sorghum, such as the ones used herein, had FI of 85 and 17.5%, respectively. A subjective endosperm texture evaluation was also performed and the results were in agreement with the observed FI and test weights. The difference in endosperm texture also affected the particle-size distribution after milling (data not shown). The sorghum control treatment yielded a flour with higher amounts of the coarse fraction (>850 μm) compared to maize (45 versus 35% respectively).

The thousand-kernel weight was severely affected by the different types of damage. This parameter is an important quality criterion because a larger grain usually contains a higher proportion of endosperm and starch. Both types of kernels were within ranges reported previously (240–370 g for maize and 23–35 g for sorghum) (Serna-Saldívar, 2010). The loss of weight for insect and sprout-damaged maize and sorghum was between 8.5 and 17.6%, indicating the effect of nutrient consumption because of the insect infestation and the activation of grain metabolism during germination. Sprout-damaged maize and sorghum lost 11 and 8.5% of the kernel weight compared to sound counterparts whereas insect-damaged maize and sorghum, 17.6 and 14.8%, respectively. The mold-infested kernels were heavier due to the purposely added conditioning moisture, even though maize and sorghum lost 3.8 and 7.9% of their dry matter weight, respectively.

The color difference, obtained as a distance in relation to control treatments in the CIE $L^*a^*b^*$ space color, was higher in the meals from sprouted kernels (Table 1). These results can be related to changes in carbohydrate composition during germination. During this physiological process, part of the starch is hydrolyzed into reducing sugars and subsequently these sugars react with simpler nitrogenous compounds forming color species (Maillard reaction). These changes are exacerbated by thermal treatments such as the ones used for drying and liquefaction.

Color changes in insect and mold-damaged treatments were not as severe as those observed for sprouted kernels. However, ground meals contaminated with mycelia from *A. flavus* had color indexes of −5.16 and −1.00 for maize and sorghum, respectively. According to Vignoni et al. (2006), these values are within the range of green to yellow-greenish, close to the characteristic or typical coloration of *A. flavus* conidia.

All hue or tone values, except the one observed for sprouted ground maize, were similar (Table 1). In contrast, chroma values were significantly lower for mold and sprout-damaged maize. In the case of sorghum, all treatments were significantly different compared to the control. Sprouting promoted the highest difference especially after drying, likely due to Maillard reaction.

Regarding color indexes, all maize treatments had similar values, ranging from green to yellow. The sprouted-maize was the only treatment with a positive color index (in the range of yellow to orange). Likewise, the control and insect-damaged sorghums had color indexes similar to the sprouted-maize. The color index of mold-infested sorghum went down to a green-yellow tone because of the presence of mycelium. The largest color index change was observed in the ground sprouted-sorghum. The corresponding value indicated colorations between orange to intense red rather than the original orange coloration.

As expected, the chemical composition was also affected by insects, molds and sprouting (Table 2). Moisture, by far the most important factor influencing the rate of deterioration, increased from 14% in the controls to 18 and 21% in mold-damaged maize and sorghum, respectively. The insect-damaged maize and sorghum

Table 1
Effects of insect, mold and sprout – damages on physical properties and color of maize and sorghum.[a]

Samples			Test weight, kg/hL[b]	Flotation index[c], %	1000 kernel weight[c], g	Color values[d]			
						Color difference, ΔE	Hue (h°)[c]	Chroma (C)[c]	Color index (CI)[c]
Maize	Control		72.43 ± 0.33a	85.00 ± 2.88a	287.50 ± 1.99a	NA	−1.42 ± 0.00a	35.15 ± 0.00a	−2.70 ± 0.00b
	Damaged	*A. flavus*	62.26 ± 0.40b	100.00 ± 0.00b	292.18 ± 2.16a	6.49 ± 2.06	−1.30 ± 0.05a	30.70 ± 1.76ab	−5.16 ± 0.89c
		S. zeamais	58.91 ± 0.40c	100.00 ± 0.00b	245.34 ± 2.71c	7.45 ± 2.34	−1.39 ± 0.01a	40.44 ± 1.88a	−3.01 ± 0.03bc
		Sprouted	58.49 ± 0.15c	100.00 ± 0.00b	257.73 ± 1.31b	14.2 ± 0.53	1.38 ± 0.00b	25.97 ± 0.81b	3.27 ± 0.09a
Sorghum	Control		75.68 ± 0.28d	17.50 ± 2.50c	24.67 ± 0.52d	NA	1.39 ± 0.00c	24.12 ± 0.00c	4.90 ± 0.00d
	Damaged	*A. flavus*	67.58 ± 0.23e	63.33 ± 6.67d	24.78 ± 0.29d	6.32 ± 1.05	−0.48 ± 1.02c	21.87 ± 0.29d	−1.00 ± 0.91e
		S. zeamais	67.54 ± 0.91e	44.43 ± 7.28d	21.39 ± 0.24e	2.36 ± 0.90	1.39 ± 0.02c	21.91 ± 0.86d	4.98 ± 0.52d
		Sprouted	72.91 ± 0.12a	63.33 ± 1.67d	20.17 ± 0.52e	11.61 ± 0.34	0.95 ± 0.02c	27.02 ± 0.31e	18.67 ± 1.05f

NA – Not apply.
[a] Means are the average of at least three replicas ± standard error.
[b] Means with different letter(s) within column are statistically different ($P < 0.05$).
[c] Means with different letter(s) within kernel type are statistically different ($P < 0.05$).
[d] Color values were estimated on ground samples. Color difference (ΔE) = $\sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$; Hue ($h^\circ$) = arctangent ($b/a$); Chroma ($C$) = $\sqrt{a^2 + b^2}$; Color index (CI) = (a^*1000/L^*b).

contained 17 and 15% moisture. In the case of sprouting, moisture was purposely increased to 41 and 35% in maize and sorghum respectively in order to activate gibberellins and enzyme activity. After achieving the desired level of sprouting, kernels were dehydrated to stop metabolic processes and stabilized kernels (Table 2). The moisture increment observed in insect or mold-infested kernels was mainly due to respiration. Serna-Saldívar (2010) indicates that insects proliferate when the grain moisture exceeds only 1.5% of the critical moisture content (14% in cereals). The degree of insect infestation is mainly affected by the grain moisture, storage conditions (temperature, RH and controlled atmosphere), length of storage and extraneous material among other factors. Insect-infested sorghum contained significantly lower moisture compared to the maize counterpart. Thus, *S. zeamais* presumably had less available water compared to counterparts developing in maize.

In the case of *A. flavus* infested kernels, both sorghum and maize, showed all the typical signs of contamination such as discoloration, formation of kernel clusters trapped with a web-like mycelium and an increase in moisture due to its own metabolic activity. It is well-known that the growth of storage fungi occurs when the grain moisture exceeds 3.5% above the critical moisture. It is likely that *A. flavus* exacerbated its growth and activity due to the generation of metabolic energy (temperature) and moisture (product of respiration).

The crude protein was higher in sound sorghum compared to maize (11.5 versus 7.9%). The protein is substrate for the production of simpler nitrogenous compounds or FAN. Despite a significant increase in FAN concentration (Table 4) in all damaged kernels, the protein content remained stable. In maize, all damaged treatments had almost twice the initial level of simpler nitrogenous compounds whereas the mold-damaged sorghum contained approximately five times more FAN compared to sound kernels. This difference is attributed to the potent mold proteolytic enzymes that hydrolyzed to a higher extent sorghum proteins compared to maize.

In relation to crude fiber (Table 2), the control kernels were within the ranges reported (2.4–3.5% in maize and 1.2–6.6% dry basis in sorghum) (Serna-Saldívar, 2010). The insect-damaged kernels contained higher crude fiber due to the reduction in starch, indicating that *S. zeamais* had preference for the starchy endosperm.

The control and mold-damaged maize treatments contained similar amounts of crude fat. These amounts are below the range indicated in the literature (3.9–5.8%) and can be related to the relative size of germ, where about 80–85% of total fat content is stored (Serna-Saldívar, 2010). The similar amounts of fat found in the mold-infested maize compared with the control treatment, indicate that this microorganism had preference for starch rather than fat as nutrient source. However, the crude fat slightly increased in insect and sprout-damaged maize kernels (3.5 and 3.1% respectively) and in both cases, these compositional changes can be associated with the analogous reduction in starch (Table 2). The crude fat in control and damaged sorghum was approximately 2%, values within the range of 0.5–5.2% reported by Serna-Saldívar (2010).

Most of the minerals are located in the pericarp, aleurone layer and germ; thus, the ash content in insect and mold-damaged kernels was expected to increase. The ash in sound maize and sorghum was around 1.5%, corresponding to values previously reported (Serna-Saldívar, 2010). The insect and mold-damaged maize contained higher amounts of minerals. However, this effect

Table 2
Effects of insect, mold and sprout – damages on the chemical properties of maize and sorghum.[a]

Samples			Moisture, %	Crude protein (N × 6.25), %	Crude fiber, %	Crude fat, %	Ash, %	Nitrogen free Extract[b], %	Total starch, %
Maize	Control		14.19 ± 0.08a	7.91 ± 0.40a	1.57 ± 0.24a	2.51 ± 0.02a	1.43 ± 0.03a	86.58 ± 0.30a	72.31 ± 3.57a
	Damaged	*A. flavus*	18.76 ± 0.02b	9.41 ± 0.25b	2.80 ± 0.45ab	2.45 ± 0.07a	1.65 ± 0.03b	83.69 ± 0.46b	71.91 ± 1.04a
		S. zeamais	17.11 ± 0.07c	9.93 ± 0.15bc	3.33 ± 0.46b	3.50 ± 0.14b	1.86 ± 0.05b	81.38 ± 0.71c	68.19 ± 1.29a
		Sprouted	8.84 ± 0.07d	8.59 ± 0.1abc	2.43 ± 0.24ab	3.16 ± 0.12b	1.32 ± 0.05a	84.50 ± 0.46ab	68.09 ± 0.10a
Sorghum	Control		14.09 ± 0.06ef	11.51 ± 0.04c	1.36 ± 0.18c	1.98 ± 0.41c	1.58 ± 0.05c	83.57 ± 0.5d	73.65 ± 0.06b
	Damaged	*A. flavus*	21.23 ± 0.17e	11.56 ± 0.15c	2.27 ± 0.21d	1.64 ± 0.20c	1.44 ± 0.06c	83.09 ± 0.36d	69.96 ± 0.81b
		S. zeamais	15.64 ± 0.02f	12.04 ± 0.25cd	3.24 ± 0.16de	2.61 ± 0.08c	1.41 ± 0.03c	80.72 ± 0.22e	70.17 ± 1.54b
		Sprouted	7.95 ± 0.01g	10.45 ± 0.39ce	2.42 ± 0.05de	1.27 ± 0.43c	1.05 ± 0.02d	84.82 ± 0.75d	68.84 ± 0.28b

[a] All values are expressed on dry matter basis and are the average of at least three replicas ± standard error of the mean. Means with different letter(s) within kernel type were statistically different ($P < 0.05$).
[b] Calculated by difference 100 – % crude protein – % crude fiber – % crude fat – % ash.

Table 3
Effects of insect, mold and sprout – damages on the amount of reducing sugars [RdS] (g/L) generated at 0, 100 and 200 min of liquefaction.[a]

Sample			Hydrolysis time (min)			Reducing sugars obtained at the end of liquefaction/Total theoretical glucose based on initial starch (%)
			0	100	200	
Maize	Control		1.44 ± 0.18a	121.03 ± 10.51ab	122.59 ± 12.89a	40.73 ± 4.28a
	Damaged	*A. flavus*	1.32 ± 0.26a	112.14 ± 5.98b	108.90 ± 7.09a	36.38 ± 2.37ab
		S. zeamais	4.44 ± 1.56ab	102.89 ± 3.23b	108.78 ± 6.16a	38.32 ± 2.17a
		Sprouted	11.05 ± 4.93b	131.06 ± 5.87ab	125.48 ± 8.46a	44.27 ± 2.98a
Sorghum	Control		0.84 ± 0.52a	124.34 ± 5.44ab	125.78 ± 6.94a	41.03 ± 2.26a
	Damaged	*A. flavus*	0.54 ± 0.45a	107.64 ± 11.36b	112.62 ± 12.22a	38.67 ± 4.20a
		S. zeamais	0.96 ± 0.69a	114.90 ± 4.68ab	126.92 ± 7.42a	43.45 ± 2.54a
		Sprouted	0.54 ± 0.36a	146.56 ± 2.68a	149.02 ± 4.36a	52.01 ± 1.52ac

[a] All values are expressed on dry matter basis and are the average of at least three replicas ± standard error of the mean. Means with different letter(s) within columns were statistically different ($P < 0.05$).

was not observed in the sorghum counterparts. The higher dry matter loss and damage observed in maize is the most reasonable explanation for the observed differences. As expected, the nitrogen free extract (NFE), highly related to starch content in cereal grains, was significantly lower in both insect-damaged maize and sorghum kernels. The highest NFE concentrations were observed in the controls and sprouted kernels.

Starch is the most important component of the grain for ethanol production and the main substrate consumed by contaminating microflora and insects and is also an indicator of intrinsic enzyme activity due to germination. For both types of kernels, all sorts of damages significantly reduced starch. The sprout-damaged kernels were the most affected with 5.8 and 6.5% less starch compared to the maize and sorghum controls, respectively. Interestingly, the starch content was inversely related to the reducing sugar concentration observed prior to liquefaction (Table 3).

3.2. Reducing sugars (RdS) and FAN during liquefaction

At the start of liquefaction, maize contained higher RdS compared to sorghum. As expected, the sprouted-maize originally contained the highest amounts of RdS (approximately seven times more compared to sound kernels). This phenomenon was not observed in sprouted-sorghum (Table 3) and can be associated with the differences in metabolic activation and sequence of the appearance of amylases during germination. Both α and β-amylases appear at the end of sprouting (after phytases, lipolytic, fibrolytic and proteolytic enzymes) (Serna-Saldívar, 2010). Yan et al. (2009) germinated high-tannin sorghum during three and four days and reported an increase of 2.2 and 4.2 times more fermentable sugars. The notorious difference between sprouted-maize and sorghum can be attributed to the low β-amylase concentration and activity generally associated with sorghum (Beta et al., 1995). This enzyme is mainly synthesized at the end of malting and its activity is mainly associated with the acrospires.

A significant increase in RdS at 100 and 200 min of liquefaction of sprouted-sorghum mashes occurred due to the intrinsic amylases or to the better accessibility of the exogenous α-amylase. The difference after 100 min liquefaction was not observed in maize, implying that sorghum sprouting can be a good pretreatment for bioethanol production.

A ratio of RdS versus total theoretical glucose was calculated as a parameter of liquefaction efficiency. The most effective treatments were the sprouted-sorghum and maize followed by insect-damaged sorghum (26, 8 and 6% more RdS compared to the controls). The lowest efficiency was observed in mold-damaged maize with 11% less RdS (Table 3).

The FAN was also evaluated at the beginning, half and last part of liquefaction. This parameter is a good indicator of the nitrogenous compounds available for yeast growth during fermentation. Nitrogen deficiencies can lead to sluggish or incomplete fermentations and in the case of maize and sorghum mashes, the main FAN sources are hydrolyzed endosperm storage proteins (Thomas and Ingledew, 1990). FAN is also an indicator of protein matrix proteolysis, event that increases the available surface area of starch granules to amylases, improving efficiency of liquefaction and saccharification (Vidal et al., 2011).

Despite having the highest protein, the control sorghum contained the lowest FAN concentration both at the start and end of liquefaction. The difference in relation to maize has been already observed by other authors (Pérez-Carrillo and Serna-Saldívar, 2007) and is attributed to the kafirin cross-linking during thermal treatments and to the higher phenol concentration generally observed in sorghum. These compounds have a high affinity for kafirins, decreasing the rate of protein hydrolysis. It is well-known that sorghum is the cereal with the lowest protein digestibility and

Table 4
Effects of insect, mold and sprout – damages on the free amino nitrogen production during liquefaction (mg/L).[a]

Sample			Hydrolysis time (min)[b,c]		
			0	100	200
Maize	Control		111.67 ± 4.86a	97.46 ± 3.68e	101.58 ± 5.55ad
	Damaged	*A. flavus*	191.67 ± 12.63c*	135.18 ± 13.6de	131.75 ± 8.89d
		S. zeamais	250.88 ± 6.94b*	174.91 ± 9.14cd	191.23 ± 11.06c
		Sprouted	248.25 ± 14.52b*	183.42 ± 6.22c	173.33 ± 7.40c
Sorghum	Control		81.84 ± 3.93a	72.54 ± 2.36a	76.84 ± 1.46a
	Damaged	*A. flavus*	389.47 ± 4.87d*	293.07 ± 7.75b	314.74 ± 5.66b
		S. zeamais	130.26 ± 4.64a	106.31 ± 2.39ade	121.84 ± 8.01ad
		Sprouted	96.05 ± 11.23a	76.84 ± 8.85ae	93.77 ± 5.90ad

[a] All values are expressed on dry matter basis and are the average of at least three replicas ± standard error of the mean.
[b] Means with different letter(s) within columns were statistically different ($P < 0.05$).
[c] Means within rows marked with an asterisk (*) are statistically different ($P < 0.05$).

that this problem is intensified by wet-cooking similar to liquefaction. Besides the interactions of protein with non-protein components such as polyphenols, the endogenous factors such as the nature of sorghum proteins and their organization within the endosperm cells seems to play an important role in the rate of digestibility. This condition can be surmounted with the use of external proteases during liquefaction (Pérez-Carrillo and Serna-Saldívar, 2007). Despite no external proteases being added in this research, the use of protease-producer organisms (*A. flavus* and *S. zeamais*), and proteases generated during sprouting, is the most logical explanation for the higher FAN assayed in damaged kernels (Table 4). The maize proteins were more prone to hydrolysis and produced more FAN compared to the sorghum treatments, except for the mold-damaged sorghum. This exception is noteworthy because it indicates the strong proteolytic activity of *Aspergillus* that together with a higher initial protein content, allowed an elevated FAN concentration which is correlated with fermentation efficiency (Pérez-Carrillo and Serna-Saldívar, 2007). At the end of liquefaction, mashes produced from mold-damaged sorghum contained the highest FAN followed by the insect and sprouted-maize samples. This finding is significant for industrial processes because feedstocks with high FAN levels prevent nitrogen supplementation before fermentation. Generally, the recommended FAN level is 150 mg/L (Thomas and Ingledew, 1990).

3.3. FAN, glucose, glycerol, ethanol and fermentation efficiency during SSF

The mashes used in SSF had an original pH of 5.6–5.9, except for the mold-damaged sorghum which was slightly more acidic (pH 5.45). This is below the average of the other treatments, probably because of the metabolites produced by *A. flavus*. This mold has potent lipases that hydrolyze stored triglycerides to fatty acids. Thus, both FAN and pH assays can be useful to evaluate the extent of grain damage.

During SSF of mashes adjusted to 15°Plato, FAN was assayed at the beginning and at the end of fermentation (Fig. 1). In the case of maize, mashes produced from damaged kernels showed a higher FAN compared to the control. This difference was not observed in sorghum except in mold-damaged kernels which contained 110 mg/L (almost twice the amount detected in the rest of the sorghum treatments). At the end of SSF, all treatments had almost the same leftover FAN (10–20 mg/L), indicating that yeast metabolized these nitrogenous compounds. Despite the differences in initial FAN, this parameter was not positively correlated with fermentation efficiencies.

The concentrations of glucose and ethanol at the beginning and end of SSF are depicted in Table 5. The initial glucose in all worts was between 130 and 140 g/L. After 72 h fermentation, all the glucose was metabolized by the fermenting yeast. This indicates a complete and high efficient fermentation and the effectiveness of the liquefaction step that rendered dextrins highly susceptible to amyloglucosidase and pullulanase. Therefore, the presence of *A. flavus*, *S. zeamais* and intrinsic enzymes did not affect yeast and α-amylase or amyloglucosidase/pullulanase activities.

The final ethanol concentration varied from 81.43 mL/L in mold-damaged maize to 92.31 and 95.49 mL/L in the sound maize and sorghum, respectively. The sprout-damaged kernels had the highest fermentation efficiencies followed by the controls, indicating the positive effect of intrinsic enzymes generated during sprouting. The processing of sprout-damaged kernels also saved liquefaction time (Table 3), increased FAN (Table 4) and produced similar amounts of ethanol compared to sound kernels. The similar fermentation efficiencies between sorghum and maize differed from previous research works (Pérez-Carrillo and Serna-Saldívar, 2007). These authors obtained 26% less ethanol from red sorghum compared to maize (279 versus 380 L/ton, respectively). Fermentation efficiencies are indeed tightly related to grain composition. Wu et al. (2007), working with different sorghum cultivars obtained efficiencies from 85.2% for high-tannin varieties to 90.2% for waxy and "high yield" samples. In maize, the detrimental influence of amylose content in fermentation has been reported. Wu et al. (2006) achieved 88.7% conversion for normal starch maize while, for a fermentation media with 35, 55 and 70% of amylose, efficiencies were 80.4, 61.8 and 52.9% respectively. Thus, the similar conversions between maize and sorghum can be related to grain composition (Table 5).

Interestingly, fermentation efficiencies (Table 5) seem to be slightly lower compared to previous reports. This can be associated with the temperature used during liquefaction. For example, Wu et al. (2006) employed a cooking temperature of 95 °C during 45 min before α-amylase addition whereas liquefaction in this study was conducted at temperatures that never exceeded 90 °C. A higher temperature enhances starch gelatinization and susceptibility to enzymatic hydrolysis and therefore is directly related to

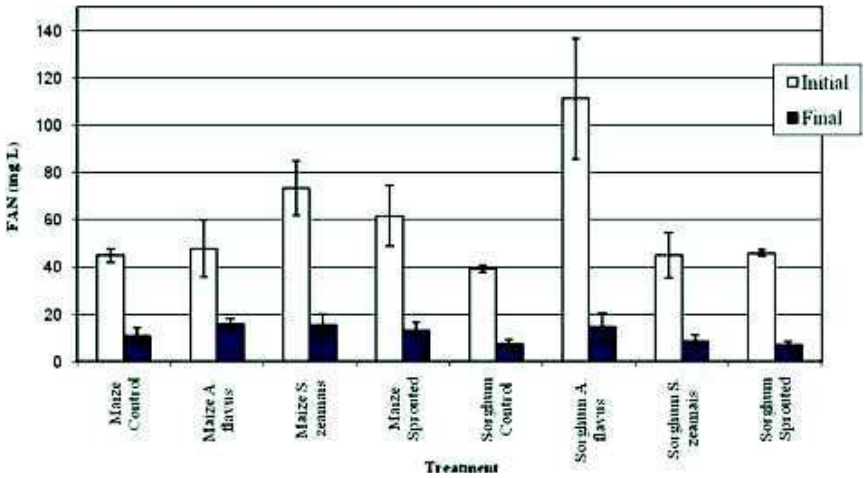


Fig. 1. Free Amino Nitrogen (FAN) concentration (mg/L) at the beginning and at the end of SSF.

Table 5
Effects of insect, mold and sprout – damages of maize and sorghum on glucose (before fermentation) and ethanol, glycerol and ethanol yields and efficiencies obtained after 72 h fermentation.[a]

Samples			Glucose (mg/mL)	Ethanol (mL/L)	Glycerol[b] (mg/mL)	Fermentation efficiency (%)	Ethanol yield[c]		
							(L/ton starch)	(L/ton of already damaged grain)	(L/ton grain)[d]
Maize	Control		133.11 ± 4.86a	92.31 ± 8.13a	9.00 ± 0.86a	84.24 ± 2.44a	520.79 ± 15.09a	376.59 ± 10.91a	376.59 ± 10.91ab
	Damaged	*A. flavus*	127.63 ± 9.95a	81.43 ± 2.87a	10.65 ± 0.23a	77.54 ± 3.84a	479.40 ± 23.71a	344.74 ± 17.05a	331.70 ± 16.41a
		S. zeamais	128.50 ± 7.68a	83.09 ± 2.51a	10.37 ± 0.17a	76.10 ± 1.63a	470.47 ± 10.10a	320.81 ± 6.89a	264.44 ± 5.68c
		Sprouted	133.56 ± 6.51a	90.11 ± 4.94a	9.73 ± 0.08a	89.04 ± 1.07a	550.48 ± 6.59a	374.82 ± 4.49a	333.67 ± 4.00a
Sorghum	Control		140.02 ± 2.30a	95.49 ± 10.02a	9.89 ± 1.03a	83.70 ± 7.86a	517.48 ± 48.57a	381.12 ± 35.77a	381.12 ± 35.77ab
	Damaged	*A. flavus*	132.56 ± 1.31a	91.65 ± 8.31a	8.74 ± 1.13a	81.83 ± 6.65a	505.95 ± 41.10a	353.96 ± 28.75a	326.00 ± 26.48a
		S. zeamais	134.89 ± 1.83a	87.14 ± 1.94a	10.92 ± 0.07a	78.72 ± 1.28a	486.67 ± 7.91a	341.50 ± 5.55a	290.75 ± 4.73abc
		Sprouted	141.2 ± 5.95a	94.12 ± 8.71a	10.78 ± 0.08a	89.90 ± 8.18a	555.78 ± 50.55a	382.60 ± 34.80a	349.85 ± 31.82a

[a] All values are expressed on dry matter basis and are the average of at least three replicas ± standard error of the mean. Means with different letter(s) within columns were statistically different ($P < 0.05$).
[b] The initial glycerol concentration was zero.
[c] Yields calculated on 14% moisture basis.
[d] Ethanol yields considering solid losses incurred during storage.

conversion efficiencies, mainly in kernels containing high amylose (Wu et al., 2006).

Glycerol, determined at the end of fermentation (Table 5), is a by-product of sugar metabolism. The role of this alcohol is to maintain the cytosolic redox balance by consuming NADH during its formation. According to Vriesekoop et al. (2009), an elevated glycerol is observed in fermentations under osmotic stress conditions, where it accumulates intracellularly acting as a stress protectant. Other authors indicate that glycerol differences are more related to yeast strain rather than compositional changes in the fermentation media (Attfield and Kletsas, 2000). As expected, there were no differences in glycerol concentration in fermented mashes and this is a sign of the adequate initial sugar concentration in worts for the fermenting yeast.

The calculation of ethanol yields/ton initial grain indicated that sprout-damaged kernels yielded similar amounts compared to sound kernels (381 and 376 L/ton of sorghum or maize grain – wet basis, respectively). For insect and mold-damaged kernels, a reduction in yield was indeed observed. A decrease of 29 and 11% was calculated in the case of insect and sprout-damaged maize, respectively. The use of insect-damaged sorghum reduced yield around 23%. These findings are crucial in geographic regions where *S. zeamais* is the principal infesting agent and indicates the robustness of sorghum against this particular insect. The negative impact of the use of insect or mold-damaged kernels is mainly due to dry matter losses incurred during storage and stresses the importance of first-rate storage practices. However, this research clearly demonstrates the use of already damaged kernels is feasible. These kernels can be acquired at a discount price by biorefineries and subsequently converted into bioethanol with similar efficiencies. Undoubtedly, the sprout-damage was the least detrimental because these kernels yielded similar amounts of ethanol compared to sound kernels. Comparatively, these kernels were more susceptible to amylases and thus can save hydrolysis and fermentation times.

4. Conclusions

This research demonstrated that there was a clear negative impact of insect, molds and intrinsic enzymes in the physical and chemical composition of maize and sorghum. Insect and mold-damaged kernels were mainly affected at the endosperm level. These kernels had reduced amounts of starch and NFE and higher levels of reducing sugars and FAN. There was no evidence of germ attack because the crude fat remained at the same concentration. Sprouted grains with high enzymatic activity had clear evidence of degradation of both starch and proteins. The use of sprouted kernels reduced hydrolysis time needed to achieve optimum reducing sugar concentration and enhanced ethanol yields. Conversions into bioethanol of insect, mold and sprout-damaged maize and sorghum were lower calculated in relation to the original grain weight, indicating that the dry matter loss incurred during storage was the main reason for the observed detrimental effect. Nevertheless, all already damaged kernels had similar fermentation efficiencies, indicating that these feedstocks are suitable for fuel ethanol production.

Acknowledgments

This research was supported by the Research Chair Funds CAT-005 from Tecnologico de Monterrey-Campus Monterrey and the PhD scholarship to C. Chuck-Hernández by CONACyT and Tecnologico de Monterrey. We acknowledge technical support from George Mahuko, Emiliano Gutiérrez and Carlos Muñoz (CIMMYT Entomology and Phytopathology Laboratory).

References

AACC, 2000. Approved Methods of the AACC, tenth ed. American Association of Cereal Chemists, St. Paul, MN.
AOAC, 1980. Official Methods of the Association of Official Analytical Chemists. AOAC, Washington, DC.
Attfield, P.V., Kletsas, S., 2000. Hyperosmotic stress response by strains of bakers' yeasts in high sugar concentration medium. Letters in Applied Microbiology 31, 323–327.
Bandyopadhyay, R., Butler, D.R., Chandrasekhar, A., Reddy, R.K., Navi, S.S., 2000. Biology, epidemiology and management of sorghum grain mold. In: Chandrashekar, A., Bandyopadhyay, R., Hall, A.J. (Eds.), Technical and Institutional Options for Sorghum Grain Mold Management: Proceedings of an International Consultation, 18–19 May 2000. ICRISAT, Patancheru, India, pp. 34–71.
Bergvinson, D.J., García-Lara, S., 2011. Synergistic effects of insect-resistant maize and *Teretrius nigrescens* on the reduction of grain losses caused by *Prostephanus truncatus* (Horn.). Journal of Stored Products Research 47, 95–100.
Beta, T., Rooney, L.W., Waniska, R.D., 1995. Malting characteristics of sorghum cultivars. Cereal Chemistry 72, 533–538.
CD (Cámara de Diputados), 2008. Ley de Promoción y Desarrollo de los Bioenergéticos Available at: http://www.diputados.gob.mx/LeyesBiblio/pdf/LPDB.pdf (accessed 05.10.11.).
Chuck-Hernández, C., Pérez-Carrillo, E., Serna-Saldívar, S.O., 2009. Production of bioethanol from steam-flaked sorghum and maize. Journal of Cereal Science 50, 131–137.
Dicko, M.H., Gruppen, H., Traoré, A.S., Voragen, A.G.J., Van Berkel, W.J.H., 2006. Phenolics compounds and related enzymes as determinants of sorghum for food use. Biotechnology and Molecular Biology Reviews 1, 21–38.
FAO, 1993. La ingeniería en el desarrollo – Manejo y tratamiento de granos poscosecha Available at: http://www.fao.org/docrep/x5041s/x5041S00.htm#Contents (accessed 05.30.11.).

García-Lara, S., Bergvinson, D.J., 2007. Programa integral para reducir pérdidas poscosecha en maíz. Agricultura Técnica en México 33, 181–189.
García-Lara, S., Bergvinson, D.J., Burt, A.J., Ramputh, A.I., Diaz-Pontones, D.M., Arnason, J.T., 2004. The role of pericarp cell wall components in maize weevil resistance. Crop Science 44, 1546–1552.
Gutiérrez-Uribe, J.A., Rojas-García, C., García-Lara, S., Serna-Saldívar, S.O., 2010. Phytochemical analysis of wastewater (nejayote) obtained after lime-cooking of different types of maize kernels processed into masa for tortillas. Journal of Cereal Science 52, 410–416.
Lozano-Alejo, N., Carrillo, G.V., Pixley, K., Palacios-Rojas, N., 2007. Physical properties and carotenoid content of maize kernels and its nixtamalized snacks. Innovative Food Science & Emerging Technologies 8, 385–389.
Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry 31, 426–428.
Murthy, G.S., Townsend, D.E., Meerdink, G.L., Bargren, G.L., Tumbleson, M.E., Singh, V., 2005. Effect of aflatoxin B1 on dry-grind ethanol process. Cereal Chemistry 82, 302–304.
Pérez-Carrillo, E., Serna-Saldívar, S.O., 2007. Effect of protease treatment before hydrolysis with α-amylase on the rate of starch and protein hydrolysis of maize, whole sorghum and decorticated sorghum. Cereal Chemistry 84, 607–613.
Ramputh, A., Teshome, A., Bergvinson, D.J., Nozzolillo, C., Arnason, J.T., 1999. Soluble phenolic content as an indicator of sorghum grain resistance to *Sitophilus oryzae* (Coleoptera: Curculionidae). Journal of Stored Products Research 35, 57–64.
RFA (Renewable Fuels Association), 2011. The Industry-Statistics Available at: http://www.ethanolrfa.org (accessed 05.10.11.).
Roselena, T., Jaccoud-Filho, D.S., Henneberg, L., Michel, M.D., Demiate, I.M., Barbosa-Pinto, A.T., Junior, M.M., Barana, A.C., 2009. Maize (*Zea Mays* L) landraces from the Southern Region of Brazil: contamination by *Fusarium* sp., zearalenone, physical and mechanical characteristics of the kernels. Brazilian Archives of Biology and Technology 52, 11–16.
Serna-Saldívar, S.O., 2010. Cereal Grains: Properties, Processing, and Nutritional Attributes, first ed. CRC Press, Boca Raton, FL.
Thomas, K.C., Ingledew, W.M., 1990. Fuel alcohol production: effects of free amino nitrogen on fermentation of very high gravity wheat mashes. Applied Environmental Microbiology 56, 2046–2050.
Vidal, B.C., Rausch, K.D., Tumbleson, M.E., Singh, V., 2011. Corn endosperm fermentation using endogenous amino nitrogen generated by a fungal protease. Cereal Chemistry 88, 117–123.
Vignoni, L.A., Césari, R.M., Forte, M., Mirábile, M.L., 2006. Determinación de índice de color en ajo picado. Información Tecnológica 17, 63–67.
Vriesekoop, F., Haass, C., Pamment, N.B., 2009. The role of acetaldehyde and glycerol in the adaptation to ethanol stress of *Saccharomyces cerevisiae* and other yeasts. FEMS Yeast Research 9, 365–371.
Walker, G.M., 2011. 125th Anniversary review: fuel alcohol: current production and future challenges. Journal of the Institute of Brewing 117, 3–22.
Wu, X., Zhao, R., Bean, S.R., Seib, P.A., McLaren, J.S., Madl, R.L., Tuinstra, M., Lenz, M.C., Wang, D., 2007. Factors impacting ethanol production from grain sorghum in the dry-grind process. Cereal Chemistry 84, 130–136.
Wu, X., Zhao, R., Wang, D., Bean, S.R., Seib, P.A., Tuinstra, M.R., Campbell, M., O'Brien, A., 2006. Effects of amylose, corn protein, and corn fiber contents on production of ethanol from starch-rich media. Cereal Chemistry 83, 569–575.
Yan, S., Wu, X., MacRitchie, F., Wang, D., 2009. Germination-improved ethanol fermentation performance of high-tannin sorghum in a laboratory dry-grind process. Cereal Chemistry 86, 597–600.
Yan, S., Wu, X., Dahlberg, J., Bean, S.R., MacRitchie, F., Wilson, J.D., Wang, D., 2010. Properties of field-sprouted sorghum and its performance in ethanol production. Journal of Cereal Science 51, 374–380.

10. REFERENCES

CHAPTER 1. INTRODUCTION

Abebe, F., Tefera, T., Mugo, S., Beyene, Y., Vidal, S., 2009. Resistance of maize varieties to the maize weevil *Sitophilus zeamais* (Motsch.) (Coleoptera: *Curculionidae*). African Journal of Biotechnology 8, 5937-5943.

Arnason, J.T., Gale, J., de Beyssac, Conilh B., Sen, A., Miller, S.S., Philogene, B., Lambert, J., Fulcher, R.G., Serratos, J., Mihm, J., 1992. The role of phenolics in resistance in maize grain to stored grain insects. Journal of Stored Products Research 28, 119-126.

Atwell, W.A., Hood, L.F., Lineback, D.R., 1988. The terminology and methodology associated with basic starch phenomena. Cereal Foods World 33, 306-311.

Awika, J.M., Rooney, L.W., 2004. Sorghum phytochemicals and their potential impact on human health. Phytochemistry 65, 1199-1221.

Awika, J.M., Rooney, L.W., Waniska, R.D., 2004a. Anthocyanins from black sorghum and their antioxidant properties. Food Chemistry 90, 293-301.

Awika, J.M., Rooney, L.W., Waniska, R.D., 2004b. Properties of 3-Deoxyanthocyanins from Sorghum. Journal of Agricultural and Food Chemistry 52, 4388-4394.

Burt, A.J., 2003. Phytochemical mediation of post-harvest insect resistance in tropical maize. M.Sc. Thesis, University of Ottawa, Ottawa-Carleton Institute of Biology, Ottawa, CA., pp. 141.

Butler, L. G., Reidl. D. J., Lebryk, D. G., Blytt, H. J., 1984. Interaction of proteins with sorghum tannin: mechanism, specificity and significance. Journal of American Oil Chemists Society 61, 916-920.

Chanapamokkhot, H., Thongngam, M., 2007. The chemical and physico-chemical properties of sorghum starch and flour. Kasetsart Journal: Natural Science 41, 343-349.

Chandrashekar, A., Mazhar, H., 1999. The Biochemical Basis and Implications of Grain Strength in Sorghum and Maize. Journal of Cereal Science 30, 193-207.

Chandrashekar, A., Satyanarayana, K.V., 2006. Disease and pest resistance in grains of sorghum and millets. Journal of Cereal Science 44, 287-304.

Chuck-Hernández, C., Peralta-Contreras, M., Bando-Carranza, G., Vera-Garcia, M., Gaxiola-Cuevas, N., Tamayo-Limon, R., Cardenas-Torres, F., Perez-Carrillo, E., Serna-Saldivar, S.,

2012. Bioconversion into ethanol of decorticated red sorghum (*Sorghum bicolor* L. Moench) supplemented with its phenolic extract or spent bran. Biotechnology Letters 34, 97–102.

Chuck-Hernández, C., Pérez-Carrillo, E., Heredia-Olea, E., Serna-Saldívar, S.O., 2011. Sorghum as a multifunctional crop for bioethanol production in Mexico: technologies, advances and improvement opportunities. Revista Mexicana de Ingeniería Química 10, 529-549.

Dicko, M.H., Gruppen, H., Traoré, A.S., Voragen, A.G.J., Van Berkel, W.J.H., 2006. Phenolics compounds and related enzymes as determinants of sorghum for food use. Biotechnology and Molecular Biology Reviews 1, 21-38.

Dobie, P., 1977. The contribution of the Tropical Stored Products Centre to the Study of insect resistance in stored maize. Tropical Stored Products Information 34, 7-22.

Duodu, K.G., Nunes, A., Delgadillo, I., Parker, M.L., Mills, E.N.C., Belton, P.S., Taylor, J.R.N., 2002. Effects of grain structure and cooking on sorghum and maize in vitro protein digestibility. Journal of Cereal Science 35, 161-174.

Duodu, K.G., Taylor, J.R.N., Belton, P.S., Hamaker, B.R., 2003. Factors affecting sorghum protein digestibility. Journal of Cereal Science 38, 117–131.

Dykes, L., Rooney, L.W., 2006. Sorghum and millet phenols and antioxidants. Journal of Cereal Science 44, 236–251.

Dykes, L., Rooney, L.W., 2007. Phenolic compounds in cereal grains and their health benefits. Cereal Foods World 52, 105-111.

Dykes, L., Rooney, L.W., Waniska, R.D., Rooney, W.L., 2005. Phenolic Compounds and Antioxidant Activity of Sorghum Grains of Varying Genotypes. Journal of Agricultural and Food Chemistry 53, 6813-6818.

Emani, C., Sunilkumar, G., Rathore, K.S., 2002. Transgene silencing and reactivation in sorghum. Plant Science 162, 181-192.

Ezeogu, L.I., Duodu, K.G., Taylor, J.R.N., 2005. Effects of endosperm texture and cooking conditions on the in vitro starch digestibility of sorghum and maize flours. Journal of Cereal Science 42: 33–44.

FAO, 1993. La ingeniería en el desarrollo - Manejo y tratamiento de granos postcosecha. Available at: http:// www.fao.org/docrep/x5041s/ x5041S00.htm#Contents.

FAO, 2009. How to feed the world in 2050. Available at: http://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf.

FAO, 2012. Food Outlook. Global Market Analysis. May 2012. Available at: http://www.fao.org/docrep/015/al989e/al989e00.pdf

FAOSTAT, 2012. Cereal production. Available from http://faostat.fao.org/

FR. Financiera Rural., 2011. Monografía del Sorgo Grano. Dirección General Adjunta de Planeación Estratégica y Análisis Sectorial. Dirección Ejecutiva de Análisis Sectorial. Avilable at: http://www.financierarural.gob.mx/informacionsectorrural/Documents/Monografias/MonografiaSorgo(jun11).pdf.

García-Lara, S., Bergvinson, D.J., Burt, A.J., Ramputh, A.I., Diaz-Pontones, D.M., Arnason, J.T., 2004. The role of pericarp cell wall components in maize weevil resistance. Crop Science 44, 1546-1552.

Gélinas, P., McKinnon, K., 2000. Fermentation and Microbiological Processes in Cereal Foods. In: Kulp, K., Ponte, J. (Eds), Chapter 26. Handbook of Cereal Science and Technology. Second Edition. Marcel Dekker., NY. USA.

Girgi, M., Breese, W.A., Lörz, H., Oldach, K.H., 2006. Rust and downy mildew resistance in pearl millet (*Pennisetum glaucum*) mediated by heterologous expression of the afp gene from *Aspergillus giganteus*. Transgenic Research 15, 313–324.

Girijashankar, V., Sharma, H.C., Sharma, K.K., Swathisree, V., Prasad, L.S., Bhat, B.V., Royer, M., Secundo, B.S., Narasu,M.L., Altosaar, I., Seetharama, N., 2005. Development of transgenic sorghum for insect resistance against the spotted stem borer (*Chilo partellus*). Plant Cell Reports 24, 513–522.

Greaves, J.P., 1963. Protein digestibility and ingestion. Nature 197, 499-500.

Guajardo-Flores, D., Cardenas-Hinojosa, A.P., Dykes, L., McDonough, C.M., Rooney, L.W., 2006. Comparison of total phenol, antioxidant activity and tannin content in different grains. AACC Annual Meeting Abstracts. Available at: http://www.wgsummit.org/program/pdfs/OralsPostersOnly.pdf

Guo, C., Cui, W., Feng, X., Zhao, J., Lu, G., 2011. Sorghum insect problems and management. Journal of Integrative Plant Biology 53, 178-192.

Hamaker, B.R., Kirleis, A.W., Butler, L.G., Axtell, J.D., Mertz, E.T., 1987. Improving the *in vitro* protein digestibility of sorghum with reducing agents. Proceedings of the National Academy of Sciences of the United States of America 84, 626-628.

Hoseney, R.C., 1998. Principles of Cereal Science and Technology, Second Edition. AACC, St. Paul, MN, USA.

Kim, M., Day, D., 2010. Composition of sugar cane, energy cane, and sweet sorghum suitable for ethanol production at Louisiana sugar mills. Journal of Industrial Microbiology & Biotechnology, Volume and number not available, pp 1-5.

King, D., Fan, M.Z., Ejeta, G., Asem, E.K., Adeola, O., 2000. The effects of tannins on nutrient utilization in the white Pekin duck. British Poultry Science 41, 629-630.

Klein, R.R., Rodriguez-Herrera, R., Schlueter, J.A., Klein, P.E., Yu, Z.H., Rooney, W.L., 2001. Identification of genomic regions that affect grain mold incidence and other traits of agronomic importance in sorghum. Theoretical Applied Genetics 102, 307–319.

Klopfenstein, C.F., Hoseney, R.C., 1995. Nutritional properties of sorghum and the millets. In: Dendy, D.A.V. (Ed.), Chapter 5 Sorghum & Millets: Chemistry and Technology. American Association of Cereal Chemists, St. Paul, MN, pp. 125-168.

Krishnaveni, S., Jeoung, J.M., Muthukrishnan, S., Liang, G.H., 2001.Transgenic sorghum plants constitutively expressing a rice chitinase gene show improved resistance to stalk rot. Journal of Genetics and Breeding 55, 151–158.

Latha, A.M., Rao, K.V., Reddy, T.P., Reddy, V.D., 2006. Development of transgenic pearl millet (*Pennisetum glaucum* (L.) R. Br.) plants resistant to downy mildew. Plant Cell Reports 29, 927–935.

Lo, S.C.C., De Verdier, K., Nicholson, R.L., 1999. Accumulation of 3-deoxyanthocyanidin phytoalexins and resistance to *Colletotrichum sublineolum* in sorghum. Physiological and Molecular Plant Pathology 55, 263-273.

MacLean, W.C., López de Romaña, G., Placko, R.P., Graham, G.G., 1981. Protein quality and digestibility of sorghum in preschool children: balance studies and plasma free amino acids. Journal of Nutrition 111, 1928-1936.

Mariotti, M., Pagani, M.A., Lucisano, M., 2006. Starch damage. An interesting index for the definition of wheat flour quality. Tecnica Molitoria 57, 397-403.

Marshall, M.R., 2010. Ash Analysis. In Nielsen, S.S. (Ed.), Chapter 7, Food Analysis. Springer, New York, USA, pp. 105-116.

O'Kennedy, M.M., Grootboom, A., Shewry P.R., 2006. Harnessing sorghum and millet biotechnology for food and health. Journal of Cereal Science 44, 224–235.

Pendleton, M., Ellis, E.A., Chitio, F., Pendleton, B., 2005. Comparison of Morphology of Sorghum Grain to Resistance to Maize Weevil *Sitophilus zeamais*. Microscopy and Microanalysis 11, 1140-1141.

Platel, K., Shurpalekar, K.S., 1994. Resistant starch content of Indian foods. Plant Foods Human Nutrition 45, 91–5.

Ramputh, A., Teshome, A., Bergvinson, D.J., Nozzolillo, C., Arnason, J.T., 1999. Soluble phenolic content as an indicator of sorghum grain resistance to *Sitophilus oryzae* (Coleoptera: *Curculionidae*). Journal of Stored Products Research 35, 57-64.

Rooney, L.W., 1978. Sorghum and pearl millet lipids. Cereal Chemistry 55, 584-590.

Rooney, L.W., 2009. Overview: sorghum and millet food research failures and successes. Available at: http://www.afripro.org.uk/papers/Paper09Rooney.pdf.

Rooney, L.W., Pflugfelder, R.L., 1986. Factors affecting starch digestibility with special emphasis on sorghum and corn. Journal of Animal Science 63, 1607–1623.

Rooney, L.W., Serna-Saldivar, S.O., 2000. Sorghum. In: Kulp, K., Ponte, J.G. (Eds.), Chapter 5, Handbook of Cereal Science & Technology. Marcel Dekker Inc., New York, USA, pp 149-175.

Sajilata, M.G., Singhal, R.S., Kulkarni, P.R., 2006. Resistant Starch, A Review. Comprehensive Reviews In Food Science And Food Safety 5, 1-17.

Salunke, D.K., Jadhav, S.J., Kadam, S.S., Chavan, J.K., 1982. Chemical, biochemical and biological significance of polyphenols in cereals and legumes. Critical Reviews in Food Science and Nutrition 17, 277-305.

Satish, K., Srinivas, G., Madhusudhana, R., Padmaja, P.G., Reddy, R., Murali, M.S., Seetharama, N., 2009. Identification of quantitative trait loci for resistance to shoot fly in sorghum [*Sorghum bicolor* (L.) Moench]. Theoretical and Applied Genetics 119, 1425–1439.

Sautier, D., O'Déyés, M., 1989. Mil, Mais, Sorgho - Techniques et alimentation au Sahel. Harmattan. Paris, France, pp. 171.

Serna-Saldívar, S.O. 2012. Tecnologías Viables De Producción De Bioetanol Para Mexico. Cuarto Curso Teórico Práctico en Operación de Bioreactores. Instituto de Biotecnología. Universidad Autónoma de Nuevo León.

Serna-Saldívar, S.O., 2010. Cereal Grains: Properties, Processing, and Nutritional Attributes, First edition. CRC Press, Boca Raton, FL., USA.

Serna-Saldivar, S.O., Rooney, L.W., 1995. Structure and chemistry of sorghum and millets. Chapter 4. In: Dendy, D.A.V. (Ed.), Sorghum & Millets: Chemistry and Technology, American Association of Cereal Chemists, St. Paul, MN, pp. 69-124.

Singh, D.N., McCain, F.S., 1963. Relationships of some nutritional properties of the corn kernel to weevil infestation. Crop Science 3, 259-261.

Subramanian, V., Hoseney, R.C., Bramel-Cox, P., 1994. Shear thinning properties of sorghum and corn starches. Cereal Chemistry 71, 272-275.

Taylor, J.R.N, Schober, T.J., Bean, S.R., 2006. Novel Food and Non-Food Uses for Sorghum and Millets. Journal of Cereal Science 44, 252-271.

Taylor, J.R.N., Belton, P.S., 2002. Sorghum. In: Belton, P.S., Taylor, J.R.N. (Eds.). Pseudocereals and less common cereals, Springer, Berlin, pp. 24-91.

Teetes, G. L., Pendleton, B.B., 2000. Insect pests of sorghum. In: Smith C. W., Frederikson R. A., (Eds.), Chapter 3.3, Sorghum: Origin, History, Technology, and Production. Wiley Series in Crop Science, New York, pp. 443-495.

Torres, J.B., Saavedra, J.L.D., Zanuncio, J.C., Waquil, J.M., 1996. Resistance of sorghum to *Sitophilus oryzae* (L.) and its association with varietal parameters. International Journal of Pest Management 42, 277-280.

USDA, 2011. Mexico. Grain and Feed Annual. Gain report number: MX1017. Available at: http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Grain%20and%20Feed%20Annual_Mexico%20City_Mexico_3-14-2011.pdf.

Van Buren, J. P., Robinson, W. B., 1969. Formation of complexes between protein and tannic acid. Journal of Agricultural and Food Chemistry 17, 772-777.

Wang, D., Bean, S., McLaren, J., Seib, P., Madl, R., Tuinstra, M., Shi, Y., Lenz, M., Wu, X., Zhao, R., 2008. Grain sorghum is a viable feedstock for ethanol production. Journal of Industrial Microbiology and Biotechnology 35, 313–320.

Waniska, R.D., Rooney, L.W., 2000. Structure and Chemistry of the Sorghum Caryopsis. In Smith, C.W., Frederikson, R.A. (Eds.), Chapter 4.1, Sorghum: Origin, History, Technology, and Production. Wiley Series in Crop Science, New York, pp 649-688.

Warsi, A.S., Wright, B.C., 1973. Effects of rates and methods of nitrogen application on the quality of sorghum grain. Indian Journal of Agricultural Science 43, 722-726.

Wong, J.H., Lau, T., Cai, N., Singh, J., Pedersen, J.F., Vensel, W.H., Hurkman, W.J., Wilson, J.D., Lemaux, P.G., Buchanan, B.B., 2009. Digestibility of protein and starch from sorghum (*Sorghum bicolor*) is linked to biochemical and structural features of grain endosperm. Journal of Cereal Science 49, 73-82.

Wu, X., Zhao, R., Bean, S. R., Seib, P. A., McLaren, J. S., Madl, R. L., Tuinstra, M., Lenz, M. C., Wang, D., 2007. Factors Impacting Ethanol Production from Grain Sorghum in the Dry-Grind Process. Cereal Chemistry 84, 130-136.

Wu, X., Zhao, R., Wang, D., Bean, S. R., Seib, P. A., Tuinstra, M. R., Campbell, M., O'Brien, A., 2006. Effects of Amylose, Corn Protein, and Corn Fiber Contents on Production of Ethanol from Starch-Rich Media. Cereal Chemistry 83, 569-575.

Wu, Y.Q., Huang, Y., 2008. Molecular mapping of QTLs for resistance to the greenbug *Schizaphis graminum* (Rondani) in *Sorghum bicolor* (Moench). Theoretical Applied Genetics 117, 117–124.

Yadav, B.S., Sharma, A., Yadav, R.B., 2010. Effect of storage on resistant starch content and in vitro starch digestibility of some pressure-cooked cereals and legumes commonly used in India. International Journal of Food Science and Technology 45, 2449–2455.

Yan, S., Wu, X., Bean, S.R., Pedersen, J.F., Tesso, T., Chen, Y.R., Wang, D., 2011. Evaluation of Waxy Grain Sorghum for Ethanol Production. Cereal Chemistry 88, 589-595.

Yan, S., Wu, X., Faubion, J., Bean, S.R., Cai, L., Shi, Y.C., Sun, X.S., Wang, D., 2012. Ethanol-Production Performance of Ozone-Treated Tannin Grain Sorghum Flour. Cereal Chemistry Journal 89, 30-37.

Yan, S., Wu, X., MacRitchie, F., Wang, D., 2009. Germination-Improved ethanol fermentation performance of high tannin sorghum in a laboratory dry-grind process. Cereal Chemistry 86, 597-600.

Zhang, C., Xie, G., Li, S., Ge, L., He, T., 2010. The productive potentials of sweet sorghum ethanol in China. Applied Energy 87, 2360-2368.

Zhao, R., Bean, S., Wu, X., Wang, D., 2008. Assessing Fermentation Quality of Grain Sorghum for Fuel Ethanol Production Using Rapid Visco-Analyzer. Cereal Chemistry 85, 830-836.

CHAPTER 2. EFFECTS OF ADDITION OF RED SORGHUM (*SORGHUM BICOLOR* L. MOENCH) PHENOLICS OR SPENT BRAN ON THE PERFORMANCE OF DECORTICATED KERNELS BIOCONVERTED INTO ETHANOL

AACC. 2000. Approved Methods of the AACC. American Association of Cereal Chemists: St. Paul, MN.

Åkerberg, C., Zacchi, G., Torto, N., and Gorton, L. 2000. A kinetic model for enzymatic wheat starch saccharification. J. Chem. Technol. Biotechnol. 75:306-314.

AOAC. 1980. Official Methods of the Association of Official Analytical Chemists. AOAC: Washington, DC.

Alves, J.S.L., Herberts, R.A., Hollatz, C., Miletti, L.C., and Stambuk, B.U. 2007. Maltose and maltotriose active transport and fermentation by *Saccharomyces cerevisiae*. J. Am. Soc. Brew. Chem. 65:99-104.

Awika, J.M., and Rooney, L. W. 2004. Sorghum phytochemicals and their potential impact on human health. Phytochemistry. 65:1199-1221.

Barnett, J.A. 1997. Sugar utilization by *Saccharomyces cerevisiae*. Pages 35-42. In: Yeast sugar metabolism. Biochemistry, genetics, biotechnology and applications. F. K. Zimmermann and K. D. Entian, eds. Technomics Publication: Lancaster, P.A.

Belton, P.S., Delgadillo, I., Halford, N.G., and Shewry, P. R. 2006. Kafirin structure and functionality. J. Cereal Sci. 44:272-286.

Boulton, C., and Quain, D. 2001. Brewing Yeast and Fermentation. First edition. Blackwell Science. Malden, MA., USA.

CD, Cámara de Diputados. 2008. Ley de Promoción y Desarrollo de los Bioenergéticos.

Cepeda, E., Hermosa, M., and Ballesteros, A. 2001. Optimization of maltodextrin hydrolysis by glucoamylase in a batch reactor. Biotech. and Bioeng. 76:70-76.

Cheynier, V. 2005. Polyphenols in foods are more complex than often thought. Am. J. Clin. Nutr. 81:223S-229S.

Chuck-Hernández, C.E. 2009. Efecto de rolado al vapor de sorgo rojo (*Sorghum bicolor* (L) Moench) y maíz (*Zea mays*) en el rendimiento y eficiencia de producción de bioetanol. Thesis. Instituto Tecnológico y de Estudios Superiores de Monterrey, Monterrey, N.L., México.

Chuck-Hernández, C., Perez-Carrillo, E., and Serna-Saldivar, S. O. 2009. Production of bioethanol from steam-flaked sorghum and maize. J. Cereal Sci. 50:131-137.

Corredor, D. Y., Bean, S. R., Schober, T., and Wang, D. 2006. Effect of decorticating sorghum on ethanol production and composition of DDGS. Cereal Chem. 83:17-21.

D'Amore, T., Russell, I., and Stewart, G. G. 1989. Sugar utilization by yeast during fermentation. J. Ind. Microbiol. Biotechnol. 4:315-324.

Daiber, K.H. 1975. Enzyme inhibition by polyphenols of sorghum grain and malt. J. Sci. Food Agric. 26:1399-1411.

Dicko, M.H., Gruppen, H., Traoré, A.S., Voragen, A.G.J., and Van Berkel, W.J.H. 2006a. Phenolics compounds and related enzymes as determinants of sorghum for food use. Biotech. Molec. Rev. 1:21-38.

Dicko, M.H., Gruppen, H., Traoré, A.S., Voragen, A.G.J., and Van Berkel, W.J.H. 2006b. Sorghum grain as human food in Africa: relevance of content of starch and amylase activities. Afr. J. Biotech. 5:384-395.

Duodu, K.G., Nunes, A., Delgadillo, I., Parker, M. L., Mills, E.N.C., Belton, P.S., and Taylor, J.R. N. 2002. Effect of grain structure and cooking on sorghum and maize in vitro protein digestibility. J. Cereal Sci. 35:161-174.

Duodu, K.G., Taylor, J.R.N., Belton, P.S., and Hamaker, B.R. 2003. Factors affecting sorghum protein digestibility. J. Cereal Sci. 38:117-131.

Dykes, L., and Rooney, L.W. 2007. Phenolic compounds in cereal grains and their health benefits. Cereal Foods World. 52:105-111.

Dykes, L., Seitz, L.M., Rooney, W.L., and Rooney, L.W. 2009. Flavonoid composition of red sorghum genotypes. Food Chem. 116:313-317.

Ezeogu, L.I., Duodu, K.G., Emmambux, M.N., and Taylor, J.R.N. 2008. Influence of cooking conditions on the protein matrix of sorghum and maize endosperm flours. Cereal Chem. 85:397-402.

EPI (Earth Police Institute). 2010. Data Center: http://www.earth-policy.org/datacenter/xls/book_pb4_ch4-5_32.xls

FAO. 2010. FAOSTAT. http://faostat.fao.org/.

Findrik, Z., Presečki, A.V. and Vasić-Rački, D. 2008. Mathematical modelling of glucoamylase catalyzed saccharification process. Pages: 1-9. In: Summaries 1. 18th International Congress of Chemical and Process Engineering. Jan Novosad (ed). Process Engineering Publisher. Prague. Czech Republic.

Garcia-Garibay, M., and Lopez-Munguia, A. 1998. Bebidas alcohólicas no destiladas. In: Biotecnología Alimentaria, 1st. M. Garcia-Garibay, R. Quintero-Ramirez and A. Lopez-Munguia, eds. Limusa Noriega Editores.

Hamaker, B.R., and Bugusu, B.A. 2003. Overview: sorghum proteins and food quality. In: Afripro. Workshop on the Proteins of Sorghum and Millets: Enhancing Nutritional and Functional Properties for Africa. P. S. Belton and J. R. N. Taylor, eds: Pretoria, South Africa.

Ingledew, W.M. 1995. The Biochemistry of Alcohol Production. Chapter 7. In: The Alcohol Textbook. Lyons, T.P., Kelsall, D.R and Murtagh, J.E, eds. First Edition. Nottingham University Press: Nottingham, United Kingdom.

Lee, C. G., Kim, C. H., and Rhee, S. K. 1992. A kinetic model and simulation of starch saccharification and simultaneous ethanol fermentation by amyloglucosidase and *Zymomonas mobilis*. Bioprocess. Biosyst. Eng. 7:335-341.

Leiper, K. A., Schlee, C., Tebble, I., and Stewart, G. G. 2006. The fermentation of beet sugar syrup to produce bioethanol. J. Inst. Brew. 112:122-133.

Lopez-Munguia, A. 1998. Edulcorantes. In: Biotecnología Alimentaria, First Edition. M. Garcia-Garibay, R. Quintero-Ramirez and A. Lopez-Munguia, eds. Limusa Noriega Editores: México, D.F.

Makkar, H.P.S., Siddhuraju, P., and Becke, K. 2007. *Alpha*-amylase inhibitor. Pages 11-14. In: Plant Secondary Metabolites. Humana Press Inc: Totowa, NJ.

McAloon, A., Taylor, F., Yee, W., Ibsen, K., and Wooley, R. 2000. Determining the cost of producing ethanol from corn starch and lignocellulosic feedstocks. Rep. U.S. Department of Agriculture and U.S. Departament of Energy: Golden, Colorado.

McDougall, G. J., Shapiro, F., Dobson, P., Smith, P., Blake, A., and Stewart, D. 2005. Different polyphenolic components of soft fruits inhibit *alpha*-amylase and *alpha*-glucosidase. J. Agric. Food Chem. 53:2760-2766.

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31:426-428.

Naidu, K., Singh, V., Johnston, D.B., Rausch, K.D., and Tumbleson, M.E. 2007. Effects of ground corn particle size on ethanol yield and thin stillage soluble solids. Cereal Chem. 84:6-9.

Novak, S., Zechner-Krpan, V., and Maric, V. 2004. Regulation of maltose transport and metabolism in *Saccharomyces cerevisiae*. Food Techn. and Biotechn. 42: 213–218.

Ponnampalam, E., Steele, D., Burgdorf, D., and McCalla, D. 2004. Effect of germ and fiber removal on production of ethanol from corn. Appl. Biochem. Biotechnol. 115:837-842.

Perez-Carrillo, E., and Serna-Saldivar, S. O. 2007. Effect of protease treatment before hydrolysis with *alpha*-amylase on the rate of starch and protein hydrolysis of maize, whole sorghum, and decorticated sorghum. Cereal Chem. 84:607-613.

Perez-Carrillo, E., Serna-Saldivar, S.O., Alvarez, M.M., and Cortes-Callejas, M.L. 2008. Effect of sorghum decortication and use of protease before liquefaction with thermoresistant *alpha*-amylase on efficiency of bioethanol production. Cereal Chem. 85:792-798.

Rautio, J., and Londesborough, J. 2003. Maltose transport by brewer's yeasts in brewer's wort. J. Inst. Brew. 109:251-261.

RFA, Renewable Fuels Association. 2011. RFA Issues Weekly Ethanol Production Statistics.http://www.grainnet.com/articles/rfa_issues_weekly_ethanol_production_statistics-103375.html

RFA, Renewable Fuels Association. 2010. The Industry-Statistics. http://www.ethanolrfa.org.

Rooney, L.W., and Serna-Saldivar, S.O. 2000. Sorghum. In: Handbook of Cereal Science and Technology. K. Kulp and J. Ponte, eds. Marcel Dekker: New York, NY.

Rooney, L.W., and Pflugfelder, R.L. 1986. Factors affecting starch digestibility with special emphasis on sorghum and corn. J. Anim. Sci. 63:1607-1623.

Roy, I., and Gupta, M.N. 2004. Hydrolysis of starch by a mixture of glucoamylase and pullulanase entrapped individually in calcium alginate beads. Enzyme Microb. Technol. 34:26-32.

Saville, B.A., Huang, C., Yacyshyn, V., and Desbarats, A. 2006 Properties and performance of glucoamylases for fuel ethanol production. Appl. Biochem. Biotechnol. 129:180-194.

Sears, A.E. 1995. The role of enzymes in alcohol production. In: The Alcohol Textbook. T. P. Lyons, D. R. Kelsall and J. E. Murtagh, eds. Nottingham University Press: Nottingham, UK.

Serna-Saldivar, S.O. 2010. Cereal Grains: Properties, Processing, and Nutritional Attributes. CRC (Taylor & Francis) Press. Boca Raton, FL.

Slominska, L., Klisowska, M., and Grzeskowiak, A. 2003. Degradation of starch granules by amylases. In: Electronic Journal of Polish Agricultural Universities.

Taylor, J.R.N., Schober, T.J., and Bean, S.R. 2006. Novel food and non-food uses for sorghum and millets. J. Cereal Sci. 44:252-271.

Vinson, J. A., Proch, J., Bose, P., and Lester, P. 2001. Determination of quantity and quality of polyphenol antioxidants in foods and beverages. Pages 103-114. In: Methods in Enzymology, Volume 335. Academic Press.

Wang, D., Bean, S., McLaren, J., Seib, P., Madl, R., Tuinstra, M., Shi, Y., Lenz, M., Wu, X., and Zhao, R. 2008. Grain sorghum is a viable feedstock for ethanol production. J. Ind. Microbiol. Biotechnol. 35:313–320.

Wang, P., Singh, V., Xu, L., Johnston, D. B., Rausch, K.D., and Tumbleson, M. E. 2005. Comparison of enzymatic (E-mill) and conventional dry-grind corn processes using a granular starch hydrolyzing enzyme. Cereal Chem. 82:734-738.

Wolfe, K., Wu, X., and Liu, R.H. 2003. Antioxidant activity of apple peels. J. Agric. Food. Chem. 51:609-614.

Wong, J.H., Lau, T., Cai, N., Singh, J., Pedersen, J.F., Vensel, W.H., Hurkman, W. J., Wilson, J.D., Lemaux, P.G., and Buchanan, B.B. 2009. Digestibility of protein and starch from sorghum (*Sorghum bicolor*) is linked to biochemical and structural features of grain endosperm. J. Cereal Sci. 49:73-82.

Wu, X., Jampala, B., Robbins, A., Hays, D., Yan, S., Xu, F., Rooney, W., Peterson, G., Shi, Y.C. and Wang, D. 2010. Ethanol fermentation performance of grain sorghums (*Sorghum bicolor*) with modified endosperm matrices. J. Agric. Food Chem. 58:9556-9562.

Wu, X., Zhao, R., Bean, S. R., Seib, P. A., McLaren, J. S., Madl, R. L., Tuinstra, M., Lenz, M.C., and Wang, D. 2007. Factors impacting ethanol production from grain sorghum in the dry-grind process. Cereal Chem. 84:130-136.

Wu, X., Zhao, R., Liu, L., Bean, S., Seib, P. A., McLaren, J., Madl, R., Tuinstra, M., Lenz, M., and Wang. D. 2008. Effects of Growing Location and Irrigation on Attributes and Ethanol Yields of Selected Grain Sorghums. Cereal Chem. 85:495-501.

Wu, X., Zhao, R., Wang, D., Bean, S. R., Seib, P. A., Tuinstra, M. R., Campbell, M., and O'Brien, A. 2006. Effects of amylose, corn protein, and corn fiber contents on production of ethanol from starch-rich media. Cereal Chem. 83:569-575.

Zhan, X., Wang, D., Tuinstra, M.R., Bean, S., Seib, P. A., and Sun, X.S. 2003. Ethanol and lactic acid production as affected by sorghum genotype and location. Ind. Crops Prod. 18:245-255.

Zhang, G., and Hamaker, B. R. 1998. Low *alpha*-amylase starch digestibility of cooked sorghum flours and the effect of protein. Cereal Chem. 75:710-713.

Zhao, R., Bean, S.R., Ioerger, B.P., Wang, D., and Boyle, D.L. 2008. Impact of mashing on sorghum proteins and its relationship to ethanol fermentation. J. Agric. Food Chem. 56:946-953.

Zhao, R., Bean, S. R., Wang, D., Park, S. H., Schober, T. J., and Wilson, J.D. 2009. Small-scale mashing procedure for predicting ethanol yield of sorghum grain. J. Cereal Sci. 49:230-238.

CHAPTER 3. CONVERSION INTO BIOETHANOL OF INSECT (*SITOPHILUS ZEAMAIS* MOTSCHULSKY), MOLD (*ASPERGILLUS FLAVUS* LINK) AND SPROUT-DAMAGED MAIZE (*ZEA MAYS* L.) AND SORGHUM (*SORGHUM BICOLOR* L. MOENCH)

AACC, 2000. Approved Methods of the AACC, 10th ed. American Association of Cereal Chemists, St. Paul, MN.

AOAC, 1980. Official Methods of the Association of Official Analytical Chemists. AOAC, Washington, DC.

Attfield, P.V., Kletsas, S., 2000. Hyperosmotic stress response by strains of bakers' yeasts in high sugar concentration medium. Letters in Applied Microbiology 31, 323-327.

Bandyopadhyay, R., Butler, D.R., Chandrasekhar, A., Reddy, R.K., Navi, S.S., 2000. Biology, Epidemiology and Management of Sorghum Grain Mold. In: Chandrashekar, A., Bandyopadhyay, R., Hall, A.J. (Eds.), Technical and institutional options for sorghum grain mold management: proceedings of an international consultation. 18-19 May 2000, ICRISAT, Patancheru, India, pp 34-71.

Bergvinson, D.J., García-Lara, S., 2011. Synergistic effects of insect-resistant maize and *Teretrius nigrescens* on the reduction of grain losses caused by *Prostephanus truncatus* (Horn.). Journal of Stored Products Research 47, 95-100.

Beta, T., Rooney, L.W., Waniska, R.D., 1995. Malting Characteristics of Sorghum Cultivars. Cereal Chemistry 72, 533-538.

CD, Cámara de Diputados, 2008. Ley de Promoción y Desarrollo de los Bioenergéticos. Available at: http://www.diputados.gob.mx/LeyesBiblio/pdf/LPDB.pdf (Accessed 05.10.11).

Chuck-Hernández, C., Pérez-Carrillo, Serna-Saldívar, S.O., 2009. Production of bioethanol from steam-flaked sorghum and maize. Journal of Cereal Science 50, 131-137.

Dicko, M.H., Gruppen, H., Traoré, A.S., Voragen, A.G.J., Van Berkel, W.J.H., 2006. Phenolics compounds and related enzymes as determinants of sorghum for food use. Biotechnology and Molecular Biology Reviews 1, 21-38.

FAO, 1993. La ingeniería en el desarrollo - Manejo y tratamiento de granos poscosecha. Available at: http://www.fao.org/docrep/x5041s/x5041S00.htm#Contents. (Accesed 05.30.11).

García-Lara, S., Bergvinson, D.J., 2007. Programa integral para reducir pérdidas poscosecha en maíz. Agricultura Técnica en México 33, 181-189.

García-Lara, S., Bergvinson, D.J., Burt, A.J., Ramputh, A.I., Diaz-Pontones, D.M., Arnason, J.T., 2004. The role of pericarp cell wall components in maize weevil resistance. Crop Science 44, 1546-1552.

Gutiérrez-Uribe, J.A., Rojas-García, C., García-Lara, S., Serna-Saldívar, S.O., 2010. Phytochemical analysis of wastewater (nejayote) obtained after lime-cooking of different types of maize kernels processed into masa for tortillas. Journal of Cereal Science 52, 410-416.

Lozano-Alejo, N., Carrillo, G.V., Pixley, K., Palacios-Rojas, N., 2007. Physical properties and carotenoid content of maize kernels and its nixtamalized snacks. Innovative Food Science & Emerging Technologies 8, 385-389.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry 31, 426-428.

Murthy, G.S., Townsend, D.E., Meerdink, G.L., Bargren, G.L., Tumbleson, M.E., Singh, V., 2005. Effect of Aflatoxin B1 on Dry-Grind Ethanol Process. Cereal Chemistry 82, 302-304.

Pérez-Carrillo, E., Serna-Saldívar, S.O., 2007. Effect of protease treatment before hydrolysis with α-amylase on the rate of starch and protein hydrolysis of maize, whole sorghum and decorticated sorghum. Cereal Chemistry 84, 607-613.

Ramputh, A., Teshome, A., Bergvinson, D.J., Nozzolillo, C., Arnason, J.T., 1999. Soluble phenolic content as an indicator of sorghum grain resistance to *Sitophilus oryzae* (Coleoptera: *Curculionidae*). Journal of Stored Products Research 35, 57-64.

RFA, Renewable Fuels Association, 2011. The Industry-Statistics Available at: http://www.ethanolrfa.org. (Accessed 05.10.11)

Roselena, T., Jaccoud-Filho, D.S., Henneberg, L., Michel, M.D., Demiate, I.M., Barbosa-Pinto, A.T., Junior, M.M., Barana, A.C., 2009. Maize (*Zea Mays* L) landraces from the Southern Region of Brazil: contamination by *Fusarium sp*, zearalenone, physical and mechanical characteristics of the kernels. Brazilian Archives of Biology and Technology 52, 11-16.

Serna-Saldívar, S.O., 2010. Cereal Grains: Properties, Processing, and Nutritional Attributes, first edition. CRC Press, Boca Raton, FL.

Thomas, K.C., Ingledew, W.M., 1990. Fuel alcohol production: Effects of free amino nitrogen on fermentation of very high gravity wheat mashes. Applied Environmental Microbiology 56, 2046-2050

Vidal, B.C., Rausch, K.D., Tumbleson, M.E., Singh, V., 2011. Corn endosperm fermentation using endogenous amino nitrogen generated by a fungal protease. Cereal Chemistry 88, 117–123.

Vignoni, L.A., Césari, R.M., Forte, M., Mirábile, M.L., 2006. Determinación de índice de color en ajo picado. Información Tecnológica 17, 63-67.

Vriesekoop, F., Haass, C., Pamment, N.B., 2009. The role of acetaldehyde and glycerol in the adaptation to ethanol stress of *Saccharomyces cerevisiae* and other yeasts. FEMS Yeast Research 9, 365-371.

Walker, G.M., 2011. 125th Anniversary Review: Fuel alcohol: current production and future challenges. Journal of the Institute of Brewing 117, 3-22.

Wu, X., Zhao, R., Bean, S.R., Seib, P.A., McLaren, J.S., Madl, R.L., Tuinstra, M., Lenz, M.C., Wang, D., 2007. Factors impacting ethanol production from grain sorghum in the dry-grind process. Cereal Chemistry 84, 130-136.

Wu, X., Zhao, R., Wang, D., Bean, S.R., Seib, P.A., Tuinstra, M.R., Campbell, M., O'Brien, A., 2006. Effects of amylose, corn protein, and corn fiber contents on production of ethanol from starch-rich media. Cereal Chemistry 83, 569-575.

Yan, S., Wu, X., MacRitchie, F., Wang, D., 2009. Germination-improved ethanol fermentation performance of high-tannin sorghum in a laboratory dry-grind process. Cereal Chemistry 86, 597-600.

Yan, S., Wu, X., Dahlberg, J., Bean, S.R., MacRitchie, F., Wilson, J.D., Wang, D., 2010. Properties of field-sprouted sorghum and its performance in ethanol production. Journal of Cereal Science 51, 374-380.

CHAPTER 4. SUSCEPTIBILITY OF DIFFERENT TYPES OF SORGHUMS DURING STORAGE TO SITOPHILUS ZEAMAIS MOTSCHULSKY

AOAC, 1980. Official Methods of the Association of Official Analytical Chemists. AOAC, Washington, DC.

Austin, D., 2008. The effects of sorghum [*Sorghum bicolor* (L.) Moench] phenolic compounds on starch digestibility of porridges. PhD Thesis, Texas A&M University, USA, pp. 119.

Awika, J.M., Yang, L., Browning, J.D., Faraj, A., 2009. Comparative antioxidant, antiproliferative and phase II enzyme inducing potential of sorghum (*Sorghum bicolor*) varieties. LWT - Food Science and Technology 42, 1041-1046.

Bergvinson, D.J., 2001. Storage pest resistance in maize. In: Maize Program Maize Research Highlights 1999-2000. CIMMYT, Mexico D.F., Mexico, pp. 32-39.

Burt, A.J., 2003. Phytochemical mediation of post-harvest insect resistance in tropical maize. M.Sc. Thesis, University of Ottawa, Ottawa-Carleton Institute of Biology, Ottawa, CA., pp. 141.

Chandrashekar, A., Satyanarayana, K.V., 2006. Disease and pest resistance in grains of sorghum and millets Journal of Cereal Science 44, 287-304.

Chiremba,C., Rooney, L.W., Taylor, J.R.N., 2011. Relationships between simple grain quality parameters for the estimation of sorghum and maize hardness in commercial hybrid cultivars. Cereal Chemistry 88, 570-575.

Chuck-Hernández, C., Pérez-Carrillo, Serna-Saldívar, S.O., 2009. Production of bioethanol from steam-flaked sorghum and maize. Journal of Cereal Science 50, 131-137.

Dicko, M.H., Gruppen, H., Traore, A.S., Van Berkel, W.J.H., Voragen, A.G.J., 2005. Evaluation of the effect of germination on phenolic compounds and antioxidant activities in sorghum varieties. Journal of Agricultural and Food Chemistry 53, 2581-2588.

Dobie, P., 1974. The laboratory assessment of the inherent susceptibility of maize varieties to post-harvest infestation by *Sitophilus zeamais* Motsch. (Coleoptera, *Curculionidae*). Journal of Stored Products Research 10, 183-197.

Doggett, H., 1982. A look back on the 70´s. In: House,L.R., Mughogho, L.K., Peacock J.M. (Eds), Sorghum in the eighties: Proc. Int. Symp on Sorghum. Int. Crops Res. Inst. Semi-Arid Tropics (ICRISAT), Patancheru, India, pp. 15-24.

FAOSTAT, 2012. Available at: http://faostat.fao.org/default.aspx?lang=en

García-Lara, S., Bergvinson, D.J., 2007. Programa integral para reducir pérdidas poscosecha en maíz. Agricultura Técnica en México 33, 181-189.

García-Lara, S., Bergvinson, D.J., Burt, A.J., Ramputh, A.I., Diaz-Pontones, D.M., Arnason, J.T., 2004. The role of pericarp cell wall components in maize weevil resistance. Crop Science 44, 1546-1552.

González, M., Guzmán, B., Rudyk, R., Romano, E., Molina, M.A.A., 2003. Spectrophotometric determination of phenolic compounds in propolis. Latin American Journal of Pharmacology 22, 243-248.

Gutiérrez-Uribe, J.A., Rojas-García, C., García-Lara, S., Serna-Saldívar, S.O., 2010. Phytochemical analysis of wastewater (nejayote) obtained after lime-cooking of different types of maize kernels processed into masa for tortillas. Journal of Cereal Science 52, 410-416.

Lozano-Alejo, N., Carrillo, G.V., Pixley, K., Palacios-Rojas, N., 2007. Physical properties and carotenoid content of maize kernels and its nixtamalized snacks. Innovative Food Science & Emerging Technologies 8, 385-389.

Perez-Carrillo, E., Serna-Saldivar, S.O., Chuck-Hernández, C., Cortes-Callejas, M.L., 2012. Addition of protease during starch liquefaction affects free amino nitrogen, fusel alcohols and ethanol production of fermented maize and whole and decorticated sorghum mashes. Biochemical Engineering Journal 67, 1-9.

Pomeranz, Y., 1986. Comparison of Screening Methods for Indirect Determination of Sorghum Hardness. Cereal Chemistry 63, 36-38.

Price, M.L., Van Scoyoc, S., Butler, L.G., 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. Journal of Agricultural and Food Chemistry 26, 1214-1218.

Prior, R.L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D., Ou, B., Jacob, R., 2003. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL)) of plasma and other biological and food samples. Journal of Agricultural and Food Chemistry 51, 3273-3279.

Ramputh, A., Teshome, A., Bergvinson, D.J., Nozzolillo, C., Arnason, J.T., 1999. Soluble phenolic content as an indicator of sorghum grain resistance to *Sitophilus oryzae* (Coleoptera: *Curculionidae*). Journal of Stored Products Research 35, 57-64.

Salinas, M.Y., Martínez, B.F., Gomez, H.J., 1992. Comparación de métodos para medirla dureza del maíz (*Zea mays* L.). Archivos Latinoamericanos de Nutrición 42, 59-63.

Serna-Saldívar, S.O., 2010. Cereal Grains: Properties, Processing, and Nutritional Attributes, First edition. CRC Press, Boca Raton, FL, USA.

Serna-Saldivar, S.O., Rooney, L.W., 1995. Structure and chemistry of sorghum and millets. In: Dendy, D.A.V. (Ed.), Chapter 4: Sorghum & Millets: Chemistry and Technology. American Association of Cereal Chemists, St. Paul, MN, pp. 69-124.

Teshome, A. 1996. Factor Maintaining Sorghum [*Sorghum bicolor* (L.) Moench] landrace diversity in north Shewa and south Welo regions of Ethiopia. PhD Thesis, Department of Biology, Carleton University, Canada, Available at: http://idl-bnc.idrc.ca/dspace/bitstream/10625/13308/1/107542.PDF.

Vignoni, L.A., Césari, R.M., Forte, M., Mirábile, M.L., 2006. Determinación de índice de color en ajo picado. Información Tecnológica 17, 63-67.

Waniska, R.D., Rooney, L.W., 2000. Structure and Chemistry of the Sorghum Caryopsis. In Smith, C.W., Frederikson, R.A. (Eds.), Chapter 4.1, Sorghum: Origin, History, Technology, and Production. Wiley Series in Crop Science, New York, USA., pp 649-688.

Waniska, R.D., Hugo, L.F., Rooney, L.W., 1992. Practical methods to determine the presence of tannins in sorghum. Journal of Applied Poultry Research 1, 122-128.

CHAPTER 5. CHANGES ELICITED BY SITOPHILUS ZEAMAIS IN STARCH AND PROTEIN FRACTIONS OF FLOUR OBTAINED FROM DIFFERENT SORGHUM CULTIVARS

AACC, 2000. Approved Methods of the AACC, 10th ed. American Association of Cereal Chemists, St. Paul, MN.

Abebe, F., Tefera, T., Mugo, S., Beyene, Y., Vidal, S., 2009. Resistance of maize varieties to the maize weevil *Sitophilus zeamais* (Motsch.) (Coleoptera: *Curculionidae*). African Journal of Biotechnology 8, 5937-5943.

Aboubacar, A., Axtell, J.D., Huang, C.P., Hamaker, B.R., 2001. A Rapid Protein Digestibility Assay for Identifying Highly Digestible Sorghum Lines. Cereal Chemistry 78, 160-165.

Aboubacar, A., Hamaker, B. R., 1999. Physicochemical properties of flours that relate to sorghum couscous quality. Cereal Chemistry 76, 308-313.

Anibaba,T.S., Osagie, A.U., 1997. Effect of Malting on Protein Metabolism in Two Varieties of Sorghum. Journal of the Science of Food and Agriculture 74, 20-24.

Anwar, M.R., Liu, D.L., Macadam, I., Kelly, G., 2012. Adapting agriculture to climate change: a review. Theoretical and Applied Climatology. In press. Published on line: 19 October 2012.

AOAC, 1980. Official Methods of the Association of Official Analytical Chemists. AOAC, Washington, DC.

Awika, J.M., Rooney, L.W., 2004. Sorghum phytochemicals and their potential impact on human health. Phytochemistry 65, 1199-1221.

Axtell, J.D., Kirleis, A.W., Hassen, M.M., D'Croz-Mason, N., Mertz, E.T., Munck. L. 1981. Digestibility of Sorghum Proteins. Proceedings of the National Academy of Sciences of the United States of America 78, 1333-1335.

Bergvinson, D.J., 2001. Storage pest resistance in maize. In: Maize Program Maize Research Highlights 1999-2000. CIMMYT, Mexico D.F., Mexico, pp. 32-39.

Campbell, A., Sinha, R. N., 1976. Damage of Wheat by Feeding of Some Stored Product Beetles. Journal of Economic Entomology 69, 11-13.

Chanapamokkhot, H., Thongngam, M., 2007. The chemical and physico-chemical properties of sorghum starch and flour. Kasetsart Journal: Natural Science 41, 343-349.

Chuck-Hernández, C., Peralta-Contreras, M., Bando-Carranza, G., Vera-Garcia, M., Gaxiola-Cuevas, N., Tamayo-Limon, R., Cardenas-Torres, F., Perez-Carrillo, E., Serna-Saldivar, S., 2012. Bioconversion into ethanol of decorticated red sorghum (*Sorghum bicolor* L. Moench) supplemented with its phenolic extract or spent bran. Biotechnology Letters 34, 97–102.

Chuck-Hernández, C., Pérez-Carrillo, E., Heredia-Olea, E., Serna-Saldívar, S.O., 2011. Sorghum as a multifunctional crop for bioethanol production in Mexico: technologies, advances and improvement opportunities. Revista Mexicana de Ingeniería Química 10, 529-549.

Dal Bello, G., Padin, S. López-Lastra, C., Fabrizio, M., 2001. Laboratory evaluation of chemical-biological control of the rice weevil (*Sitophilus oryzae* L.) in stored grains. Journal of Stored Products Research 37, 77-84.

Dicko, M.H., Gruppen, H., Traoré, A.S., Voragen, A.G.J., Van Berkel, W.J.H., 2006a. Phenolics compounds and related enzymes as determinants of sorghum for food use. Biotechnology and Molecular Biology Reviews 1, 21-38.

Dicko, M.H., Gruppen, H., Traoré, A.S., Voragen, A.G.J., Van Berkel, W.J.H., 2006b. Sorghum grain as human food in Africa: relevance of content of starch and amylase activities. African Journal of Biotechnology 5, 384-395.

Dobie, P., 1974. The laboratory assessment of the inherent susceptibility of maize varieties to post-harvest infestation by *Sitophilus zeamais* Motsch. (Coleoptera, *Curculionidae*). Journal of Stored Products Research 10, 183-197.

Duodu, K.G., Nunes, A., Delgadillo, I., Parker, M.L., Mills, E.N.C., Belton, P.S., Taylor, J.R.N., 2002. Effects of grain structure and cooking on sorghum and maize in vitro protein digestibility. Journal of Cereal Science 35, 161-174.

Dykes, L., Rooney, L.W., Waniska, R.D., Rooney, W.L., 2005. Phenolic Compounds and Antioxidant Activity of Sorghum Grains of Varying Genotypes. Journal of Agricultural and Food Chemistry 53, 6813-6818.

FAOSTAT, 2012. Cereal production. Available from http://faostat.fao.org/

Guo, C., Cui, W., Feng, X., Zhao, J., Lu, G., 2011. Sorghum insect problems and management. Journal of Integrative Plant Biology 53, 178-192.

Hamaker, B.R., Kirleis, A.W., Butler, L.G., Axtell, J.D., Mertz, E.T., 1987. Improving the in vitro protein digestibility of sorghum with reducing agents. Proceedings of the National Academy of Sciences of the United States of America 84, 626-628.

Jackson, R.D. 1986. Remote Sensing of Biotic and Abiotic Plant Stress. Annual Review of Phytopathology 24, 265-287.

Jood, S., Kapoor, A.C., Singh, R., 1996. Chemical composition of cereal grains as affected by storage and insect infestation. Tropical Agriculture 73, 161-164.

Jood,S., Kapoor, A.C., 1992. Effect of storage and insect infestation on protein and starch digestibility of cereal grains. Food Chemistry 44, 209-212.

Kim, M., Day, D., 2010. Composition of sugar cane, energy cane, and sweet sorghum suitable for ethanol production at Louisiana sugar mills. Journal of Industrial Microbiology & Biotechnology, Volume and number not available, pp 1-5.

Maclean, W.C., López de Romaña, G., Placko, R.P., Graham, G.G., 1981. Protein quality and digestibility of sorghum in preschool children: balance studies and plasma free amino acids. Journal of Nutrition 111, 1928-1936.

Novozymes. 2007. Product Data Sheet. Liquozyme SC DS.

Pant, K.C., Susheela, T. P., 1977. Effect of storage and insect infestation on the chemical composition and nutritive value of grain sorghums. Journal of the Science of Food and Agriculture 28, 963- 970.

Park, S.O., Arthur, F.H., Bean, S.R., Schober, T.J., 2008. Impact of differing population levels of *Rhyzopertha dominica* (F.) on milling and physicochemical properties of sorghum kernel and flour. Journal of Stored Products Research 44, 322-327.

Platel, K., Shurpalekar, K.S., 1994. Resistant starch content of Indian foods. Plant Foods Human Nutrition 45, 91–5.

Pushparaj, F.S., Urooj, A., 2011. Influence of Processing on Dietary Fiber, Tannin and in Vitro Protein Digestibility of Pearl Millet. Food and Nutrition Sciences 2, 895-900.

Ranhotra, G. S., Gelroth, J. A., Eisenbraun, G. J., 1993. Correlation between Chopin and AACC methods of determining damaged starch. Cereal Chemistry 70, 236-237.

Rooney, L.W., Pflugfelder, R.L., 1986. Factors affecting starch digestibility with special emphasis on sorghum and corn. Journal of Animal Science 63, 1607–1623.

Rooney, L.W., Serna-Saldivar, S.O., 2000. Sorghum. In: Kulp, K., Ponte, J.G. (Eds.), Chapter 5, Handbook of Cereal Science & Technology. Marcel Dekker Inc., New York, USA, pp 149-175.

Serna Saldivar, S.O., Rooney, L.W., 1995. Structure and chemistry of sorghum and millets. In: Dendy, D.A.V. (Ed.), Chapter 4: Sorghum & Millets: Chemistry and Technology. American Association of Cereal Chemists, St. Paul, MN, pp. 69-124.

Serna-Saldívar, S.O., 2010. Cereal Grains: Properties, Processing, and Nutritional Attributes, First edition. CRC Press, Boca Raton, FL, USA.

Serna-Saldívar, S.O., 2012. Cereal Grains: laboratory reference and procedures manual, First edition. CRC Press, Boca Raton, FL. USA.

Taylor, J.R.N, Schober, T.J., Bean, S.R., 2006. Novel Food and Non-Food Uses for Sorghum and Millets. Journal of Cereal Science 44, 252-271.

Wang, D., Bean, S., McLaren, J., Seib, P., Madl, R., Tuinstra, M., Shi, Y., Lenz, M., Wu, X., Zhao, R., 2008. Grain sorghum is a viable feedstock for ethanol production. Journal of Industrial Microbiology and Biotechnology 35, 313–320.

Weaver,C.A., Hamaker, B.R., Axtell, J.D., 1998. Discovery of Grain Sorghum Germ Plasm with High Uncooked and Cooked In Vitro Protein Digestibilities Cereal Chemistry 75, 665-670.

Wu, X., Zhao, R., Bean, S. R., Seib, P. A., McLaren, J. S., Madl, R. L., Tuinstra, M., Lenz, M. C., Wang, D., 2007. Factors Impacting Ethanol Production from Grain Sorghum in the Dry-Grind Process. Cereal Chemistry 84, 130-136.

Yan, S., Wu, X., Bean, S.R., Pedersen, J.F., Tesso, T., Chen, Y.R., Wang, D., 2011. Evaluation of Waxy Grain Sorghum for Ethanol Production. Cereal Chemistry 88, 589-595.

Yan, S., Wu, X., MacRitchie, F., Wang, D., 2009. Germination-Improved ethanol fermentation performance of high tannin sorghum in a laboratory dry-grind process. Cereal Chemistry 86, 597-600.

CHAPTER 6. COMMON CHARACTERISTICS OF SORGHUM GRAIN INVOLVED IN FUEL ETHANOL PRODUCTION AND RESISTANCE TO MAIZE WEEVIL (SITOPHILUS ZEAMAIS).

AACC, 2000. Approved Methods of the AACC, 10th ed. American Association of Cereal Chemists, St. Paul, MN.

Abebe, F., Tefera, T., Mugo, S., Beyene, Y., Vidal, S., 2009. Resistance of maize varieties to the maize weevil *Sitophilus zeamais* (Motsch.) (Coleoptera: *Curculionidae*). African Journal of Biotechnology 8, 5937-5943.

Anglani, C., 1998. Sorghum carbohydrates: A review. Plant Foods for Human Nutrition 52, 77-83.

AOAC, 1980. Official Methods of the Association of Official Analytical Chemists. AOAC, Washington, DC.

Attfield, P.V., Kletsas, S., 2000. Hyperosmotic stress response by strains of bakers' yeasts in high sugar concentration medium. Letters in Applied Microbiology 31, 323-327.

Axtell, J.D., Kirleis, A.W., Hassen, M.M., D'Croz-Mason, N., Mertz, E.T., Munck. L., 1981. Digestibility of Sorghum Proteins. Proceedings of the National Academy of Science of the United States of America 78, 1333-1335.

Bergvinson, D.J., 2001. Storage pest resistance in maize. In: Maize Program Maize Research Highlights 1999-2000. CIMMYT, Mexico D.F., Mexico, pp. 32-39.

Bideaux, C., Alfenore, S., Cameleyre, X., Molina-Jouve, C., Uribelarrea, J.L., Guillouet, S.E., 2006. Minimization of Glycerol Production during the High-Performance Fed-Batch Ethanolic Fermentation Process in *Saccharomyces cerevisiae*, Using a Metabolic Model as a Prediction Tool. Applied And Environmental Microbiology 72, 2134-2140.

Brumm, P. J., Hebeda, R. E., 1988. Glycerol production in industrial alcohol fermentations. Biotechnology Letters 10, 677-682.

Buffo, R.A., Weller, C.L., Parkhurst, A.M. 1998. Wet-milling factors of sorghum and relationship to grain quality. Journal of Cereal Science 27, 327-334.

Chanapamokkhot, H., Thongngam, M., 2007. The chemical and physico-chemical properties of sorghum starch and flour. Kasetsart Journal: Natural Science 41, 343-349.

Chuck-Hernández, C., García-Lara, S., Serna-Saldívar, S.O., 2012a. Conversion into bioethanol of insect (*Sitophilus zeamais* Motschulsky), mold (*Aspergillus flavus* Link) and sprout-damaged maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench). Journal of Cereal Science 55, 285-292.

Chuck-Hernández, C., Peralta-Contreras, M., Bando-Carranza, G., Vera-Garcia, M., Gaxiola-Cuevas, N., Tamayo-Limon, R., Cardenas-Torres, F., Perez-Carrillo, E., Serna-Saldivar, S., 2012b. Bioconversion into ethanol of decorticated red sorghum (*Sorghum bicolor* L. Moench) supplemented with its phenolic extract or spent bran. Biotechnology Letters 34, 97–102.

Chuck-Hernández, C., Pérez-Carrillo, Serna-Saldívar, S.O., 2009. Production of bioethanol from steam-flaked sorghum and maize. Journal of Cereal Science 50, 131-137.

Duodu, K.G., Taylor, J.N.R., Belton, P.S., Hamaker, B.R., 2003. Factors affecting sorghum protein digestibility. Journal of Cereal Science 38: 117-131.

Elmaki, H.B., Babiker, E.E., El Tinay, A.H., 1999. Changes in chemical composition, grain malting, starch and tannin contents and protein digestibility during germination of sorghum cultivars. Food Chemistry 64, 331-336.

FAO, 2003. Food Energy: Methods of Analysis and Conversion Factors. FAO Food and Nutrition Paper 77. Electronic version available at: http://www.fao.org/docrep/006/Y5022E/y5022e00.htm#Contents

FAOSTAT, 2012. Cereal production. Available from http://faostat.fao.org/

FR. Financiera Rural., 2011. Monografía del Sorgo Grano. Dirección General Adjunta de Planeación Estratégica y Análisis Sectorial. Dirección Ejecutiva de Análisis Sectorial. Avilable at: http://www.financierarural.gob.mx/informacionsectorrural/Documents/Monografias/MonografiaSorgo(jun11).pdf.

García-Lara, S., Bergvinson, D.J., 2007. Programa integral para reducir pérdidas poscosecha en maíz. Agricultura Técnica en México 33, 181-189.

Gélinas, P., McKinnon, K., 2000. Fermentation and Microbiological Processes in Cereal Foods. In: Kulp, K., Ponte, J. (Eds), Chapter 26. Handbook of Cereal Science and Technology. Second Edition. Marcel Dekker., NY. USA pp. 741-754.

Gurrea, M.T. 2005. Análisis de Componentes Principales. Universidad Abierta de Cataluña. Disponible en: http://www.uoc.edu/in3/emath/docs/Componentes_principales.pdf

Gutiérrez-Uribe, J.A., Rojas-García, C., García-Lara, S., Serna-Saldívar, S.O., 2010. Phytochemical analysis of wastewater (nejayote) obtained after lime-cooking of different types of maize kernels processed into masa for tortillas. Journal of Cereal Science 52, 410-416.

Hamaker, B.R., Kirleis, A.W., Butler, L.G., Axtell, J.D., Mertz, E.T., 1987. Improving the in vitro protein digestibility of sorghum with reducing agents. Proceedings of the National Academy of Science of the United States of America 84, 626-628.

Kim, M., Day, D., 2010. Composition of sugar cane, energy cane, and sweet sorghum suitable for ethanol production at Louisiana sugar mills. Journal of Industrial Microbiology & Biotechnology, Volume and number not available, pp 1-5.

Lacerenza, J. A., Martin, J. M., Talbert, L. E., Lanning, S. P., Giroux M. J., 2008. Relationship of Ethanol Yield to Agronomic and Seed Quality Characteristics of Small Grains. Cereal Chemistry Journal 5, 322-328.

Lee, W.J., Pedersen, J.F., Shelton, D.R., 2002. Relationship of Sorghum kernel size to physiochemical, milling, pasting, and cooking properties. Food Research International 35, 643-649.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry 31, 426-428.

Mullins, J. T., NeSmith, C., 1986. Ethanol production from bird-resistant and non-bird resistant grain sorghum. In: Smith, W.H. (Ed.), Biomass Energy Development. Plenum Press, New York USA, pp. 541-550.

Munck, L., 1995. New milling technologies and products: whole plant utilization by milling and separation of the botanical and chemical components. In: Dendy, D.A.V. (Ed.), Chapter 8: Sorghum & Millets: Chemistry and Technology. American Association of Cereal Chemists, St. Paul, MN, pp. 223-281.

Murty, D.S., Singh, U., Suryaprakash, S., Nicodemus, K.D., 1985. Soluble sugars in five endosperm types of sorghum. Cereal Chemistry 62: 150-152.

Novozymes. No Date. Efficient liquefaction of starch. Available at: http://www.bimber.info/files/thermamyl-aplication.pdf

Olsen, H.S., 2002. Enzymes in Starch Modification. Chapter 9. In: Whitehurst, R.J., Law, B.A. (Eds). Enzymes in Food Technology. CRC Press. USA, pp. 255.

Perez-Carrillo, E., Serna-Saldivar, S. O., Alvarez, M. M., Cortes-Callejas, M. L., 2008. Effect of sorghum decortication and use of protease before liquefaction with thermoresistant *alpha*-amylase on efficiency of bioethanol production. Cereal Chemistry 85, 792-798.

Prior, R.L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D., Ou, B., Jacob, R., 2003. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL)) of plasma and other biological and food samples. Journal of Agricultural and Food Chemistry 51, 3273-3279.

Ramputh, A., Teshome, A., Bergvinson, D.J., Nozzolillo, C., Arnason, J.T., 1999. Soluble phenolic content as an indicator of sorghum grain resistance to *Sitophilus oryzae* (Coleoptera: *Curculionidae*). Journal of Stored Products Research 35, 57-64.

Rooney, L.W. 2003. Sorghum and millet food research failures and successes. Workshop on the proteins of sorghum and millets : enhancing nutritional and functional properties for Africa. 2-4 April 2003. Available at: http://www.afripro.org.uk/papers/Paper09Rooney.pdf

Rooney, L.W., Pflugfelder, R.L., 1986. Factors affecting starch digestibility with special emphasis on sorghum and corn. Journal of Animal Science 63, 1607–1623.

Salinas, M.Y., Martínez, B.F., Gomez, H.J., 1992. Comparación de métodos para medir la dureza del maíz (*Zea mays* L.). Archivos Latinoamericanos de Nutrición 42, 59-63.

Salunke, D.K., Jadhav, S.J., Kadam, S.S., Chavan, and J.K., 1982. Chemical, biochemical and biological significance of polyphenols in cereals and legumes. Critical Reviews in Food Science and Nutrition 17, 277-305.

Scanes, K.T., Hohmann, S., Prior, B.A., 1998. Glycerol production by the yeast *Saccharomyces cerevisiae* and its relevance to wine: a review. South African Journal for Enology and Viticulture 19, 18-24.

Serna-Saldívar, S.O., 2010. Cereal Grains: Properties, Processing, and Nutritional Attributes, First edition. CRC Press, Boca Raton, FL., USA.

Serna-Saldívar, S.O., 2012. Cereal Grains: laboratory reference and procedures manual, First edition. CRC Press, Boca Raton, FL. USA.

Serna-Saldivar, S.O., Rooney, L.W., 1995. Structure and chemistry of sorghum and millets. Chapter 4 In: Dendy, D.A.V. (Ed.), Sorghum & Millets: Chemistry and Technology, American Association of Cereal Chemists, St. Paul, MN, pp. 69-124.

Smith, L.I., 2002. A tutorial on Principal Components Analysis. Available at: http://www.cs.otago.ac.nz/cosc453/student_tutorials/principal_components.pdf. pp. 26.

Sun, J., Zhao, R., Zeng, J., Li, G., Li, X., 2010. Characterization of Dextrins with Different Dextrose Equivalents. Molecules 15: 5162-5173.

Taylor, J.R.N., Belton, P.S., 2002. Sorghum. In: Belton, P.S., Taylor, J.R.N. (Eds.). Pseudocereals and less common cereals, Springer, Berlin, pp. 24-91.

Teetes, G. L., Pendleton, B.B., 2000. Insect pests of sorghum. In: Smith C. W., Frederikson R. A., (Eds.), Chapter 3.3, Sorghum: Origin, History, Technology, and Production. Wiley Series in Crop Science, New York, pp. 443-495.

Vignoni, L.A., Césari, R.M., Forte, M., Mirábile, M.L., 2006. Determinación de índice de color en ajo picado. Información Tecnológica 17, 63-67.

Vriesekroop, F., Haass, C., Pamment, N.B., 2009. The role of acetaldehyde and glycerol in the adaptation to ethanol stress of *Saccharomyces cerevisiae* and other yeasts. FEMS Yeast Research 9, 365-371.

Wang, D., Bean, S., McLaren, J., Seib, P., Madl, R., Tuinstra, M., Shi, Y., Lenz, M., Wu, X., Zhao, R., 2008. Grain sorghum is a viable feedstock for ethanol production. Journal of Industrial Microbiology and Biotechnology 35, 313–320.

Waniska, R.D., Rooney, L.W., 2000. Structure and Chemistry of the Sorghum Caryopsis. In Smith, C.W., Frederikson, R.A. (Eds.), Chapter 4.1, Sorghum: Origin, History, Technology, and Production. Wiley Series in Crop Science, New York, USA., pp 649-688.

Watson S.A., Hirata Y. 1960. Carbohydrates in grain sorghum kernels. Newsletter 3, 6–7.

Wrolstad, R.E., 2012. Food Carbohydrate Chemistry. Wiley-Blackwell. IFT Press Series, pp. 256.

Wu, X., Jampala, B., Robbins, A., Hays, D., Yan, S., Xu, F., Rooney, W., Peterson, G., Shi, Y., Wang, D., 2010. Ethanol fermentation performance of grain sorghum (*Sorghum bicolor*) with modified endosperm matrices. Journal of Agricultural and Food Chemistry 58, 9556-9562.

Wu, X., Zhao, R., Bean, S.R., Seib, P.A., McLaren, J.S., Madl, R.L., Tuinstra, M., Lenz, M.C., Wang, D., 2007. Factors impacting ethanol production from grain sorghum in the dry-grind process. Cereal Chemistry 84, 130-136.

Wu, X., Zhao, R., Liu, L., Bean, S., Seib, P.A., McLaren, J., Madl, R., Tuinstra, M., Lenz, M., Wang, D. 2008. Effects of growing location and irrigation on attributes and ethanol yields of selected grain sorghums. Cereal Chemistry 85, 495-501.

Wu, X., Zhao, R., Wang, D., Bean, S. R., Seib, P. A., Tuinstra, M. R., Campbell, M., O'Brien, A., 2006. Effects of Amylose, Corn Protein, and Corn Fiber Contents on Production of Ethanol from Starch-Rich Media. Cereal Chemistry 83, 569-575.

Yan, S., Wu, X., Bean, S. R., Pedersen, J.F., Tesso, T., Chen, Y., Wang, D., 2011. Evaluation of Waxy Grain Sorghum for Ethanol Production. Cereal Chemistry 88, 589-595.

Yan, S., Wu, X., Dahlberg, J., Bean, S.R., MacRitchie, F., Wilson, J.D., Wang, D., 2010. Properties of field-sprouted sorghum and its performance in ethanol production. Journal of Cereal Science 51, 374-380.

Yan, S., Wu, X., MacRitchie, F., Wang, D., 2009. Germination-improved ethanol fermentation performance of high-tannin sorghum in a laboratory dry-grind process. Cereal Chemistry 86, 597-600.

Zhan, X., Wang, D., Tuinstra, M.R., Bean, S., Seib, P.A., Sun, X.S., 2003. Ethanol and lactic acid production as affected by sorghum genotype and location. Industrial Crops and Products 18, 245-255.

Zhang, G., Hamaker, B. R., 1998. Low alpha-amylase starch digestibility of cooked sorghum flours and the effect of protein. Cereal Chemistry 75, 710-713.

Zhao, R., Bean, S. R., Ioerger, B. P., Wang, D., Boyle, D. L., 2008. Impact of mashing on sorghum proteins and its relationship to ethanol fermentation. Journal of Agricultural and Food Chemistry 56, 946-953.

Zhao, R., Bean, S. R., Wang, D., Park, S. H., Schober, T. J., Wilson, J. D., 2009. Small-scale mashing procedure for predicting ethanol yield of sorghum grain. Journal of Cereal Science 49, 230-238.