FERMENTATION OF MELON AND FLUTED PUMPKIN SEEDS FOR "OGIRI" PRODUCTION USING *Bacillus subtilis*: A RESPONSE SURFACE APPROACH.

BY

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Fermentation of melon and fluted pumpkin seeds for "ogiri" production using *bacillus subtilis*: a response surface approach. By Okoli, A.I. (2015) is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

CERTIFICATION

This is to certify that this project on the *"Fermentation Of Melon And Fluted Pumpkin Seeds For 'Ogiri' Production Using A Response Surface Approach"* was carried out by Okoli, Innocentia Ada Reg No: 20094699018 in partial fulfilment of the requirements for the award of M.Sc degree in Food Science and Technology, Federal University of Technology, Owerri, Imo State.

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DEDICATION

This work is dedicated to God Almighty, the God of Impossibilities who has seen me through this period of study. Also to my loving husband Chukwuemeka Monu and Our son Ugochukwu, thank you for being there for me.

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ABSTRACT

Fermentation of melon and fluted pumpkin seeds for ogiri production using *Bacillus subtilis* as starters and using the response surface approach were studied. The seeds were fermented for 96 hours and free fatty acid, total titratable acidity (TTA), amino nitrogen, pH and peroxide values were determined during the fermentation using standard analytical methods. Free fatty acids increased as fermentation progressed with highest values (4.74mg/g) and (4.26mg/g) obtained from sample 5 (fermented at 65% RH, 35°C and 50µm) and sample 3 (fermented at 65% RH, 40°C and 70µm) for both pumpkin and melon seeds respectively. The TTA increased with increase in time of fermentation for both the fluted pumpkin and melon seeds. Sample 12 (fermented at 85% RH, 40°C and 90µm) produced the highest TTA value(1.56%)for pumpkin while sample 3 (fermented at 65% RH, 40°C and 70µm) with 1.24% had the highest for melon seeds. Peroxide values obtained for the two fermented seeds were below 30 meq/kg which is the upper limit recommended for peroxide value for oily foods. The highest peroxide values obtained from sample 2 (fermented at 85% RH, 30°C and 70µm) was 4.85 meq.kg for fluted pumpkin seeds while sample 6 (fermented at 85% RH, 35°C and 50µm) with 4.08 meq/kg was obtained for melon seeds. pH values obtained during the fermentation of both seeds increased from start of fermentation to the 96th hour tending towards the alkaline range. Samples 3(fermented at 65% RH, 40°C and 70µm) and 4 (fermented at 85% RH, 40°C and 70µm) had the highest pH values of 8.23 and 8.15 for fluted pumpkin and melon seeds respectively. Amino nitrogen increased throughout the fermentation period with the highest values obtained from samples 4 (fermented at 85% RH, 40°C and 70µm) with a value of 8.33 mgN/g for fluted pumpkin seeds, and sample 9 (fermented at 65% RH, 30°C and 50µm) with 6.77mgN/g for melon seeds. Daily amino nitrogen determinations showed that fluted pumpkin fermentation got to its peak at 72h while fermentation of melon seeds got to its peak at 48h. The response surface analysis showed that the amino nitrogen during melon seed fermentation could be predicted using the quadratic model. The R^2, adjusted R^2 and predicted R^2 were 0.9956, 0.9877 and 0.9076 respectively.

Key words: *Amino nitrogen, Bacillus subtilis, fermentation, ogiri, starters, fluted pumpkin, melon.*

TABLE OF CONTENTS

Title page	i
Certification	ii
Dedication	iii
Acknowledgement	iv
Abstract	v
Table of contents	vi-ix
List of Tables	x
List of Figures	xi
CHAPTER ONE – INTRODUCTION	1
1.1 Background information	1-3
1.2 Problem statement	3
1.3 Objectives	3-4
1.4 Justification	4
1.5 Scope	4
CHAPTER TWO -LITERATURE REVIEW	5
2.1 Historical Background of Melon (*Citrullus vulgaris)*	5
2.1.2 Nutritional composition of melon seeds	5-7
2.1.3. Uses of melon	7
2.2 Traditional processing of ogiri egusi	7-8
2.3 Biochemical changes accompanying fermentation	8
2.3.1 Changes in carbohydrates	8-9

2.3.2 Changes in amino acids 9-10

2.3.3 Changes in lipids 10

2.3.4 Changes in vitamins and minerals 11

2.3.5 Changes in titratable acidity, pH and temperature 11

2.4 Predominant microbes involved in ogiri egusi 11

2.5 Use of starter cultures in ogiri egusi fermentation 11-12

2.6 Safety of ogiri egusi fermentation 12

2.7 Description and origin of fluted pumpkin (*Telfairia occidentalis* Hook f.)13-15

2.8 Brief historical background of the response surface methodology (RSM) 15

CHAPTER THREE - MATERIALS AND METHODS 16

3.1 Source of materials 16

3.2 Methods 16

3.2.1 Media preparation 16

3.2.1.1 Preparation of Tryptone soy agar and Tryptone soy broth 16

3.2.1.2 Preparation of Nutrient agar 16-17

3.2.1.3 Preparation of peptone water 17

3.2.1.4 Preparation of Tryptone soy agar slant 17

3.2.2 Generation of relative humidity 17

3.2.3 Isolation of pure cultures of *Bacillus subtilis* 17-18

3.2.4 Identification of isolated cultures 18

3.2.4.1 Gram staining test 18-19

3.2.4.2 Endospore staining test 19

3.2.4.3 Catalase test 19

3.2.4.4 Oxidase test 19-20

3.2.4.5 Starch hydrolysis 20

3.2.5 Standardization of pure culture 20-21

3.2.6 Traditional production of ogiri egusi 21

3.2.6.1 Traditional production of ogiri ugu 22

3.2.7 Production of ogiri egusi and ogiri ugu

using pure culture of *Bacillus subtilis* 23

3.2.8 Experimental design 24

3.2.9 Determination of free fatty acids 25

3.2.10 Determination of peroxide value 26

3.2.11 Determination of total titratable acidity 26

3.2.12 pH Determination 26-27

3.2.13 Determination of Amino nitrogen 27-28

3.2.14 Statistical analysis determination 28

CHAPTER FOUR - RESULTS AND DISCUSSION 29

4.1 Results on fermented pumpkin and melon seeds 29

4.2 Discussion 29

4.2.1 Effect of variables on the fermented fluted pumpkin 46-51

4.2.2 Effect of variables on the fermented melon seeds 51-57

CHAPTER FIVE - CONCLUSIONS AND RECOMMENDATIONS 58

5.1 Conclusions 58

5.2 Recommendations 58-59

5.3 Contributions to knowledge 59

References 60-69

Appendix 1 – Response graph for ogiri ugu production 70-75

Appendix 2- statistical analysis of fluted pumpkin 76-113

LIST OF TABLES

Table 3.1: Independent variables and levels 24

Table 3.2: Combination of the independent variables 25

Table 4.1 Free fatty acids levels during fermentation offluted pumpkin seeds (mg/g) 30

Table 4.2: Total titratable acidity levels during fermentation offluted pumpkin seeds (%) 31

Table 4.3: Peroxide values during fermentation of fluted pumpkin seeds (Meq/kg) 32

Table 4.4: Amino nitrogen levels during fermentation of fluted pumpkin seeds (MgN/g) 33

Table 4.5: pH levels during fermentation of fluted pumpkin seeds 34

Table 4.6: Free fatty acids levels during fermentation of melon seeds (mg/g) 35

Table 4.7: Total titratable acidity levels during fermentation of melon seeds (%) 36

Table 4.8: Peroxide values during fermentation of melon seeds (Meq/kg) 37

Table 4.9: Amino nitrogen levels during fermentation of melon seeds (mgN/g) 38

Table 4.10: pH levels during fermentation of melon seeds 39

4.11: Model summary statistics for amino nitrogen 40

4.12: Anova table for response surface quadratic model (Amino nitrogen) during melon seeds fermentation 41

4.13: Daily amino nitrogen production for pumpkin seeds (mgN/g) 42

4.14: Daily amino nitrogen production for melon seeds (mgN/g) 43

LIST OF FIGURES

Figure 3.1: Flow diagram for the production of ogiri egusi 22

Figure 3.2: Flowchart for production of ogiri ugu 23

Figure 4.1: Effect of temperature and relative humidity on amino nitrogen content of ogiri egusi. 44

Figure 4.2: Effect of pore size and relative humidity on the amino nitrogen content of ogiri egusi. 44

Figure 4.3: Effect of pore size and temperature on the amino nitrogen content of ogiri egusi. 45

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND INFORMATION

Ogiri is an oily paste produced from oil seeds in West Africa. They are also used as soup condiments having a strong smell (Akinyele and Oloruntoba, 2013). It is also a food condiment prepared by traditional methods of uncontrolled solid state fermentation of melon seeds *(Citrullus vulgaris*) (Asagbra *et al.*, 2012).The food flavouring condiments are prepared by traditional methods of uncontrolled solid substrate fermentation resulting in extensive hydrolysis of the protein and carbohydrate components (Achi, 2005). Apart from increasing the shelf life, and reducing anti nutritional factors, fermentation also improves digestibility, nutritive value and flavours of the raw seeds (Achi, 2005). Ogiri is produced from various plant materials including fluted pumpkin (*Telfaira occidentalis*) for 'ogiri ugu', castor oil seeds (*Ricinus communis)* for Ógiri igbo', melon seeds (*Citrullus vulgaris*) 'Ógiri egusi' and groundnut seeds (*Arachis hypogea*) ógiri isi', Iru from African locust bean (*Parkia biglobosa),* Ówoh from cotton seeds (*Gossypium hirsitum*) and African yam beans (*Stenophylis stenocarpa*), and 'daddawa' from soya bean (*Glycine* max) (Achi, 2005).

These soup condiments are known to have high protein content, thus serving a dual purpose of flavouring as well as sources of protein supplements (Abaelu *et al.*, 1990).

Melon (*Citrullus vulgaris*) is a legume and an annual crop grown during the early period of the rainy season. It is mostly cultivated in the southern part of Nigeria and is inter-planted with yam and cassava because of the creeping nature of the stem and mostly used as a cover crop (Asagbra *et al.*, 2012). The seeds may account for up to 80% dietary protein and may be the only source of protein for

some groups, thus it can be used as substitute for fish or meat (Asagbra *et al.,* 2012).

Ogiri egusi is a food flavouring condiment prepared by traditional methods of uncontrolled solid substrate fermentation of melon seeds (*Citrullus vulgaris*) (Achi, 2005). The fermentation usually takes 3-5days at the prevailing ambient temperature (Barber and Achinewhu, 1992; Omafvube et *al.,* 2004).

Ogiri egusi appears locally in a mashed whitish or ash coloured form (Achi, 2005). During its production, melon seeds are boiled till they are soft. These are wrapped tightly in leaves and left to ferment for about five days at the prevailing ambient temperature (Ihekoronye and Ngoddy, 1985).

Melon seeds are made up of 30-40% protein, 52% oil, 3.6% ash and 15.5% crude fibre (Omafuvbe *et al.*, 2004). The seeds contain some amount of non-digestible carbohydrates which may include arabinogalactan, stachyose and raffinose (Odunfa, 1983).

Various workers have identified different microorganisms in fermented melon seeds. These microorganisms include *Bacillus sp*, *E.coli, Proteus sp, Pediococcus sp* and *Alcaligenes sp* (Barimalaa *et al.*, 1989; Barber and Achinwehu, 1992; Sanni *et .al*, 2000). However, *B. subtilis* and *B. lichenformis* have been identified as the main bacteria present (Barimalaa *et al.,* 1989).

Fluted pumpkin seed (*Telfairia occidentalis Hook, F.*) is widely consumed in Nigeria, especially in southern part of Nigeria. The seeds can be used in cookie formulations and marmalade manufacturing (Giami and Barber, 2004; Fagbemi *et .al*, 2005) and it is also a good source of edible oil. The seed has been compared with some common plant foods in Nigeria and has shown that *Telfairia*

occidentalis Hook, F. is high in carbohydrate, fat and phosphorus (Agatemor, 2007).

1.2 PROBLEM STATEMENT

The use of chance fermentation as practiced traditionally makes the fermentation difficult to control and results in the contamination of products with pathogens or other microorganisms capable of producing toxins and those that cause off flavours (Ogueke and Nwagwu, 2007). Chance fermentation also results in variation in the product quality. Attempts have been made by Ogueke and Nwagwu (2007) to produce ogiri egusi using pure cultures of the bacteria isolated from previous ogiri egusi thus trying to introduce the use of starter cultures.

There is also need to predict and optimize the fermentation process with the view to industrializing the production. However, before this can be achieved there is need to study all the fermentation variables as they affect the fermentation process and the final product quality using pure cultures of the major bacterium involved in the fermentation. This will also enable one to understand how the variables interplay during the fermentation.

1.3 OBJECTIVES

The main objective of the research work is the fermentation of melon and fluted pumpkin seeds for ogiri production using *B. subtilis* as starters and using response surface method to predict the fermentation process.

The specific objectives of the work are as follows;

1. To isolate *Bacillus subtilis* from a previous ogiri egusi and ogiri ugu samples.
2. To use the isolates to ferment melon and fluted pumpkin seeds at different temperatures, relative humidity and pore size of wrapping material.

3. To monitor free fatty acid, total titratable acid, amino nitrogen, pH and peroxide value on 24 hour basis throughout the fermentation process.
4. To determine the effect of relative humidity, temperature and pore size of wrapping material on the fermentation process.
5. Attempt on prediction of process using the response surface methodology.

1.4 JUSTIFICATION

The results of this work will show the effect of the interaction of the variables on the fermentation process, thus making it possible for the process to be predicted and possibly optimized. This will also eliminate the use of chance fermentation which usually introduce pathogenic and/or spoilage microorganisms. Thus, this will ultimately guarantee quality of product at all times and will also pave way for large scale industrial production of the product.

1.5 SCOPE OF THE WORK

Egusi and fluted pumpkin seeds were used for this work. They were fermented using starter culture of *Bacillus subtilis* for 96 hours using different pore sizes of polyethene at varying relative humidity and temperatures. At every 24 hour interval, the fermenting seeds were analysed for Free Fatty Acids, Total Titratable Acidity, peroxide values, pH and amino nitrogen. The response surface method was used to predict the amino nitrogen of the fermenting seeds.

CHAPTER TWO

LITERATURE REVIEW

2.1 HISTORICAL BACKGROUND OF MELON (*Citrullus vulgaris)*

Melon (*Citrullus vulgaris*) belongs to the family *Curcurbicaceae.* It is a legume and an annual crop grown during the early period of the rainy season. It is cultivated mostly in the southern part of Nigeria and is inter-planted with cassava and yam because of the creeping nature of its stem and mostly used as a cover crop (Asagbra *et al.*, 2012). The melon plant produces about 15-30 round fruits, 7-10cm in diameter, green in colour with undulated yellow strips on its body and this becomes yellow throughout when it is dry. The content is slimy to touch when the pods are broken.

Flowering occurs between May and August and the seeds are small (6mm in length), brownish in colour and smooth when ripe (Ogueke and Nwagwu, 2007). Melon seeds originated from tropical Asia and Africa and widely distributed in the Sahara-Arabian phyto geographical region in Africa and the Mediterranean region. They serve as both food and as source of income for millions of people, and are grown not for their pulp which is bitter but for their seeds which are particularly rich in protein and oils (Voet and Voet, 1995).

2.1.2 Nutritional composition of melon seed

Melon seeds are very high in nutritional value. It has about 8% carbohydrate and a good dietary source of arginine, tryptophan, methionine, vitamin B1, Vitamin B2, niacin and various trace elements. Its oil is cholesterol free; 78% of the fat is unsaturated fatty acid which is protective to the heart (Obeta and Abiriba, 1994). In terms of vitamins, it contains alpha-tocopherol, a component of vitamin E that helps in maintaining smooth young skin and good fertility (Omafuvbe *et al.*, 2004). It also contains palmitic, stearic, linoleic and oleic acids important in protecting

the heart too, and very small amount of carbohydrate and calcium (Omafuvbe *et al.*, 2004).

The seeds contain some amount of non-digestible carbohydrate which may include arabinogalactan, stachyose, and raffinose (Odunfa, 1983). These carbohydrates are associated with abdominal distension and flatulence in humans (Barber and Achinewhu, 1992).

The ash content of raw melon seed has been found to be 3.3%. Boiling and fermentation did not seem to have any significant effect on the ash content (Omafuvbe *et al.*, 2004).

Omafuvbe *et al.,* (2004) also reported the crude fibre content of raw melon seeds to be 15.8% and that the crude fibre content of melon seeds increased by 31% after boiling process, this probably is due to the soluble compounds in the seeds being leached out. But fermentation after twenty-four hours decreased the crude fibre content by 22% probably due to the production of extracellular enzymes. Achinewhu (1983) also found some minerals like calcium, magnesium, phosphorus and potassium as nutritional components of ogiri egusi. Achinewhu (1983) reported a slight decrease in minerals and fibre content of ogiri egusi when compared with the data on raw melon seeds. The ether extract of raw melon seeds decreased after boiling and thereafter increased by 3-5% every 24 hours during fermentation (Omafuvbe *et al.*, 2004). Adewusi *et al.*, 1992 reported crude protein content to be 19.3% for raw melon seeds while that of fermented melon seeds was close to 18.4%. Achinwehu (1983) reported the increase in crude protein indicating that the protein has been pre-digested during fermentation and thus improve the availability of amino acids. Omafuvbe *et al.* (2004) reported fermentation of melon seeds samples to have led to an increased pH as earlier observed (Odunfa, 1981). Acid and iodine values of melon seeds increased with

prolongation of the fermentation period. Heat treatment employed in the production of ogiri egusi reduced the iodine value (Omafuvbe *et al.*, 2004).

Omafuvbe *et al.*, 2004 reported increase in reducing sugars due to fermentation of ogiri egusi and that ogiri egusi contains maltose, sucrose, fructose, galactose and other unidentified sugars.

No wonder the centre of cover crops information and seed exchange in Africa decries the gross under research and utility of egusi. It could greatly help to wipe off malnutrition (Obeta and Abiriba, 1994).

2.1.3 Uses

Melon seeds have shown a high degree of utilization in areas like processing into other edible forms and medicinal benefits. The seeds can be processed into flour and meal and also made into patties used to substitute meal and used as meat extenders when defatted. Melon seeds are used locally to prepare foods like igbo cake (Úsu'). Due to the high protein percentage, the seeds could be processed to protein concentrates and hydrolysates and used in the supplementation of low protein foods. Melon flours are used to supplement cereals in the meat industry to produce cheap nutrient meat in areas where meat supply is small and expensive (Ogueke and Nwagwu, 2007). Melon seeds are roasted and eaten as snacks or prepared as salad with oil bean seed (ugba) when boiled. The seeds have emulsifying property and as such are used as a thickener in soup preparation. Medicinally, the melon fruits are used especially to curb stomach pains due to the glycoside content such as colocynthin of the pulp; it is a laxative (Burkill, 1985).

2.2 Traditional processing of ogiri egusi

Ogiri egusi is a food flavouring condiment prepared by traditional methods of uncontrolled solid substrate fermentation of melon seeds (*Citrullus vulgaris*)

(Achi, 2005). Ogiri egusi appears locally in a mashed whitish or ash coloured form. It is prepared conventionally by rinsing cleaned shelled melon seeds with warm water twice and boiled to soften. The boiled water is drained and the melon seeds are thereafter transferred into a clay pot and covered with plantain leaves put over flame for minutes, wrapped in sack cloth for three days. The fermented product is then ground with stone, mortar and pestle to almost a smooth paste with ash added and sold as ogiri egusi (Achi, 2005).

In traditional production of ogiri egusi as reported by Steinkraus (1995), Barber and Achinewhu, (1992), Iwuoha and Eke (1996) and Omafuvbe et al, (2004), dry melon seeds are moistened and dehulled. The bad seeds and foreign materials are removed. The cotyledons are then boiled in water for 5-6h and the water drained out. The boiled seeds are wrapped tightly in layers of blanched plantain leaves and are left to ferment at the prevailing ambient temperature for 3-5days. At the end of the fermentation period, the seeds are ground into a paste with ash from burnt oil palm leaves. This adds grey colours to the paste. The paste is distributed in small portions into plantain leaves, wrapped up and left near the fire place until sold or used.

2.3 Biochemical changes accompanying fermentation

2.3.1 Changes in carbohydrates.

It has been reported that *Bacillus spp* produce certain enzymes such as amylases, galactamase, galactosidase, glucosidase and fructo furanosidase which are involved in the degradation of carbohydrate (Aderibegbe and Odunfa, 1990). Microbial amylase hydrolyses carbohydrates into sugars which are then readily digestible by humans.

Similarly, galactanase softens the texture of the seeds and liberate sugars for digestion. Omafuvbe *et al.,* (2004) reported that fermentation of melon seeds resulted in substantial decrease in the total sugar. This was probably due to the

removal of the hulls that contain higher level of sugar. The cooking stage of the seeds have been reported as the most important step in the preparation of raw materials for fermentation which have also resulted in partial loss of soluble solids in the cooking water as reported by Omafuvbe *et al.,* (2004). He reported further decrease in total soluble sugar level with fermentation of melon seeds. Omafuvbe *et al.*, (2004) reported that the reducing sugar level decreased during processing but fluctuated during fermentation. The decreased level of reducing sugar is a reflection of the activities of amylase and sucrase in the fermenting seeds. Omafuvbe *et al.*, (2004) also reported that alpha amylase increased with fermentation while sucrase increase reached its peak at the 48th hour and then dropped. The fluctuation in the level of sugar with fermentation may be related to its utilization by the fermenting microorganisms for their metabolic activities. George (1979) reported that soluble sugars decreased initially but increased later to exceed the initial concentration, hence producing little alcohol in the course of fermentation. It was observed that soluble sugars like maltose, sucrose, galactose, fructose and other unidentified sugars were reported to be contained in ogiri egusi (Achinewhu, 1983).

2.3.2 Changes in amino acid

Achinewhu *et al.,* (1991) reported that free amino nitrogen (FAN) and total microbial counts increased during the fermentation of melon seeds (Ogiri egusi) which weremaximum at 35°C. However, Ogueke and Nwagwu (2007) observed a slight decrease during fermentation with pure cultures of bacteria isolated from previous ogiri egusi. They attributed this slight decrease to the strong proteases deaminase activity of *Bacillus spp* which resulted in ammonia production and their ability to utilize amino acids as carbon and energy sources.

Barber and Achinewhu (1992) observed an increase in pH up to 8.1 during the fermentation of ogiri egusi. The high pH values of fermented legumes compared

to other materials under similar conditions have been attributed to their high protein content. In most fermented high protein products, the extent of protein hydrolysis is one of the most important factors in the changes in texture and flavour (Whitaker, 1978). Ammonia produced because of protein metabolism are responsible for the gradual pH increase and levelling off towards 7.5-8.0 (Barimalaa *et al.*, 1989; Achi 1992; Barber and Achinewhu, 1992). The increase in pH due to the formation of ammonia from amino acids may consequently encourage growth of spoilage organisms such as *Lactobacilli* and *Pediococci.* Free amino acids increased, but longer fermentation results in losses of lysine or other essential amino acids (Winarno and Reddy, 1986). Omafuvbe *et al.,* (2004) reported that fermentation of melon seeds resulted in increase in total free amino acids. Thus rapid increase in total free amino acids is a reflection of the increased protease activity which has been reported to be abundant in the fermentation of similar protein rich foods (Sarkar *et al*, 1993). George (1979) reported that ogiri egusi foul smell is due to the production of hydrogen sulphide and ammonia gases during the proteolytic breakdown of the protein. Specifically, he found the proteolytic activity to be at maximum after 12 hours of fermentation. Also, the free amino nitrogen increased during fermentation.

2.3.3 Changes in Lipids

The significant lipolysis of the seeds yields predominantly oleic, linoleic and linolenic acids (Achinewhu, 1983). Free fatty acids, particularly oleic, linoleic and linolenic acids were associated with non-specific antitryptic activity (Winarno and Reddy, 1986) as extensive hydrolysis could diminish the nutritional quality. Although oils constitute up to 40% of the seeds, extensive lipolysis does not take place (Achi, 2005). Odunfa (1983) reported low levels of lipase in melon seed fermentation. Darweesh *et al* (1991) as cited by Achi (2005) have reported that aldehydes resulting from oxidation of lipids cause off flavour and odour problems.

However, organic solvent treatment on the objectionable odour of the fermented melon seed did not show increase in the odour.

2.3.4 Changes in vitamins and minerals

Other changes include that of vitamin. Fermentation improved the vitamin content of ogiri egusi (Lopez Paredes and Henry, 1988). Achinewhu (1983) reported that niacin, riboflavin and thiamine were produced during the fermentation of ogiri egusi.

Also increase in ash content was reported in the fermented product (Enujiugha, 2003). This was attributed to the increased metabolic activities of the fermenting microorganisms since some of the biosynthetic mechanism; especially those involving *Bacillus species* were capable of synthesizing divalent metals (Moat, 1979).

2.3.5 Changes in titratable acidity, pH and Temperature

Ogiri egusi fermentation is particularly sensitive to temperature and pH of fermentation. The optimum condition for performance are 3days at 29-30°C with pH of 6.5-8.4. During fermentation, fermented melon seeds have a higher pH and titratable acidity (David and Aderibigbe, 2010).

2.4 Predominant microbes involved in ogiri egusi

Various bacteria have been identified to be involved in ogiri egusi fermentation. These include *Bacillus sp, Proteus sp, Pediococcus sp* and *Lactobacillus sp* (Ogueke and Nwagwu, 2007). Barimalaa *et al.*, 1989; Barber and Achinewhu, 1992; Sanni *et al.,* 2000 have identified *Bacillus sp, E.coli, Proteus sp* and *Pediococus* and *Alcaligenes.* However, *B.subtilis* and *B, lichenformis* have been identified as the main bacteria present (Barimaalaa *et al.*, 1989).

2.5 Use of starter cultures in ogiri egusi fermentation

The problem with fermentation of Ogiri egusi is variability and stability of the nutritional quality of the fermented product (Ouoba *et al.,* 2008). The use of well

selected starter cultures based on defined identification and characteristics can help solve this problem.

Ogueke and Nwagwu (2007) have used pure cultures of the various bacteria isolated from ogiri egusi for the fermentation, and the sample that fermented with pure cultures of *Bacillus sp* produced a product very similar to that produced from natural fermentation. A well-adapted fermentation starter (which also include a mixed microbial population) can provide strong process control with minimal need for control instrumentation and is most stable not only for the 'low-tech' environment of developing countries but for the more sophisticated environment of developed countries as well (Philips *et al.*, 2005)

Also Adeola *et al.,* (1990) have fermented melon seeds using pure cultures of singular microorganisms isolated from fermented oil beans which include; *Bacillus lichenformis*, *Bacillus subtilis*, and *Bacillus pumulis*.

The use of these starter cultures will eliminate the incidence of food contamination and food poisoning by other spoilage microorganism and also lead to the production of a product with uniform and standard quality.

2.6 Safety of ogiri egusi fermentation

During natural fermentation of ogiri egusi, food poisoning microorganism may grow along with the *Bacillus spp* more so when the sanitary conditions of the fermenting food is poor. These microorganisms need to be eliminated to make fermented foods safe for consumption. Reports has it that ogiri egusi has a high mycotoxin contamination (Odunfa, 1985). Despite all these factors, several other factors contribute to the safety of ogiri egusi. For instance, ogiri egusi fermentation is carried out by acid producing microorganisms, thus producing some organic acids such as lactic, acetic, furmaric acids which act as preservatives. Ogiri egusi is also usually prepared with the addition of salt which can act as a preservative (Nwosu and Ojimelukwe, 2000).

2.7 Description and Origin of Fluted pumpkin (*Telfairia occidentalis* Hook f)

Telfaira occidentalis commonly known as fluted pumpkin belongs to the family *Cucurbitaceae* a tropical vine grown in West Africa (Kajihausa *et al* 2010). It is a creeping vegetative shrub that spreads low on the ground with large lobed leave and long twisting tendrils (Horsfall and Spiff, 2005; Christian 2006; Ojiako and Igwe, 2008). The plant is a dioecious, perennial, drought tolerant and usually grows trellised (Eroarame, 2012).The vegetable prefers to grow on loose, friable humus rich land under shade. It is one of the locally available, under-exploited but potentially high protein food sources in Nigeria. In addition to its importance as an oilseed (54%), it is a valuable source of protein (27%) with a fairly well balanced amino acid composition.

Harvesting of fluted pumpkin takes place 120-150 days after planting. The vegetable is of commercial importance across the low land humid tropic in West Africa with Nigeria, Ghana and Sierra Leone being the major producers (Amanabo *et al.,* 2011).

Young shoots and leaves are the main parts used in soup. The common names of the plant in Nigeria include fluted gourd, fluted pumpkin, iroko and ugu (Eroarame, 2012). The vines of the plant will climb to 1.5m and the flowers are white and dark purple. Sex of fluted pumpkin is difficult to identify until about 4months after planting when it produces flowers, a major obstacle to its production (Eroarame, 2012). Housewives prefer the female leaves leading to higher demand.

The young shoots and leaves of the female plant form main ingredients in edikang ikong, a soup favoured by people in Cross River and Akwa Ibom states of Nigeria (Eroarame, 2012).

T. occidentalis like other leafy vegetables also contains considerable levels of certain anti nutrients and toxic substances which have been shown to have negative effect on animal and human health at high concentrations (Ekpedema *et al*., 2000; Ojokoh *et al.,* 2002). The plant contains considerable amount of anti-nutrients like phytic acid, tannin, oxalic acids, hydrocyanic acid and saponins (Ladeji *et al.*, 1995; Ajibade *et al.,* 2006; Kajihausa *et al.*, 2010). For instance, oxalate, in addition to the formation of kidney stone, also reduces the bioavailability of mineral elements (Nakata, 2003; Shingeru *et al*., 2003; Antia *et al*., 2006; Proph *et al*., 2006). Cyanide is a potent respiratory poison (Ames *et al*., 1981, Ellenborn and Barcelonx, 1988) while nitrate causes methaemoglobinemia and cancer (Galler, 1997; Amanabo *et al.*, 2011). However, the content of nutrients and toxic substances in this vegetable like others are known to be influenced by their developmental phases.

It has been reported that derivable nutritional benefits of *Telfairia occidentalis* can be fully harnessed by inclusion of the vegetable in our meal at vegetative phase (Amanabo *et al.*, 2011). This practice will reduce the health problems associated with high intake of cyanide and oxalate.

The plant produces luxuriant edible green leaves, which are rich in iron and water soluble vitamins such as vitamin C, riboflavin and thiamine.

The leaves and edible shoots of fluted pumpkin together contain 85% moisture, while the dry portion of what is usually consumed contains 11% crude protein, 25% carbohydrate, 3.0% fat and 0.58% phosphorus (Oyolu, 1980). According to Akwaowo *et al.,* (2000), the older leaves of fluted pumpkin, the higher in percentage crude protein, crude fat, ash and crude fibre while the younger leaves are higher in moisture content and carbohydrate. The older leaves contain 39.4% crude protein in comparison to 22.4% for younger leaves. The leaves are also rich

in iron, thus used to cure anaemia (Ajibade *et al.,* 2006). These anti-nutrients are widely distributed in some vegetable crops with notable effects on other nutrients especially reducing bio-availability of some metals like calcium, iron etc (Hurrel *et al*., 1992). The leaves are rich source of protein, oil, vitamins and minerals, low in crude fibre but rich source of folic acid, calcium, zinc, potassium, cobalt, copper, iron, vitamin A, C and K. Relative to most vegetable, its protein content is top (Ladeji *et al*., 1995; Ajibade *et al*., 2006; Eroarame, 2012).

The tender leaves are consumed by man as vegetable as well as by livestock (sheep, pigs and goat) as forage, while the young seeds are eaten as human food (Akoroda, 1990; Badifu and Ogunsua, 1999; Akwaowo *et al.*, 2000; Kajihausa *et al.,* 2010). It has medicinal value due to its rich source of vitamins and some minerals (Ladeji *et al.*, 1995; *Ajibade et al.*, 2006; Eroarame, 2012).

2.8 Brief historical background of the response surface methodology (RSM)

Response surface methodology is a collection of mathematical and statistical techniques for empirical model building. By careful design of experiments, the objective is to optimize a response (output variable) which is influenced by several independent variables (input variables). RSM explores the relationships between several explanatory variables and one or more response variables (Wikipedia, 2015). The method was introduced by G. E.P Box and K.B. Wilson in 1951. RSM has an effective track record of helping researchers to improve products and services (Wikipedia 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Source of materials

Ogiri egusi samples, dehulled melon seeds and fluted pumpkin seeds were bought from Ekenonwa market Owerri and Umuahia respectively. Tryptone Soy agar, Tryptone Soy Broth, Peptone Buffered water (Merk KGaA, Germany), Nutrient Agar, Nutrient Broth (Merck KGaA, Germany), distilled water, ethanol, simple sugar, Aluminium foils and other equipment used were obtained from NAFDAC laboratory, Port Harcourt and National Root Crop Research Institute, Umudike. The low density and high density polyethylene wrapping materials were obtained from a reputable store in Lagos. All the chemicals and reagents used in the study were of analytical grade.

3.2 Methods

3.2.1 Media preparation

The media used include Nutrient agar (NA), Tryptone Soy Agar (TSA), Tryptone Soy broth (TSB) and Peptone Buffered water.

3.2.1.1 Preparation of Tryptone Soy Agar and Tryptone Soy Broth

These were prepared according to the Manufacturer's instruction. Forty (40) grams of Tryptone Soy Agar powder was suspended in 1litre of distilled water, heated to boil to dissolve completely and sterilized by autoclaving at 121°C for 15 minutes while the Tryptone Soy Broth (TSB) was prepared by dissolving thirty (30) grams of TSB in 1litre of water and distributed into 250ml conical flask which was further sterilized by autoclaving at 121°C for 15 minutes.

3.2.1.2 Preparation of Nutrient Agar

Nutrient Agar (NA) was also prepared according to the manufacturer's instruction. Twenty (20) grams of the powder was dispensed in 1litre of water, allowed to

dissolve for 10 minutes, swirled to mix and then sterilized for 15 minutes at 121°C and allowed to cool to 47°C.

3.2.1.3 Preparation of Peptone water

Peptone water was prepared according to the manufacturer's instruction. 25.5 grams of peptone water powder was suspended in 1litre of distilled water. The mixture was then dispensed into test tubes, closed with cotton wool and aluminium foil and autoclaved for 15minutes at 121°C.

3.2.1.4 Preparation of Tryptone Soy Agar Slant

Tryptone Soy Agar Slant was prepared by dissolving 3.42g of powdered Tryptone Soy Agar into 150ml of distilled water with slight heating to enable complete dissolution. Then 15ml of the solution was poured into Mac Cartney bottles which were slanted and allowed to cool and solidify in that position.

3.2.2 Generation of Relative Humidity

The relative humidity (RH) was generated as described by Ogueke *et al.,* (2013):

i. 65% RH was generated by dissolving 50g of anhydrous $NaNO_3$ in 33.3ml of hot water.
ii. 75% RH was generated by dissolving 20g of Sodium Chloride (NaCl) in 50ml of Hot water.
iii. 85% RH was generated by dissolving 25g of Potassium Chloride (KCl) in 50ml of hot water.

3.2.3 Isolation of pure cultures of *Bacillus subtilis*

Stock solution of the ogiri egusi samples were prepared by blending 1 gram of sample in 9ml of peptone water. Serial dilutions were made from the stock solution as follows; 1ml of stock was pipetted into 9ml of peptone water to give a 10^{-1} dilution. This was vigorously shaken to mix well. Then 1ml of the 10^{-1} dilution was pipetted into another 9ml of sterile peptone water in another test tube to

obtain 10^{-2} dilution. Further dilutions were prepared until a 10^{-6} dilution was obtained. A 0.1ml portion of each of the dilutions was used to inoculate already labelled plates (in triplicates) of TSA and NA using the spread plate method (Ogueke and Nwagwu, 2007).

After incubation, plates with distinct colony growth were selected. Pure cultures were isolated from these selected plates. This was done as follows; with a sterile inoculating loop, distinct colonies were picked and streaked on the surface of fresh TSA or NA plates. Each plate was used for a different microorganism. The plates were appropriately labelled and incubated at 37°C for 24hours. The various bacteria were then transferred to agar slant as follows; with a sterile wire loop a loop full of the microorganism was aseptically taken and streaked on the surface of the slant. After streaking the bacteria on the surface of the TSA (taking care not to cut into the TSA), the bottle was loosely closed and incubated at 37°C for 24 hours. After incubation the bottles were tightly closed, foiled and refrigerated at 4°C until required.

3.2.4 Identification of isolated cultures

Bacterial identification was based on colony characteristics, cellular morphology and biochemical reaction as described by Omafuvbe *et al.,* (2004). The bacterial isolates were subjected to the following biochemical tests; Gram staining, Endospore stain, Catalase test, Oxidase test, Gelatin hydrolysis test and Starch hydrolysis test. The *Bacillus species* were further identified using the PCR method as described by Ouoba *et al.,* (2008).

3.2.4.1 Gram staining test

The smear of the isolates was each prepared on clean glass slides. They were heat-fixed by passing them over Bunsen flame three times. The smear on the slide were then flooded with crystal violet stain for 60 seconds. This was washed off under running tap water. The smear were then flooded with iodine solution for 30

seconds and rinsed with running water, and then decolorized with 95% ethyl alcohol. After washing off the alcohol, the smears were counter stained with safranin for 30 seconds and washed off under running water. They were blotted dry with clean blotting paper and examined under oil immersion microscope. Purple colouration of bacteria indicated that the microorganism was Gram positive.

3.2.4.2 Endospore staining test

The spore staining is used to detect the presence of spores. Smears were prepared on separate slides. They were allowed to dry and then were fixed by passing them over hot water bath until large water droplets condensed on the other side of the slide. The smears were flooded with malachite green and allowed to stand for 60seconds. The slides were again washed under running water, air dried and viewed under an oil immersion high power microscope. Bright red substance indicates presence of spore while the remaining of bacteria appears blue.

3.2.4.3 Catalase test

This test is to determine microorganism that contain catalase enzymes capable of releasing oxygen when mixed with hydrogen peroxide (H_2O_2). A drop of 3% hydrogen peroxide was placed on a clean glass slide and a wooden application stock was used to pick a colony from the stock culture and dipped into the hydrogen peroxide. The evolution of gas bubbles indicates positive catalase.

3.2.4.4. Oxidase test

This test is used to differentiate the bacteria isolates in their ability to oxidize or ferment carbohydrates. Two (2) grams of peptone, 2 grams of sodium chloride, 0.2 grams of dipotassium hydrogen phosphate, 3 grams of Agar-Agar (Oxide) and 3ml of 1% aqueous solution of bromocresol ingredient were dissolved in 100ml of

distilled water by boiling. The mixture was dispensed into test tubes (9ml portion) and autoclaved at 121°C for 15 minutes.

Ten (10) per cent of glucose solution was prepared separately and autoclaved at 10psi for 10 minutes, 1ml portion of the glucose solution was added aseptically into the test tubes. The solidified medium was incubated at 37°C for 24 hours to check for contamination. Each isolate was inoculated in duplicate by stabbing, and the tubes appropriately labelled. After inoculum, a layer of sterile petroleum jelly was poured over the surface of the medium in one of each set of tubes to minimize any oxidative activity. The tubes were then incubated at 37°C for a week. Change from blue colour of the medium to yellow indicates acid production. The yellow colour appears only in the open tube.

3.2.4.5 Starch hydrolysis

A nutrient agar medium with 0.4% gelatin was prepared. It was autoclaved at 121°C for 15 minutes, cooled and poured into sterile petridishes. The plates were inoculated along with a plate which served as control and all sample plates were incubated at 30°C for seven days. After the incubation period, the plates were flooded with 8ml of HgCl solution. The mercuric chloride (HgCl) solution was prepared by dissolving 7.5g of mercuric chloride in 50ml of distilled water and added 15ml of concentrated hydrochloric acid. Unhydrolyzed gelatin in the plate formed a white opaque precipitate with reagent HgCl solution while hydrolyzed gelatin appears as clear.

The isolates identified as *B.subtilis* were used for the study.

3.2.5 Standardization of pure culture

The pure culture of *B. subtilis* was standardized as follows:

With a sterile inoculating loop an inoculum of the pure culture was taken from the stock culture. This was streaked on the surface of the solidified TSA plates and incubated at 37°C for 24 hours. After then, a sterilized wire loop was used to

transfer some inoculum from the petri dish into TSB in test tubes and incubated at 37°C. At every hour of growth in the incubator, 1ml of the broth was taken, the optical density (OD) of the culture determined at 550nm wavelength using a UV-Visible spectrophotometer and serially diluted, and plated out on petri dishes. The plates were incubated at 37°C for 24 hours. This procedure was repeated at every hour for 6 hours. At the end of incubation, the plates were retrieved and the number of colonies that formed counted and the colony forming unit (cfu) calculated. Then a graph of OD against $\log_{10}$ cfu of cells was plotted to obtain the standard curve.

3.2.6 TRADITIONAL PRODUCTION OF OGIRI

3.2.6.1 Traditional production of ogiri egusi

The traditional method of production of ogiri egusi was used. The dehulled egusi was sorted and 500 grams was weighed out. It was washed with water and boiled until it softened (brownish colour) and was allowed to cool and the water drained. Then leaves obtained from "Mkpodu"(*Napoleona vagelii*)were placed over fire, turned at interval and allowed to cool. Then 40 grams of egusi was weighed using a top loading balance and placed in the layered leaves, then tied with rope. The wraps were punctured so that water can sip in and returned to the pot with enough water covering the wraps. These were boiled until the water covering the wraps dried up after which it was left to ferment for five days (5 days). After fermentation, they were pounded with mortar, re-wrapped in small portions and hung over a fire place to further dry and prevent flies.

The production process is shown in the Figure 3.1

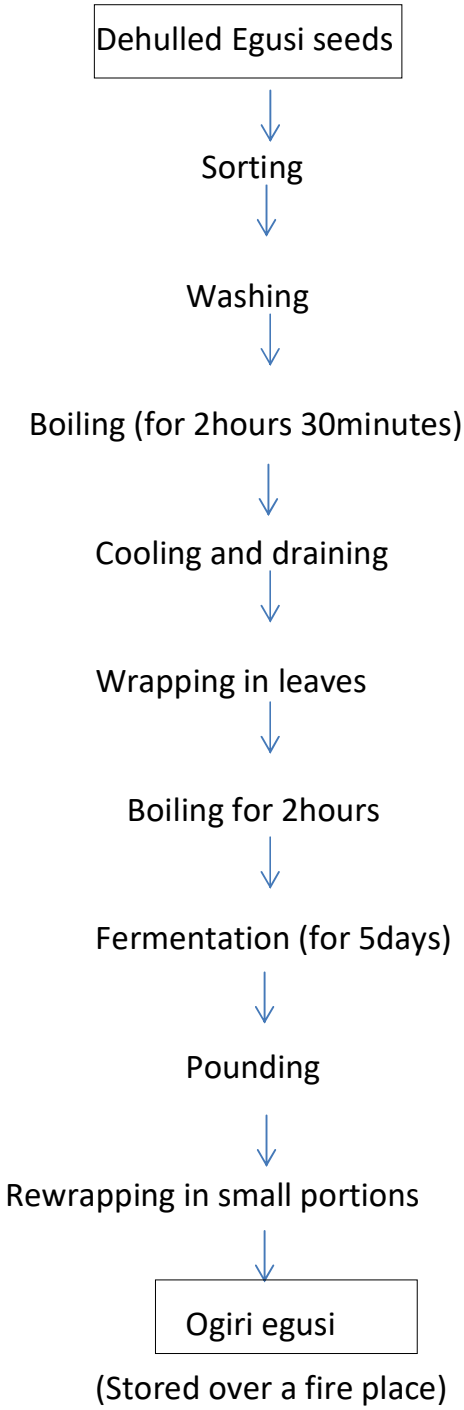

Figure 3.1: Flow diagram for the production of ogiri egusi

Source: Achi 2005

3.2.6.2 Traditional production of ogiri ugu

The pumpkin was bought with its pulp and opened to expose the seed. The pumpkin was washed and weighed on a top loading balance. The seeds weighing 2.12kg was boiled with three litres of water for one hour and allowed to cool. The

coatings were removed and the whitish cotyledons washed and put into a pot and covered with leaves for three days. It was furthermore removed after three days, ground, rewrapped with leaves and placed over a fire.

The flow chart for ogiri ugu is shown in Figure 3.2

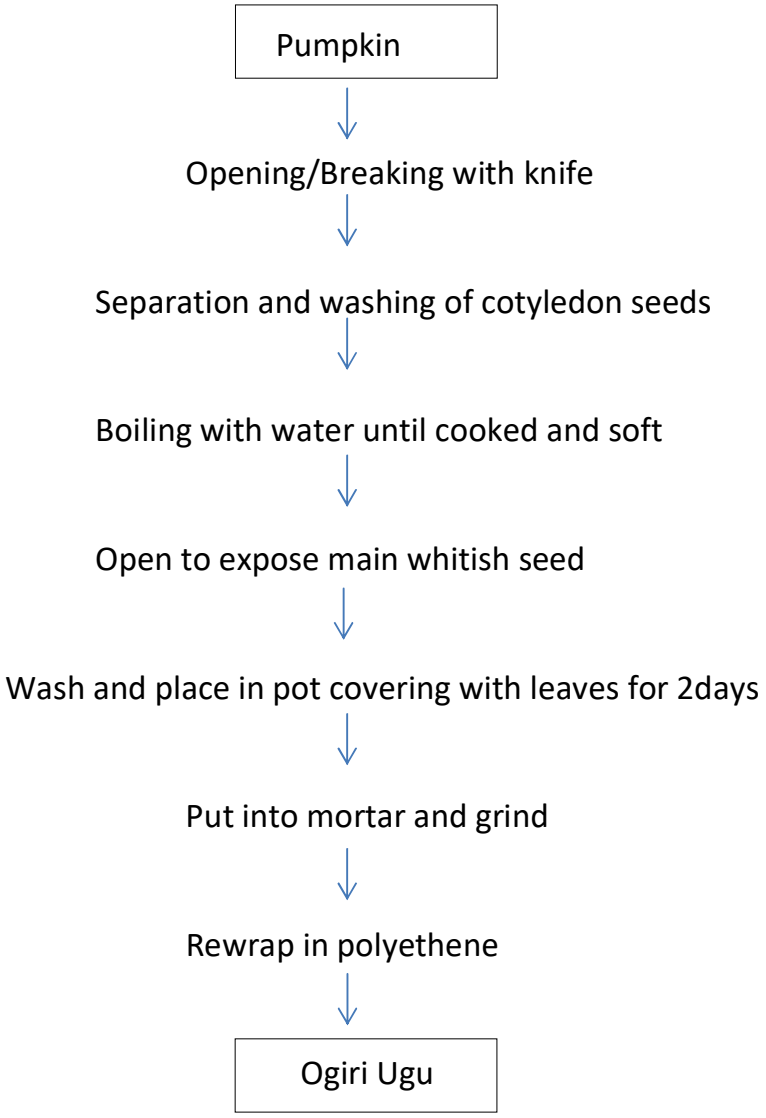

Figure 3.2: Flowchart for production of ogiri ugu

Source: Achi 2005

3.2.7 PRODUCTION OF OGIRI EGUSI AND OGIRI UGU USING PURE CULTURE OF *Bacillus subtilis*

Ogiri egusi and ogiri ugu were prepared in the laboratory as describe in sections 3.2.6.1 and 3.2.6.2 without allowing the samples to ferment naturally and

wrapping with different pore sizes of polyethene. Tryptone soy broth (TSB) was prepared in test tubes and inoculated with a loop full of *B.subtilis.* The tubes were incubated at 37°C and the OD (Optical Density) was monitored at intervals of 30 minutes until an OD of 0.04 (approximately 10^5 cells) was obtained. One millilitre (1ml) of the broth culture was taken out and distributed evenly into 40 grams of the samples, mixed and tied to ferment for five days at different temperatures, relative humidity and pore size of wrapping materials as shown in the experimental design below;

3.2.8 EXPERIMENTAL DESIGN

Box-Behuken rotatable response (Lawson and Madrigal, 1994) for k=3 was used to study the linear, interactive and quadratic effects of the independent variables. The variables were of three levels as shown below;

TABLE 3.1: INDEPENDENT VARIABLES AND LEVELS

INDEPENDENT VARIABLES	**VARIABLES LEVELS**		
	-1	0	1
Relative humidity (%) X_1	65	75	85
Temperature (^{o}C) X_2	30	35	40
Pore size (µm) X_3	50 LD	70 HD	90 HD

- LD –Low Density Polyethene
- HD- High Density Polyethene

The combinations of the independent variables are shown in Table 3.2 below.

TABLE 3.2: Combination of the independent variables

S/N	RUN	X_1	X_2	X_3
1	1	-1	-1	0
2	2	+1	-1	0
3	3	-1	+1	0
4	4	+1	+1	0
5	5	-1	0	-1
6	6	+1	0	-1
7	7	-1	0	+1
8	8	+1	0	+1
9	9	-1	-1	-1
10	10	0	+1	-1
11	11	0	-1	+1
12	12	+1	+1	+1
13	13	0	0	0

Samples were collected from the fermenting materials at 24 hour interval and analysed for pH, amino nitrogen, total titratable acidity, free fatty acid and peroxide value.

3.2.9 DETERMINATION OF FREE FATTY ACIDS

The method described by Pearson (1996) was used. One gramme (1g) of the egusi and pumpkin were measured into the conical flask. Twenty five millilitre (25ml) of diethyl ether and 25ml alcohol were added and 1ml of phenolphthalein indicator was used for titration against 0.01N NaOH, a persistent pink colour was obtained

$$\text{Acid Value} = \frac{\text{Titre} \times 5.61}{\text{Weight of sample}} \qquad 3.1$$

3.2.10 DETERMINATION OF PEROXIDE VALUE

It was determined using the method as described by Onwuka (2005). One gramme (1g) of the sample was measured inside a conical flask with 1g of powdered potassium iodide (KI) added. Twenty millilitres (20ml) of solvent mixture was also added and boiled for thirty (30) seconds vigorously. The content was later poured inside a flask containing 20ml potassium iodide solution (5%) and 25ml of H_2O was added twice for washing. The solution was later titrated against 0.01N sodium thiosuplhate using starch indicator.

Calculation

$$\text{Peroxide Value (PV)} = \frac{T \times 2}{W} \qquad 3.2$$

Where T is Titre Value

W is weight of sample used

3.2.11 DETERMINATION OF TOTAL TITRATABLE ACIDITY (TTA)

This was determined in accordance with the method described by A.O.A.C (1995). Distilled water (100ml) was put into a conical flask. One gramme (1g) of the sample and 5drops of phenolphthalein indicator were added to the distilled water in the conical flask. The mixture was titrated against 0.01N solution sodium hydroxide. The endpoint was reached when change of colour was observed after adding a drop of the sodium hydroxide solution.

$$\text{\% Titrable acidity} = \text{Average titre} \times 0.900 \qquad 3.3$$

3. 2. 12 pH DETERMINATION

The pH was measured directly using a pH digital meter as described by Ogueke *et al.*, (2013).

Five grammes (5g) of fermenting seeds were taken from the bulk and mashed in 45ml of sterile deionized water. The pH was measured using a standardized (pH

4.0-9.0) digital pH meter (Jenway pH/MV/Temperature meter, Model 3510, Essex, Uk).

3.2.13 DETERMINATION OF AMINO NITROGEN

This was determined by the method described in Asagbra *et al.,* 2012. It was done using spectrophotometric method.

The analysis involved different stages as shown below.

Stage 1: Preparation of sample solution

This involves measuring out 1g of the sample and diluting with water in order to obtain 1 to 3 mg free amino nitrogen per litre. Then 2ml of the diluted solution was pipetted into test tube and 1ml of the ninhydrin colour reagent was added, covered and heated for 15minutes in a boiling water bath before cooling for 20minutes. The absorbance of the solution was later determined at wavelength of 570nm against distilled water.

Stage 2: Preparation of the standard

The sample procedure above was repeated using glycine standard solution. Also 1ml of ninhydrin colour reagent was added before absorbance reading was taken at 570nm.

Calculation

Free Amino Acid (mg/L) = $\frac{(A-B-D)\,C}{A_s-A_b} \times D_f$ 3.4

Where A= Absorbance of sample solution

B= Absorbance of blank solution

C= Concentration of standard

D= Absorbance of correction factor

A_s= Absorbance of standard

A_b= Absorbance of blank

D_f= Dilution factor

3.2.14 STATISTICAL ANALYSIS

The data obtained from the study were analysed statistically using Analysis of Variance (ANOVA). Statistical analysis was performed using Analyse-it® Method Validation version 3.0 statistical software for Microsoft Excel. The means were separated using Fisher's Least Significant Difference (LSD). Microsoft Excel 2007 was used in processing the data. The response surface plots and analysis were carried out using Design Expert software version 9®.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS ON FERMENTED PUMPKIN AND MELON

Tables 4.1 to 4.5 show the values obtained for Free Fatty acid (FFA), Total Titratable Acidity (TTA), peroxide value (PV), amino nitrogen and pH during the fermentation of fluted pumpkin seeds for ogiri production, while Tables 4.6 to 4.12 present the values obtained for these parameters and the response surface analysis during fermentation of melon seeds over a period of 96 hours.

Tables 4.13 and 4.14 show the daily amino nitrogen production for fluted pumpkin and melon seeds.

Figures 4.1 to 4.3 show the response surface plots obtained for amino nitrogen during fermentation of melon seeds for ogiri production.

Table 4.1: Free Fatty acids levels during fermentation of fluted pumpkin seeds (mg/g).

Period of fermentation (hours)

Sample	*0*	*24*	*48*	*72*	*96*	*LSD*
1	$^{1}1.76^{a}$	$^{1}2.26^{b}$	$^{1}2.94^{c}$	$^{1}3.24^{d}$	$^{1}3.75^{e}$	**0.62**
2	$^{1}1.85^{a}$	$^{2}2.38^{b}$	$^{2}3.08^{c}$	$^{2}4.18^{d}$	$^{2}4.68^{e}$	**0.93**
3	$^{1}1.84^{a}$	$^{3}2.32^{b}$	$^{3}3.06^{c}$	$^{3}4.05^{d}$	$^{3}4.57^{e}$	**0.88**
4	$^{1}1.84^{a}$	$^{4}2.35^{b}$	$^{4}3.10^{c}$	$^{4}4.06^{d}$	$^{4}4.45^{e}$	**0.86**
5	$^{1}1.84^{a}$	$^{5}2.36^{b}$	$^{3}3.06^{c}$	$^{5}4.24^{d}$	$^{5}4.74^{e}$	**0.95**
6	$^{1}1.72^{a}$	$^{6}2.24^{b}$	$^{1}2.95^{c}$	$^{6}3.16^{d}$	$^{6}3.85^{e}$	**0.65**
7	$^{1}1.65^{a}$	$^{7}2.17^{b}$	$^{5}2.84^{c}$	$^{7}3.18^{d}$	$^{7}3.59^{e}$	**0.59**
8	$^{1}1.91^{a}$	$^{8}2.42^{b}$	$^{6}3.17^{c}$	$^{8}4.12^{d}$	$^{8}4.60^{e}$	**0.88**
9	$^{1}1.84^{a}$	$^{4}2.35^{b}$	$^{7}3.70^{c}$	$^{9}3.85^{d}$	$^{9}3.90^{e}$	**0.71**
10	$^{1}1.81^{a}$	$^{9}1.96^{b}$	$^{8}2.65^{c}$	$^{10}3.42^{d}$	$^{10}3.42^{e}$	**0.60**
11	$^{1}1.72^{a}$	$^{10}2.79^{b}$	$^{9}3.44^{c}$	$^{11}3.81^{d}$	$^{11}3.93^{e}$	**0.70**
12	$^{1}1.68^{a}$	$^{11}2.16^{b}$	$^{8}2.65^{c}$	$^{12}3.06^{d}$	$^{12}3.56^{e}$	**0.62**
13	$^{1}1.91^{a}$	$^{12}3.28^{b}$	$^{5}2.84^{c}$	$^{13}3.14^{d}$	$^{13}3.40^{e}$	**0.50**
LSD	**0.04**	**0.10**	**0.09**	**0.21**	**0.23**	

abc…. Values on the same row with the same superscript are not significantly different (p<0.05)

1,2,3…Values on the same column with different superscript are significantly different (p<0.05)

Sample 1 - fermented at 65% RH, 30°C and 70µm pore size
Sample 2 –fermented at 85% RH, 30°C and 70µm pore size
Sample 3 – fermented at 65% RH, 40°C and 70µm pore size
Sample 4 – fermented at 85% RH, 40°C and 70µm pore size
Sample 5 – fermented at 65% RH, 35°C and 50µm pore size
Sample 6 – fermented at 85% RH, 35°C and 50µm pore size
Sample 7 – fermented at 65% RH, 35°C and 90µm pore size
Sample 8 – fermented at 85% RH, 35°C and 90µm pore size
Sample 9 – fermented at 65% RH, 30°C and 50µm pore size
Sample 10 – fermented at 75% RH, 40°C and 50µm pore size
Sample 11 – fermented at 75% RH, 30°C and 90µm pore size
Sample 12 – fermented at 85% RH, 40°C and 90µm pore size
Sample 13 – fermented at 75% RH, 35°C and 70µm pore size

Table 4.2: Total Titratable Acidity levels during fermentation of fluted pumpkin seeds (%).

Period of fermentation (hours)

Sample	*0*	*24*	*48*	*72*	*96*	*LSD*
1	$^{1}0.84^{a}$	$^{1}1.06^{b}$	$^{1}1.25^{bc}$	$^{1}1.35^{c}$	$^{1}1.40^{c}$	**0.71**
2	$^{1}0.96^{a}$	$^{1}1.17^{b}$	$^{1}1.36^{bc}$	$^{1}1.47^{c}$	$^{1}1.53^{c}$	**0.75**
3	$^{1}0.84^{a}$	$^{1}1.03^{b}$	$^{1}1.34^{c}$	$^{1}1.45^{d}$	$^{1}1.51^{d}$	**0.87**
4	$^{1}0.83^{a}$	$^{1}0.95^{a}$	$^{1}1.28^{b}$	$^{1}1.34^{bc}$	$^{1}1.42^{c}$	**0.88**
5	$^{1}0.79^{a}$	$^{1}0.92^{a}$	$^{1}1.17^{b}$	$^{1}1.27^{b}$	$^{1}1.32^{b}$	**0.50**
6	$^{1}0.80^{a}$	$^{1}1.03^{b}$	$^{1}1.24^{c}$	$^{1}1.34^{cd}$	$^{1}1.40^{d}$	**0.98**
7	$^{1}0.85^{a}$	$^{1}0.98^{a}$	$^{1}1.18^{b}$	$^{1}1.27^{b}$	$^{1}1.34^{b}$	**0.89**
8	$^{1}0.82^{a}$	$^{1}1.06^{b}$	$^{1}1.25^{c}$	$^{1}1.40^{c}$	$^{1}1.40^{c}$	**0.61**
9	$^{1}0.90^{a}$	$^{1}1.07^{ab}$	$^{1}1.20^{bc}$	$^{1}1.36^{c}$	$^{1}1.42^{c}$	**0.73**
10	$^{1}0.72^{a}$	$^{1}1.13^{b}$	$^{1}1.24^{c}$	$^{1}1.39^{d}$	$^{1}1.43^{d}$	**0.92**
11	$^{1}0.79^{a}$	$^{1}1.24^{b}$	$^{1}1.30^{b}$	$^{1}1.45^{c}$	$^{1}1.50^{c}$	**0.97**
12	$^{1}0.80^{a}$	$^{1}1.08^{b}$	$^{1}1.44^{c}$	$^{1}1.52^{d}$	$^{1}1.56^{d}$	**0.61**
13	$^{1}1.06^{a}$	$^{1}1.36^{abc}$	$^{1}1.17^{ab}$	$^{1}1.46^{bc}$	$^{1}1.52^{c}$	**0.79**
LSD	**0.09**	**0.08**	**0.96**	**0.71**	**0.79**	

abc…Values on the same row with the same superscript are not significantly different ($p<0.05$)

1,2,3…Values on the same column with different superscript are significantly different ($p<0.05$)

Sample 1 - fermented at 65% RH, 30°C and 70µm pore size
Sample 2 –fermented at 85% RH, 30°C and 70µm pore size
Sample 3 – fermented at 65% RH, 40°C and 70µm pore size
Sample 4 – fermented at 85% RH, 40°C and 70µm pore size
Sample 5 – fermented at 65% RH, 35°C and 50µm pore size
Sample 6 – fermented at 85% RH, 35°C and 50µm pore size
Sample 7 – fermented at 65% RH, 35°C and 90µm pore size
Sample 8 – fermented at 85% RH, 35°C and 90µm pore size
Sample 9 – fermented at 65% RH, 30°C and 50µm pore size
Sample 10 – fermented at 75% RH, 40°C and 50µm pore size
Sample 11 – fermented at 75% RH, 30°C and 90µm pore size
Sample 12 – fermented at 85% RH, 40°C and 90µm pore size
Sample 13 – fermented at 75% RH, 35°C and 70µm pore size

Table 4.3: Peroxide values during fermentation of fluted pumpkin seeds (Meq/kg)

Period of fermentation (hours)

Sample	*0*	*24*	*48*	*72*	*96*	*LSD*
1	$^{1}2.84^{a}$	$^{1}3.02^{b}$	$^{1}3.45^{c}$	$^{1}3.60^{d}$	$^{1}4.12^{e}$	**0.40**
2	$^{1}2.94^{a}$	$^{2}3.27^{b}$	$^{1}3.64^{c}$	$^{1}4.35^{d}$	$^{2}4.85^{e}$	**0.61**
3	$^{1}2.91^{a}$	$^{3}3.41^{b}$	$^{1}3.81^{c}$	$^{1}4.12^{d}$	$^{3}3.82^{e}$	**0.38**
4	$^{1}3.12^{a}$	$^{2}3.28^{b}$	$^{1}3.65^{c}$	$^{1}3.70^{d}$	$^{4}3.90^{e}$	**0.24**
5	$^{1}2.94^{a}$	$^{4}3.17^{b}$	$^{1}3.58^{c}$	$^{1}3.78^{d}$	$^{5}4.17^{e}$	**0.38**
6	$^{1}2.85^{a}$	$^{5}3.12^{b}$	$^{1}3.51^{c}$	$^{1}3.75^{d}$	$^{6}3.80^{e}$	**0.33**
7	$^{1}2.77^{a}$	$^{6}2.91^{b}$	$^{1}3.42^{c}$	$^{1}3.62^{d}$	$^{7}3.70^{e}$	**0.30**
8	$^{1}2.78^{a}$	$^{6}2.94^{b}$	$^{1}3.39^{c}$	$^{1}3.58^{d}$	$^{8}3.65^{e}$	**0.29**
9	$^{1}3.06^{a}$	$^{2}3.28^{b}$	$^{1}3.65^{c}$	$^{1}3.84^{d}$	$^{9}3.93^{e}$	**0.33**
10	$^{1}2.65^{a}$	$^{6}2.91^{b}$	$^{1}3.41^{c}$	$^{1}3.67^{d}$	$^{10}3.74^{e}$	**0.38**
11	$^{1}3.20^{a}$	$^{7}3.45^{b}$	$^{1}3.62^{c}$	$^{1}3.86^{d}$	$^{11}4.05^{e}$	**0.27**
12	$^{1}2.92^{a}$	$^{8}3.57^{b}$	$^{1}3.46^{c}$	$^{1}3.60^{d}$	$^{12}3.79^{e}$	**0.26**
13	$^{1}3.04^{a}$	$^{9}3.75^{b}$	$^{1}3.62^{c}$	$^{1}3.86^{d}$	$^{13}3.96^{e}$	**0.29**
LSD	**0.07**	**0.10**	**0.06**	**0.10**	**0.14**	

abc..Values on the same row with the same superscript are not significantly different ($p<0.05$)

1,2,3…Values on the same column with different superscript are significantly different ($p<0.05$)

Sample 1 - fermented at 65% RH, 30°C and 70µm pore size
Sample 2 –fermented at 85% RH, 30°C and 70µm pore size
Sample 3 – fermented at 65% RH, 40°C and 70µm pore size
Sample 4 – fermented at 85% RH, 40°C and 70µm pore size
Sample 5 – fermented at 65% RH, 35°C and 50µm pore size
Sample 6 – fermented at 85% RH, 35°C and 50µm pore size
Sample 7 – fermented at 65% RH, 35°C and 90µm pore size
Sample 8 – fermented at 85% RH, 35°C and 90µm pore size
Sample 9 – fermented at 65% RH, 30°C and 50µm pore size
Sample 10 – fermented at 75% RH, 40°C and 50µm pore size
Sample 11 – fermented at 75% RH, 30°C and 90µm pore size
Sample 12 – fermented at 85% RH, 40°C and 90µm pore size
Sample 13 – fermented at 75% RH, 35°C and 70µm pore size

Table 4.4: Amino Nitrogen levels during fermentation of fluted pumpkin seeds (mgN/g)

Period of fermentation (hours)

Sample	*0*	*24*	*48*	*72*	*96*	*LSD*
1	$^{1}3.76^{a}$	$^{1}3.97^{b}$	$^{1}4.92^{c}$	$^{1}6.45^{d}$	$^{1}7.61^{e}$	**1.29**
2	$^{2}3.49^{a}$	$^{2}3.71^{b}$	$^{2}4.95^{c}$	$^{2}6.80^{d}$	$^{2}7.96^{e}$	**1.52**
3	$^{3}3.62^{a}$	$^{3}3.85^{b}$	$^{3}4.91^{c}$	$^{3}6.93^{d}$	$^{3}7.82^{e}$	**1.46**
4	$^{4}4.38^{a}$	$^{4}4.56^{b}$	$^{4}4.71^{c}$	$^{4}7.16^{d}$	$^{4}8.33^{e}$	**1.31**
5	$^{5}3.45^{a}$	$^{5}3.67^{b}$	$^{5}4.72^{c}$	$^{2}6.80^{d}$	$^{5}8.16^{e}$	**1.60**
6	$^{6}4.36^{a}$	$^{6}4.54^{b}$	$^{6}5.62^{c}$	$^{5}7.81^{d}$	$^{6}8.24^{e}$	**1.42**
7	$^{7}3.70^{a}$	$^{7}3.94^{b}$	$^{2}4.95^{c}$	$^{6}6.95^{d}$	$^{7}7.16^{e}$	**1.28**
8	$^{8}4.67^{a}$	$^{8}4.78^{b}$	$^{7}5.84^{c}$	$^{7}7.82^{d}$	$^{8}8.05^{e}$	**1.29**
9	$^{9}4.16^{a}$	$^{9}4.34^{b}$	$^{8}5.30^{c}$	$^{8}7.18^{d}$	$^{9}8.19^{e}$	**1.40**
10	$^{10}3.83^{a}$	$^{10}4.12^{b}$	$^{9}5.18^{c}$	$^{9}7.40^{d}$	$^{10}7.85^{e}$	**1.46**
11	$^{11}4.25^{a}$	$^{12}4.49^{b}$	$^{10}5.61^{c}$	$^{10}7.58^{d}$	$^{11}8.27^{e}$	**1.38**
12	$^{12}3.95^{a}$	$^{13}4.09^{b}$	$^{11}5.42^{c}$	$^{11}7.60^{d}$	$^{12}7.92^{e}$	**1.45**
13	$^{13}3.84^{a}$	$^{14}5.17^{b}$	$^{12}5.85^{c}$	$^{12}7.43^{d}$	$^{10}7.85^{e}$	**1.44**
LSD	**0.14**	**0.16**	**0.15**	**0.18**	**0.17**	

abc.. Values on the same row with the same superscript are not significantly different ($p<0.05$)

1,2,3…Values on the same column with different superscript are significantly different ($p<0.05$)

Sample 1 - fermented at 65% RH, 30°C and 70µm pore size
Sample 2 –fermented at 85% RH, 30°C and 70µm pore size
Sample 3 – fermented at 65% RH, 40°C and 70µm pore size
Sample 4 – fermented at 85% RH, 40°C and 70µm pore size
Sample 5 – fermented at 65% RH, 35°C and 50µm pore size
Sample 6 – fermented at 85% RH, 35°C and 50µm pore size
Sample 7 – fermented at 65% RH, 35°C and 90µm pore size
Sample 8 – fermented at 85% RH, 35°C and 90µm pore size
Sample 9 – fermented at 65% RH, 30°C and 50µm pore size
Sample 10 – fermented at 75% RH, 40°C and 50µm pore size
Sample 11 – fermented at 75% RH, 30°C and 90µm pore size
Sample 12 – fermented at 85% RH, 40°C and 90µm pore size
Sample 13 – fermented at 75% RH, 35°C and 70µm pore size

Table 4.5: pH levels during fermentation of fluted pumpkin seeds

	Period of fermentation (hours)					
Sample	***0***	***24***	***48***	***72***	***96***	***LSD***
1	$^{1,3}6.45^{a}$	$^{1}7.15^{b}$	$^{1,4}7.48^{c}$	$^{1}7.92^{d}$	$^{1,2}8.05^{e}$	**0.61**
2	$^{2}6.68^{a}$	$^{2}7.32^{b}$	$^{2}7.65^{c}$	$^{2}8.06^{d}$	$^{1,2}8.14^{e}$	**0.50**
3	$^{1,5}6.32^{a}$	$^{3}7.04^{b}$	$^{3,6}7.35^{c}$	$^{1,4}7.85^{d}$	$^{1}8.23^{e}$	**0.57**
4	$^{2}6.71^{a}$	$^{4}7.41^{b}$	$^{4}7.56^{c}$	$^{2}8.03^{d}$	$^{1,2}8.12^{e}$	**0.44**
5	$^{1}6.45^{a}$	$^{1}7.15^{b}$	$^{1,3}7.45^{c}$	$^{3}7.65^{d}$	$^{1,2}8.16^{e}$	**0.49**
6	$^{2}6.68^{a}$	$^{2,6}7.29^{b}$	$^{3,6}7.39^{c}$	$^{1,4}7.82^{d}$	$^{1,2}8.05^{e}$	**0.41**
7	$^{2}6.62^{a}$	$^{5}6.78^{b}$	$^{5}7.09^{c}$	$^{1}7.91^{d}$	$^{2}7.91^{e}$	**0.59**
8	$^{2}6.65^{a}$	$^{6}7.25^{b}$	$^{4}7.57^{c}$	$^{4}7.80^{d}$	$^{2}7.94^{e}$	**0.46**
9	$^{2}6.63^{a}$	$^{7}6.85^{b}$	$^{5}7.15^{c}$	$^{1}7.92^{d}$	$^{1,2}8.10^{e}$	**0.40**
10	$^{2}6.46^{a}$	$^{8}6.94^{b}$	$^{6}7.26^{c}$	$^{4}7.81^{d}$	$^{2}7.90^{e}$	**0.58.**
11	$^{2}6.60^{a}$	$^{9}6.77^{b}$	$^{5,7}7.06^{c}$	$^{1}7.92^{d}$	$^{2}8.16^{e}$	**0.47**
12	$^{4}5.92^{a}$	$^{10}6.58^{b}$	$^{7}6.94^{c}$	$^{5}7.16^{d}$	$^{3}7.48^{e}$	**0.52**
13	$^{5}6.27^{a}$	$^{8}6.91^{b}$	$^{6}7.26^{c}$	$^{3}7.60^{d}$	$^{4}7.81^{e}$	**0.50**
LSD	**0.11**	**0.10**	**0.10**	**0.11**	**0.24**	

abc.. Values on the same row with the same superscript are not significantly different (p<0.05)

1,2,3…Values on the same column with different superscript are significantly different (p<0.05)

Sample 1 - fermented at 65% RH, 30°C and 70µm pore size
Sample 2 –fermented at 85% RH, 30°C and 70µm pore size
Sample 3 – fermented at 65% RH, 40°C and 70µm pore size
Sample 4 – fermented at 85% RH, 40°C and 70µm pore size
Sample 5 – fermented at 65% RH, 35°C and 50µm pore size
Sample 6 – fermented at 85% RH, 35°C and 50µm pore size
Sample 7 – fermented at 65% RH, 35°C and 90µm pore size
Sample 8 – fermented at 85% RH, 35°C and 90µm pore size
Sample 9 – fermented at 65% RH, 30°C and 50µm pore size
Sample 10 – fermented at 75% RH, 40°C and 50µm pore size
Sample 11 – fermented at 75% RH, 30°C and 90µm pore size
Sample 12 – fermented at 85% RH, 40°C and 90µm pore size
Sample 13 – fermented at 75% RH, 35°C and 70µm pore size

Table 4.6: Free fatty acids levels during fermentation of melon seeds (mg/g)

Period of fermentation (hours)

Sample	***0***	***24***	***48***	***72***	***96***	*LSD*
1	$^{1}1.08^{a}$	$^{1}1.14^{b}$	$^{1}1.94^{c}$	$^{1}2.29^{d}$	$^{1}3.48^{e}$	0.56
2	$^{1}1.27^{a}$	$^{1}1.35^{b}$	$^{2}2.92^{c}$	$^{2}3.17^{b}$	$^{2}4.26^{e}$	0.72
3	$^{1}1.15^{a}$	$^{1}1.22^{b}$	$^{3}2.15^{c}$	$^{3}2.18^{b}$	$^{3}2.65^{e}$	0.54
4	$^{1}1.28^{a}$	$^{1}1.30^{b}$	$^{4}2.85^{c}$	$^{4}3.05^{b}$	$^{4}3.12^{e}$	0.45
5	$^{1}1.12^{a}$	$^{1}1.19^{b}$	$^{5}1.98^{c}$	$^{5}2.76^{bc}$	$^{5}2.95^{e}$	0.50
6	$^{1}1.25^{a}$	$^{1}1.30^{b}$	$^{6}2.85^{c}$	$^{6}3.12^{b}$	$^{6}4.24^{e}$	0.55
7	$^{1}1.18^{a}$	$^{1}1.24^{b}$	$^{7}2.45^{c}$	$^{7}2.60^{b}$	$^{7}3.72^{e}$	0.80
8	$^{1}1.30^{a}$	$^{1}1.36^{b}$	$^{8}2.75^{c}$	$^{4}3.05^{b}$	$^{8}4.17^{e}$	0.60
9	$^{1}1.29^{a}$	$^{1}1.32^{b}$	$^{9}2.60^{c}$	$^{8}3.02^{b}$	$^{9}4.12^{e}$	0.88
10	$^{1}1.18^{a}$	$^{1}1.20^{b}$	$^{10}2.29^{c}$	$^{9}3.78^{b}$	$^{10}3.55^{e}$	0.71
11	$^{1}1.28^{a}$	$^{1}1.29^{b}$	$^{11}2.55^{c}$	$^{10}2.71^{b}$	$^{11}3.75^{e}$	0.60
12	$^{1}1.31^{a}$	$^{1}1.32^{b}$	$^{12}2.58^{c}$	$^{11}2.95^{bd}$	$^{12}3.06^{e}$	0.74
13	$^{1}1.28^{a}$	$^{1}1.25^{b}$	$^{13}2.46^{c}$	$^{12}2.64^{b}$	$^{13}3.75^{e}$	0.40
LSD	0.05	0.90	0.73	0.25	0.32	

abc.. Values on the same row with the same superscript are not significantly different ($p<0.05$)

1,2,3…Values on the same column with different superscript are significantly different ($p<0.05$)

Sample 1 - fermented at 65% RH, 30°C and 70µm pore size
Sample 2 –fermented at 85% RH, 30°C and 70µm pore size
Sample 3 – fermented at 65% RH, 40°C and 70µm pore size
Sample 4 – fermented at 85% RH, 40°C and 70µm pore size
Sample 5 – fermented at 65% RH, 35°C and 50µm pore size
Sample 6 – fermented at 85% RH, 35°C and 50µm pore size
Sample 7 – fermented at 65% RH, 35°C and 90µm pore size
Sample 8 – fermented at 85% RH, 35°C and 90µm pore size
Sample 9 – fermented at 65% RH, 30°C and 50µm pore size
Sample 10 – fermented at 75% RH, 40°C and 50µm pore size
Sample 11 – fermented at 75% RH, 30°C and 90µm pore size
Sample 12 – fermented at 85% RH, 40°C and 90µm pore size
Sample 13 – fermented at 75% RH, 35°C and 70µm pore size

Table 4.7: Total Titratable Acidity levels during fermentation of melon seeds (%)

Period of fermentation (hours)

Sample	*0*	*24*	*48*	*72*	*96*	*LSD*
1	$^{1}0.68^{ab}$	$^{1}0.62^{a}$	$^{1}0.72^{ab}$	$^{1}0.76^{ab}$	$^{1}1.08^{b}$	**0.77**
2	$^{1}0.75^{a}$	$^{1}0.78^{a}$	$^{1}0.82^{a}$	$^{1}0.95^{a}$	$^{1}1.16^{a}$	**0.78**
3	$^{1}0.62^{a}$	$^{1}0.65^{a}$	$^{1}0.70^{a}$	$^{1}0.74^{a}$	$^{1}1.24^{b}$	**0.48**
4	$^{1}0.73^{a}$	$^{1}0.76^{a}$	$^{1}0.79^{a}$	$^{1}0.90^{a}$	$^{1}0.98^{a}$	**0.83**
5	$^{1}0.60^{a}$	$^{1}0.64^{a}$	$^{1}0.65^{a}$	$^{1}0.75^{a}$	$^{1}1.02^{a}$	**0.62**
6	$^{1}0.70^{a}$	$^{1}0.77^{a}$	$^{1}0.80^{a}$	$^{1}0.82^{a}$	$^{1}0.95^{a}$	**0.88**
7	$^{1}0.72^{a}$	$^{1}0.75^{a}$	$^{1}0.80^{a}$	$^{1}0.82^{a}$	$^{1}0.86^{a}$	**0.78**
8	$^{1}0.64^{a}$	$^{1}0.69^{a}$	$^{1}0.74^{a}$	$^{1}0.76^{a}$	$^{1}0.91^{a}$	**0.84**
9	$^{1}0.65^{a}$	$^{1}0.75^{a}$	$^{1}0.79^{a}$	$^{1}0.84^{a}$	$^{1}1.08^{a}$	**0.52**
10	$^{1}0.68^{a}$	$^{1}0.71^{a}$	$^{1}0.79^{a}$	$^{1}0.82^{a}$	$^{1}0.92^{a}$	**0.87**
11	$^{1}0.65^{a}$	$^{1}0.69^{a}$	$^{1}0.71^{a}$	$^{1}0.75^{a}$	$^{1}1.03^{a}$	**0.69**
12	$^{1}0.62^{a}$	$^{1}0.65^{a}$	$^{1}0.73^{a}$	$^{1}0.84^{a}$	$^{1}0.91^{a}$	**0.73**
13	$^{1}0.71^{a}$	$^{1}0.72^{a}$	$^{1}0.83^{a}$	$^{1}0.86^{a}$	$^{1}1.05^{a}$	**0.65**
LSD	**0.10**	**0.23**	**0.10**	**0.21**	**0.25**	

abc.. Values on the same row with the same superscript are not significantly different ($p<0.05$)

1,2,3…Values on the same column with different superscript are significantly different ($p<0.05$)

Sample 1 - fermented at 65% RH, 30°C and 70µm pore size
Sample 2 –fermented at 85% RH, 30°C and 70µm pore size
Sample 3 – fermented at 65% RH, 40°C and 70µm pore size
Sample 4 – fermented at 85% RH, 40°C and 70µm pore size
Sample 5 – fermented at 65% RH, 35°C and 50µm pore size
Sample 6 – fermented at 85% RH, 35°C and 50µm pore size
Sample 7 – fermented at 65% RH, 35°C and 90µm pore size
Sample 8 – fermented at 85% RH, 35°C and 90µm pore size
Sample 9 – fermented at 65% RH, 30°C and 50µm pore size
Sample 10 – fermented at 75% RH, 40°C and 50µm pore size
Sample 11 – fermented at 75% RH, 30°C and 90µm pore size
Sample 12 – fermented at 85% RH, 40°C and 90µm pore size
Sample 13 – fermented at 75% RH, 35°C and 70µm pore size

Table 4.8: Peroxide values during fermentation of melon seeds (Meq/kg)

Period of fermentation (hours)

Sample	***0***	***24***	***48***	***72***	***96***	*LSD*
1	$^{1}2.65^{a}$	$^{1,3}2.90^{b}$	$^{1}3.06^{c}$	$^{1}3.25^{d}$	$^{1}3.75^{e}$	0.50
2	$^{1}2.80^{a}$	$^{2}3.37^{b}$	$^{2}3.63^{c}$	$^{2}4.16^{d}$	$^{2}3.81^{e}$	0.70
3	$^{1}2.69^{a}$	$^{1}2.72^{b}$	$^{3}3.92^{c}$	$^{3}4.09^{d}$	$^{3}3.52^{e}$	0.40
4	$^{1}2.76^{a}$	$^{2,4}3.24^{b}$	$^{4}3.94^{c}$	$^{4}3.45^{d}$	$^{4}3.51^{e}$	0.35
5	$^{1}2.85^{a}$	$^{1,3}2.94^{b}$	$^{5}2.95^{b}$	$^{5}3.57^{c}$	$^{5}3.62^{d}$	0.40
6	$^{1}2.70^{a}$	$^{2,3}3.20^{b}$	$^{6}3.25^{c}$	$^{6}3.60^{d}$	$^{6}4.08^{e}$	0.42
7	$^{1}2.90^{a}$	$^{1,3}2.94^{a}$	$^{7}3.09^{b}$	$^{6}3.60^{c}$	$^{7}3.31^{d}$	0.46
8	$^{1}2.85^{a}$	$^{2,3}3.15^{b}$	$^{8}3.22^{c}$	$^{7}3.39^{d}$	$^{8}3.82^{e}$	0.29
9	$^{1}3.12^{a}$	$^{2,3}3.17^{a}$	$^{9}3.19^{a}$	$^{8}3.24^{a}$	$^{9}2.86^{a}$	0.34
10	$^{1}2.95^{a}$	$^{1,4}2.97^{a}$	$^{9}3.19^{ab}$	$^{9}3.30^{b}$	$^{10}3.45^{b}$	0.26
11	$^{1}2.65^{a}$	$^{1,4}2.98^{b}$	$^{1}3.06^{b}$	$^{10}3.18^{c}$	$^{11}3.40^{d}$	0.26
12	$^{1}3.04^{a}$	$^{2,3,4}3.10^{a}$	$^{10}3.12^{a}$	$^{11}3.22^{a}$	$^{12}3.29^{a}$	0.29
13	$^{1}2.64^{a}$	$^{1}2.73^{b}$	$^{11}2.81^{c}$	$^{12}3.06^{d}$	$^{10}3.45^{e}$	0.30
LSD	0.05	0.08	0.08	0.12	0.16	

abc…Values on the same row with the same superscript are not significantly different (p<0.05)

1,2,3…Values on the same column with different superscript are significantly different (p<0.05)

Sample 1 - fermented at 65% RH, 30°C and 70µm pore size
Sample 2 –fermented at 85% RH, 30°C and 70µm pore size
Sample 3 – fermented at 65% RH, 40°C and 70µm pore size
Sample 4 – fermented at 85% RH, 40°C and 70µm pore size
Sample 5 – fermented at 65% RH, 35°C and 50µm pore size
Sample 6 – fermented at 85% RH, 35°C and 50µm pore size
Sample 7 – fermented at 65% RH, 35°C and 90µm pore size
Sample 8 – fermented at 85% RH, 35°C and 90µm pore size
Sample 9 – fermented at 65% RH, 30°C and 50µm pore size
Sample 10 – fermented at 75% RH, 40°C and 50µm pore size
Sample 11 – fermented at 75% RH, 30°C and 90µm pore size
Sample 12 – fermented at 85% RH, 40°C and 90µm pore size
Sample 13 – fermented at 75% RH, 35°C and 70µm pore size

Table 4.9: Amino Nitrogen levels during fermentation of melon seeds (mgN/g)

Period of fermentation (hours)

Sample	*0*	*24*	*48*	*72*	*96*	*LSD*
1	$^{1}3.28^{a}$	$^{1}3.52^{b}$	$^{1}4.75^{c}$	$^{1}5.20^{d}$	$^{1}5.82^{e}$	**0.98**
2	$^{2}2.98^{a}$	$^{2}3.51^{b}$	$^{2}4.55^{c}$	$^{2}4.80^{d}$	$^{2}5.45^{e}$	**0.88**
3	$^{3}3.12^{a}$	$^{3}3.65^{b}$	$^{3}4.42^{c}$	$^{3}4.85^{d}$	$^{3}6.31^{e}$	**0.80**
4	$^{4}3.85^{a}$	$^{4}4.26^{b}$	$^{4}4.70^{c}$	$^{4}5.72^{d}$	$^{4}6.24^{e}$	**0.75**
5	$^{5}2.96^{a}$	$^{5}3.45^{b}$	$^{5}4.42^{c}$	$^{5}4.61^{d}$	$^{5}5.13^{e}$	**0.77**
6	$^{6}3.82^{a}$	$^{6}4.35^{b}$	$^{6}5.64^{c}$	$^{6}5.77^{d}$	$^{6}6.28^{e}$	**1.20**
7	$^{7}3.24^{a}$	$^{1}3.52^{b}$	$^{7}4.60^{c}$	$^{7}5.11^{d}$	$^{7}5.17^{e}$	**1.15**
8	$^{8}4.06^{a}$	$^{7}4.36^{b}$	$^{8}5.65^{c}$	$^{4}5.72^{d}$	$^{8}6.23^{e}$	**1.42**
9	$^{9}3.65^{a}$	$^{8}4.17^{b}$	$^{9}5.16^{c}$	$^{8}5.65^{d}$	$^{9}6.77^{e}$	**1.08**
10	$^{10}3.34^{a}$	$^{9}3.75^{b}$	$^{10}4.94^{c}$	$^{9}5.18^{d}$	$^{10}5.46^{e}$	**0.99**
11	$^{11}3.17^{a1}$	$^{10}4.08^{b}$	$^{11}5.48^{c}$	$^{10}5.75^{d}$	$^{1}5.82^{e}$	**1.50**
12	$^{12}3.58^{a}$	$^{11}3.84^{b}$	$^{12}5.28^{c}$	$^{11}5.46^{d}$	$^{11}6.18^{e}$	**1.42**
13	$^{13}3.38^{a}$	$^{12}3.78^{b}$	$^{13}4.92^{c}$	$^{12}5.14^{d}$	$^{10}5.46^{e}$	**1.44**
LSD	**0.14**	**0.20**	**0.10**	**0.20**	**0.17**	

abc.. Values on the same row with the same superscript are not significantly different (p<0.05)

1,2,3…Values on the same column with different superscript are significantly different (p<0.05)

Sample 1 - fermented at 65% RH, 30°C and 70µm pore size
Sample 2 –fermented at 85% RH, 30°C and 70µm pore size
Sample 3 – fermented at 65% RH, 40°C and 70µm pore size
Sample 4 – fermented at 85% RH, 40°C and 70µm pore size
Sample 5 – fermented at 65% RH, 35°C and 50µm pore size
Sample 6 – fermented at 85% RH, 35°C and 50µm pore size
Sample 7 – fermented at 65% RH, 35°C and 90µm pore size
Sample 8 – fermented at 85% RH, 35°C and 90µm pore size
Sample 9 – fermented at 65% RH, 30°C and 50µm pore size
Sample 10 – fermented at 75% RH, 40°C and 50µm pore size
Sample 11 – fermented at 75% RH, 30°C and 90µm pore size
Sample 12 – fermented at 85% RH, 40°C and 90µm pore size
Sample 13 – fermented at 75% RH, 35°C and 70µm pore size

Table 4.10: pH levels during fermentation of melon seeds

Period of fermentation (hours)

Sample	*0*	*24*	*48*	*72*	*96*	*LSD*
1	$^{1}5.94^{a}$	$^{1}6.08^{b}$	$^{1}6.13^{c}$	$^{1}6.65^{d}$	$^{1}7.16^{e}$	**0.38**
2	$^{1}6.15^{a}$	$^{1}6.32^{b}$	$^{2}7.18^{c}$	$^{2}7.41^{d}$	$^{2}8.15^{e}$	**0.45**
3	$^{1}5.82^{a}$	$^{1}5.90^{b}$	$^{3}7.15^{c}$	$^{3}6.82^{d}$	$^{3}7.31^{e}$	**0.50**
4	$^{1}6.19^{a}$	$^{1}6.25^{b}$	$^{4}6.24^{c}$	$^{4}7.61^{d}$	$^{4}8.16^{e}$	**0.54**
5	$^{1}5.96^{a}$	$^{1}6.12^{b}$	$^{5}6.15^{c}$	$^{5}6.78^{d}$	$^{5}7.28^{e}$	**0.54**
6	$^{1}6.18^{a}$	$^{1}6.25^{b}$	$^{6}6.35^{c}$	$^{6}7.21^{d}$	$^{6}7.92^{e}$	**0.45**
7	$^{1}6.12^{a}$	$^{1}6.22^{b}$	$^{7}6.29^{c}$	$^{7}7.16^{d}$	$^{7}7.56^{e}$	**0.49**
8	$^{1}6.14^{a}$	$^{1}6.16^{b}$	$^{8}7.13^{c}$	$^{2}7.40^{d}$	$^{8}7.90^{e}$	**0.51**
9	$^{1}6.14^{a}$	$^{1}6.24^{b}$	$^{9}7.08^{c}$	$^{8}7.36^{d}$	$^{9}7.86^{e}$	**0.41**
10	$^{1}5.96^{a}$	$^{1}6.16^{b}$	$^{10}6.32^{c}$	$^{9}6.94^{d}$	$^{10}7.47^{e}$	**0.60**
11	$^{1}6.11^{a}$	$^{1}6.21^{b}$	$^{11}6.28^{c}$	$^{6}7.21^{d}$	$^{11}7.81^{e}$	**0.51**
12	$^{1}6.18^{a}$	$^{1}6.24^{b}$	$^{12}6.91^{c}$	$^{10}7.29^{d}$	$^{12}7.79^{e}$	**0.47**
13	$^{1}6.05^{a}$	$^{1}6.17^{b}$	$^{13}6.25^{c}$	$^{9}6.95^{d}$	$^{13}7.45^{e}$	**0.47**
LSD	**0.09**	**0.10**	**0.11**	**0.12**	**0.08**	

abc…Values on the same row with the same superscript are not significantly different ($p<0.05$)

1,2,3…Values on the same column with different superscript are significantly different ($p<0.05$)

Sample 1 - fermented at 65% RH, 30°C and 70µm pore size
Sample 2 –fermented at 85% RH, 30°C and 70µm pore size
Sample 3 – fermented at 65% RH, 40°C and 70µm pore size
Sample 4 – fermented at 85% RH, 40°C and 70µm pore size
Sample 5 – fermented at 65% RH, 35°C and 50µm pore size
Sample 6 – fermented at 85% RH, 35°C and 50µm pore size
Sample 7 – fermented at 65% RH, 35°C and 90µm pore size
Sample 8 – fermented at 85% RH, 35°C and 90µm pore size
Sample 9 – fermented at 65% RH, 30°C and 50µm pore size
Sample 10 – fermented at 75% RH, 40°C and 50µm pore size
Sample 11 – fermented at 75% RH, 30°C and 90µm pore size
Sample 12 – fermented at 85% RH, 40°C and 90µm pore size
Sample 13 – fermented at 75% RH, 35°C and 70µm pore size

Table 4.11: Model Summary Statistics for amino nitrogen

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS
Linear	0.075	0.5502	0.4276	0.3208	0.093
2FI	0.087	0.5591	0.2284	-0.2611	0.17
Quadratic	0.011	0.9956	0.9877	0.9076	0.013
Cubic	2.306E-003	0.9999	0.9995		

"Model Summary Statistics": Focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared".

Table 4.12: ANOVA table for response surface quadratic model (Amino nitrogen) during melon seeds fermentation

Source	Sum of	Df	Mean square	F – value	p – value Prob F
Model	0.14	9	0.015	125.74	0.0001
A-Relative Humidity	0.036	1	0.036	301.07	0.0001
B-Temperature	4.948E-005	1	4.948E-005	0.41	0.5504
C-Pore size	0.012	1	0.012	96.72	0.0002
AB	8.473E-005	1	8.473E-005	0.70	0.4405
AC	8.344E-004	1	8.344E-004	6.91	0.0466
BC	4.243E-003	1	4.243E-003	35.12	0.0020
A^2	0.025	1	0.025	208.53	0.0001
B^2	3.218E-003	1	3.218E-003	26.64	0.0036
C^2	9.703E-003	1	9.703E-003	80.32	0.0003
Residual	6.040E-004	5	1.208E-004		
Lack of fit	5.934E-004	3	1.978E-004	37.20	0.0263
Pure error	1.063E-005	2	5.316E-006		
Cor Total	0.14	14			

Table 4.13: Daily amino nitrogen production for pumpkin seeds (mg N/g)

	Period of fermentation (hours)				
Sample	***0***	***24***	***48***	***72***	***96***
1	0	0.21	0.95	1.53	1.16
2	0	0.22	1.24	1.85	1.16
3	0	0.23	1.06	2.02	0.89
4	0	0.18	0.15	2.45	1.17
5	0	0.22	1.05	2.08	1.36
6	0	0.18	1.08	2.19	0.43
7	0	0.24	1.01	2.00	0.21
8	0	0.11	1.06	1.98	1.01
9	0	0.18	0.96	1.88	1.01
10	0	0.29	1.06	2.22	0.45
11	0	0.24	1.12	1.97	0.69
12	0	0.14	1.33	2.18	0.32
13	0	1.33	0.08	1.58	0.42

Table 4.14: **Daily amino nitrogen production for melon seeds (mg N/g)**

	Period of fermentation (hours)				
Sample	***0***	***24***	***48***	***72***	***96***
1	0	0.24	1.23	0.45	0.62
2	0	0.53	1.04	0.25	0.65
3	0	0.53	0.77	0.43	1.46
4	0	0.41	0.44	1.02	0.52
5	0	0.49	0.97	0.19	0.52
6	0	0.53	1.29	0.13	0.51
7	0	0.28	1.08	0.51	0.06
8	0	0.30	1.29	0.07	0.51
9	0	0.52	0.99	0.49	1.12
10	0	0.41	1.19	0.24	0.28
11	0	0.91	1.40	0.27	0.07
12	0	0.26	1.44	0.18	0.72
13	0	0.40	1.14	0.22	0.32

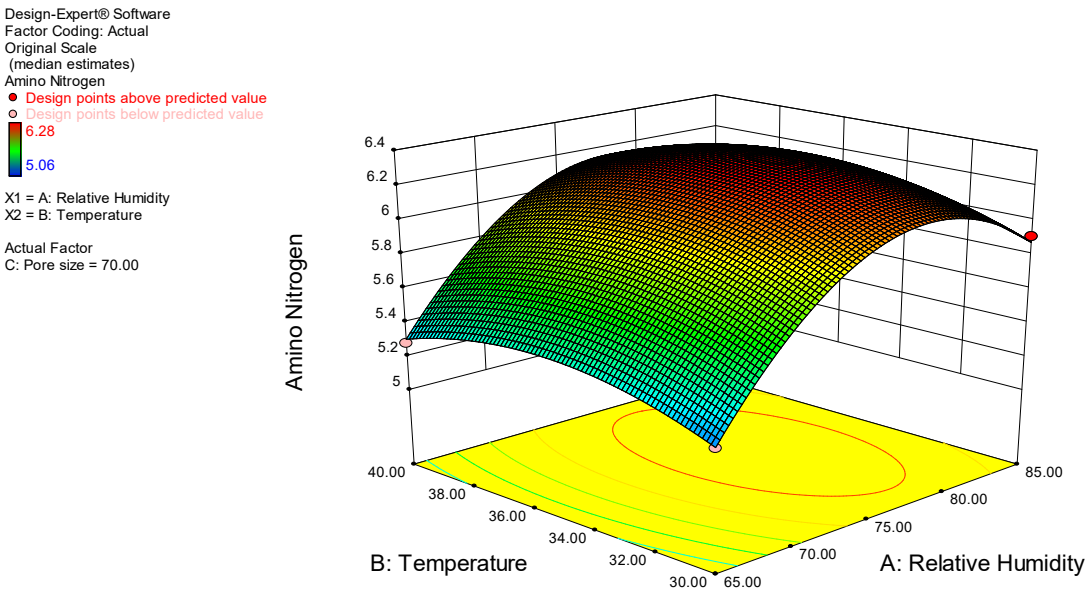

Figure 4.1: Effect of temperature and relative humidity on amino nitrogen content of ogiri egusi.

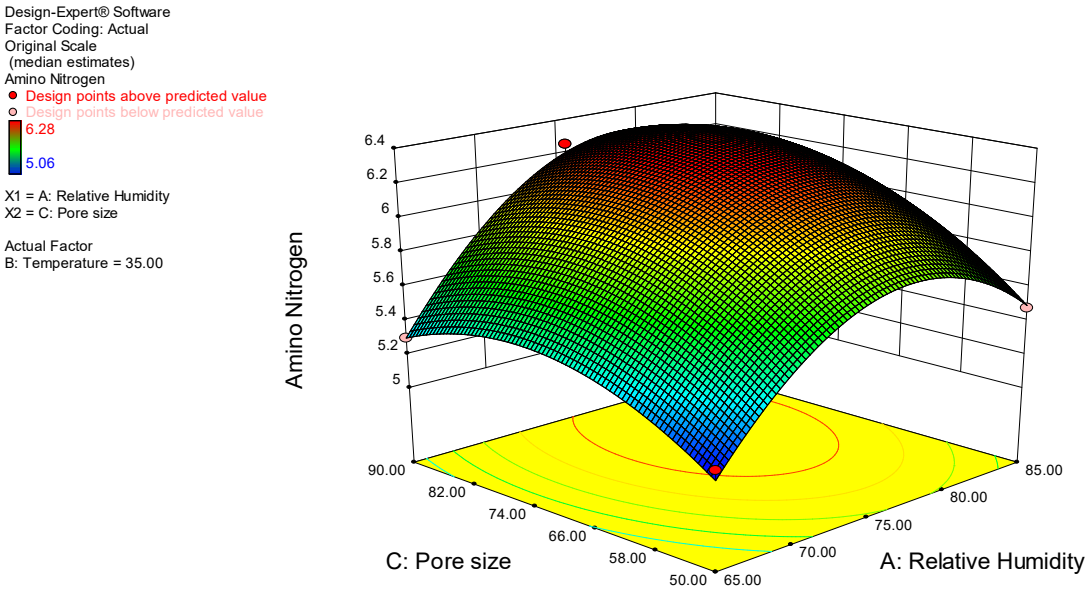

Figure 4.2: Effect of pore size and relative humidity on the amino nitrogen content of ogiri egusi.

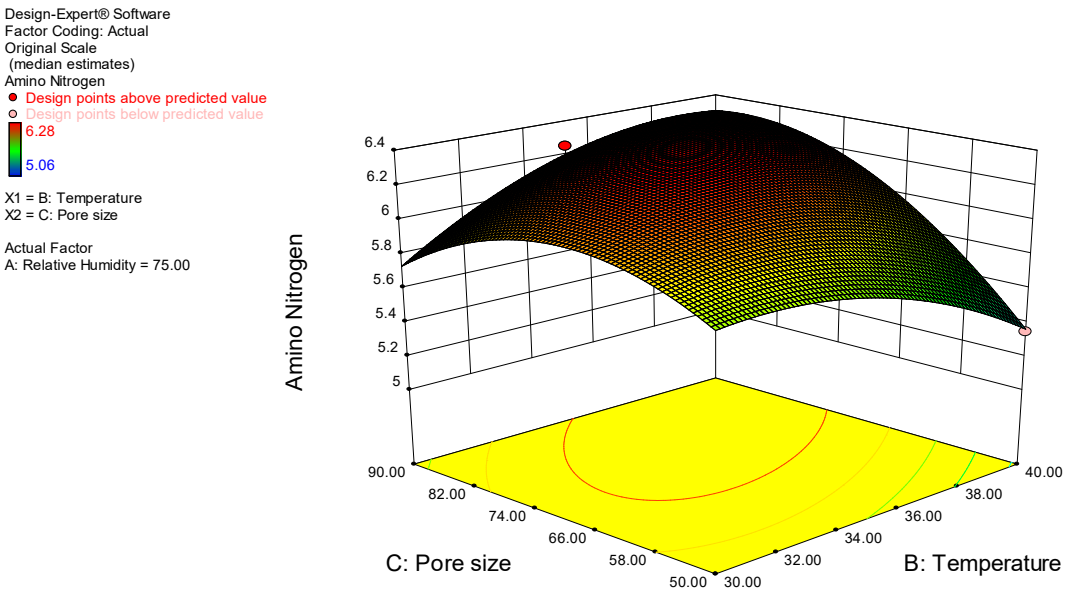

Figure4.3: Effect of pore size and temperature on the amino nitrogen content of ogiri egusi.

4.2 DISSCUSSION

4.2.1 Effect of variables on the fermented fluted pumpkin

Table 4.1 shows the free fatty acids (FFA) values of the fermenting fluted pumpkin seeds over a period of 96 hours. The results showed that FFA increased with increase in fermentation time in all the samples. These increase were shown to be significantly different ($p<0.05$) as indicated in Table 4.1. The highest value of FFA recorded at 96 hours was obtained from sample 5 (fermented at 65% RH, 35^0C and 50µm pore size of polyethene).

At zero (0) hour, it was observed that sample 8 (fermented at 85% RH, 35^0C and 90µm pore size) and sample 13 (fermented at 75% RH, 35^oC and 70µm pore size) had the highest value of 1.9mg/g while sample 7 (fermented at 65% RH, 35^oC and 90µm pore size) had the lowest value of 1.65mg/g. After 24 hours, sample 13 (fermented at 75% RH, 35^oC and 70µm pore size) had the highest value (3.28mg/g) while sample 10 (fermented at 75% RH, 40^oC and 50µm pore size) had the lowest value (1.96mg/g).

At 48 hours, samples 10 (fermented at 75% RH, 40^oC and 50µm pore size) and 12 (fermented at 85% RH, 40^oC and 90µm pore size) had the lowest FFA value of 2.65mg/g while sample 11 (fermented at 75% RH, 30^oC and 90µm pore size) had the highest value (3.44mg/g). Looking further at 72 hour fermentation, sample 12 (fermented at 85% RH, 40^oC and 90µm pore size) had the lowest FFA value (3.06mg/g) while sample 5 (fermented at 65% RH, 35^oC and 50µm pore size) had the highest value of 4.24mg/g. At 96 hours of fermentation, sample 5 (fermented at 65% RH, 35^oC and 50µm pore size) recorded the highest FFA value of 4.74mg/g while sample 13 (fermented at 75% RH, 35^oC and 70µm pore size) had the lowest value (3.40mg/g).

The trend which the free fatty acids followed has been observed by Abaelu *et al*., (1990) during fermentation of melon seeds. The increase in free fatty acid is an indication of lipase production by the fermenting bacterium. Lipase production results in fermenting unsaturated fatty acids to saturated fatty acids (Abaelu *et al.*,

1990). This transformation is of interest since it would decrease the process of rancidity and thereby increase the shelf-life of the fermented product. Free fatty acid is a shelf life indicator test for hydrolytic rancidity, while acid value refers to a measure of free fatty acid present in fat.

Table 4.2 shows the values obtained for total titratable acidity (TTA) during the fermentation of fluted pumpkin seeds for ogiri ugu production. The table showed that TTA values increased with increase in fermentation time with least values obtained at zero hour and the highest value obtained at 96 hours of fermentation. The highest value of the TTA at 96 hours was obtained from sample 12 (fermented at 85% RH, temperature of 40°C and 90µm pore size of wrapping material.

At zero (0) hour of fermentation, the TTA was observed to be highest in sample 13 (fermented at 75% RH, 35°C and 70µm) with a value of 1.06% and lowest in sample 10 (fermented at 75% RH, 40°C and 50µm) with 0.72%. At 24 hours of fermentation, sample 13 (fermented at 75% RH, 35°C and 70µm) had the highest value (1.36%) while sample 5 (fermented at 65%, 35°C and 50µm) had the least value (0.92%). At 48 hours of fermentation, sample 12 (fermented at 85% RH, 40°C and 90µm) had the highest value of fermentation and sample 13 (fermented at 75% RH, 35°C and 70µm) and sample 5 (fermented at 65%, 35°C and 50µm) had the least values (1.17%) respectively. At 72 hours of fermentation, sample 5 (fermented at 65%, 35°C and 50µm) and 7 (fermented at 65% RH, 35°C and 90µm) had the least value (1.27%) respectively while the highest value was recorded in sample 2 (fermented at 85% RH, 30°C and 70µm pore size) with 1.47%. Finally, at 96 hours of fermentation, it was observed that sample 12 (fermented at 85% RH, 40°C and 90µm) had the highest TTA value (1.56%) while sample 5 (fermented at 65%, 35°C and 50µm) had the lowest value (1.32%). The variations in the fermenting conditions may have resulted in increased TTA values. Ogueke and Nwagwu (2007) have reported similar increase in TTA during fermentation of melon seeds using *Proteus vulgaris* as the fermenting organism.

Also Moat (1979) reported that fermentations which involve *Bacillus species* usually result in production of primary organic acids especially butyric acid. These may be responsible for the increase in TTA during the fermentation.

Table 4.3 shows the results of peroxide values (PV) obtained during fermentation of fluted pumpkin seeds for the period of 96 hours. The peroxide values showed significant difference in all the samples. From the result, the PV values increased with increase in fermentation time, however, the highest value was obtained at 96 hours in sample 2 which was fermented at 85% RH, temperature 40°C and 70µm pore size of wrapping material. At zero hour, it was observed that PV was highest in sample 11 (fermented at 75% RH, 30°C and 90µm) with a value of 3.20meq/kg and lowest in sample 10 (fermented at 75% RH, 40°C and 50µm) with a value of 2.65meq/kg. At 24 hours of fermentation, sample 13 (fermented at 75% RH, 35°C and 70µm) had the highest PV value of 3.75meq/kg while samples 7 (fermented at 65% RH, 35°C and 90µm) and 10 (fermented at 75% RH, 40°C and 50µm) had the least value of 2.91meq/kg. At 48 hours of fermentation, sample 3 (fermented at 65% RH, 40°C and 70µm) recorded the highest PV value (3.81 meq/kg) while sample 8 (fermented at 85% RH, 35°C and 90µm) had the lowest value of 3.39meq/kg. At 72 hours of fermentation, sample 2 (fermented at 85% RH, 30°C and 70µm) had the highest PV value (4.35meq/kg) while sample 8 (fermented at 85% RH, 35°C and 90µm) had the least value of 3.58meq/kg. At 96 hours of fermentation, it was observed that sample 2 (fermented at 85% RH, 30°C and 70µm) had the highest PV (4.85meq/kg) while sample 8 (fermented at 85% RH, 35°C and 90µm) recorded the least PV value of 3.65meq/kg.

Peroxide value is used as a measure of the extent to which rancidity can occur in foods (Ihekoronye and Ngoddy, 1985). It is also an indicator of autoxidation (oxidative rancidity). However, since the peroxide values obtained in the study were below the threshold value of 30meq/kg of oil in oil rich foods (Gotoh and Wada, 2006), it implies that the possibility of the samples getting rancid is very low,

thus they cannot introduce off flavours to soups and stews prepared with the condiment.

Table 4.4 shows the result of amino nitrogen values (AN) obtained during the fermentation of fluted pumpkin seeds for ogiri ugu production within 96 hours fermentation period. The result showed a significant difference in all the samples and an increase in the production of amino nitrogen. The highest AN value was obtained in sample 4 fermented at 85% RH, temperature 30°C and 70µm pore size at 96 hours.

At zero hour, sample 5 (fermented at 65%, 35°C and 50µm) had the least AN value (3.45mgN/g) while sample 8(fermented at 85% RH, 35°C and 90µm) had the highest value (4.67mgN/g). Similarly, at 24 hours of fermentation, sample 13 (fermented at 75% RH, 35°C and 70µm) recorded the highest AN value (5.17mgN/g) while sample 5 (fermented at 65%, 35°C and 50µm)had the least AN value (3.67mgN/g). Subsequently, at 48 hours of fermentation sample 13 (fermented at 75% RH, 35°C and 70µm) was recorded as the sample with the highest AN value (5.85mgN/g) while sample 4 (fermented at 85% RH, 30°C and 70µm) had the least value (4.71mgN/g). At the 72 hours, sample 8 (fermented at 85% RH, 35°C and 90µm) had the highest amino nitrogen production (7.82mgN/g) while sample 1 (fermented at 65% RH, 40°C and 70µm) recorded the least value of 6.45mgN/g. At the 96 hour fermentation, sample 4 (fermented at 85% RH, 30°C and 70µm) had the highest AN value(8.33mgN/g) and sample 7 (fermented at 65% RH, 35°C and 90µm) the least AN value (7.16mgN/g).

The results obtained agrees with the findings of Sarkar *et al* (1993) who showed that there was a rapid increase in total free amino acids which is a reflection of the increased protease activity. Increased amino nitrogen production may be due to breakdown of proteins to amino acids by proteases. The increased levels in amino nitrogen could be as a result of increased cell density per unit of fermenting mash as suggested by Isu and Ofuya (2000). It was observed that there were general daily

increase in AN in all samples. In fact, it has been suggested that amino nitrogen can be used as an index of fermentation efficiency during fermentation of proteinaceous food materials (Enujiugha, 2003).

Table 4.5 presents the pH values obtained during fermentation of fluted pumpkin seeds for ogiri production. The period of fermentation showed a significant difference in all the samples. Looking at the periods of fermentation for the various samples, it was observed that the pH values increased as fermentation progressed. The values for all samples gradually increased from acidic range to alkaline range within the period of fermentation. The changes were observed to be significant ($P < 0.05$) in all samples irrespective of the varying conditions of fermentation. The highest pH value was obtained in sample 3 fermented at 65%RH, 30°C and 70µm pore size of polyethene. The pH values of most of the samples at 96 hours were tending towards the alkaline region (greater than 8.0). Sample 4 (fermented at 85% RH, 30°C and 70µm) had the highest pH value (6.71) and sample 12 (fermented at 85% RH, 40°C and 90µm) had the least value (5.92) at zero hour. The 24 hours fermentation presents sample 4 (fermented at 85% RH, 30°C and 70µm) as the sample with the highest value of 7.41 while sample 12 (fermented at 85% RH, 40°C and 90µm) had the least value (6.58). It was observed that sample 2 (fermented at 85% RH, 30°C and 70µm) produced the highest pH value (7.65) while sample 12 (fermented at 85% RH, 40°C and 90µm) had the least value (6.94) at the 48 hours of fermentation. Similarly, sample 2 (fermented at 85% RH, 30°C and 70µm) also recorded the highest pH value (8.06) at 72 hours of fermentation while sample 12 (fermented at 85% RH, 40°C and 90µm) had the least pH value (7.16). At 96 hours of fermentation, sample 3 (fermented at 65%RH, 30°C and 70µm) produced the highest pH value of 8.23 while sample 12 (fermented at 85% RH, 40°C and 90µm) had the least pH value (7.48).

This agrees with the findings of other workers (Odunfa and Oyeyiola, 1985; Njoku and Okemadu, 1989; Ikenebomeh *et al.*, 1986; Ogueke and Aririatu, 2004) who also

observed that fermentations of proteinous oily seeds usually results in increase in pH to the alkaline region. The pH increase is as a result of the activities of the proteases and deaminase enzymes produced by the fermenting bacterium (*B. subtilis*) resulting in the release of ammonia which increased pH (Njoku and Okemadu, 1989; Achi, 2005).

4.2.2 Effect of variables on the fermented melon seeds

The free fatty acid (FFA) values obtained during fermentation of melon seeds are shown in Table 4.6. The results showed that FFA values increased as fermentation progressed. There were significant differences in the samples at $p<0.05$ irrespective of the different fermentation conditions.

The zero hour recorded sample 12 (fermented at 85% RH, 40°C and 90µm) as the sample with the highest FFA value (1.31mg/g) while sample 1 (fermented at 65% RH, 30°C and 70µm) had the least value of 1.08mg/g. As fermentation progressed into 24 hours, sample 8 (fermented at 85% RH, 35°C and 90µm) produced the highest FFA value (1.36mg/g) while sample 1 (fermented at 65% RH, 30°C and 70µm) had the least FFA value (1.14mg/g). Similarly, sample 2 (fermented at 85% RH, 30°C and 70µm) had the highest FFA value (2.92mg/g) at 48 hours of fermentation with sample 1 (fermented at 65% RH, 30°C and 70µm) recording the lowest value of 1.94mg/g. The end of the 72 hour fermentation produced sample 10 (fermented at 75% RH, 40°C and 50µm) as the sample with the highest FFA value (3.78mg/g) while sample 3 (fermented at 65% RH, 40°C and 70µm) had the least value (2.18mg/g). At 96 hours, sample 2 (fermented at 85% RH, 30°C and 70µm) was observed to have the highest value of 4.26mg/g while sample 3 (fermented at 65% RH, 40°C and 70µm) had the least value of 2.65mg/g.

Similar trend has been observed by Abaelu *et al.,* (1990) during fermentation of melon seeds. The increase in free fatty acid is an indication of lipase production by the fermenting bacterium. Low free fatty acids in the samples indicate stability of the oil (Abiodun and Adeleke, 2010).

Table 4.7 shows the results obtained from total titratable acidity (TTA) during the fermentation of melon seeds. The total titratable acidity showed no significant difference in all the samples and in the period of fermentation. From the table, it was observed that TTA increased throughout the period of fermentation. It was observed that sample 3 had the highest value of TTA (1.24%) while sample 7 had the least value (0.86%).

Sample 2 (fermented at 85% RH, 30°C and 70µm) was seen to have the highest value of TTA (0.75%) while sample 5 (fermented at 65%, 35°C and 50µm)had the least value of TTA (0.60%) at the zero hour. Similarly, sample 2 (fermented at 85% RH, 30°C and 70µm) also produced the highest value of TTA (0.78%) at 24 hours of fermentation. The end of 48 hours produced sample 13 (fermented at 75%, 35°C and 70µm)as the sample with the highest TTA value (0.83%) and sample 5 (fermented at 65%, 35°C and 50µm)had the least value (0.65%).As fermentation progressed into 72 hours, sample 2 (fermented at 85%, 30°C and70um) produced the highest value (0.95%) of TTA while sample 3 (fermented at 65%, 40°C and 70µm) had the least value (0.74%) of TTA.

The increase in TTA agrees with the work reported by Ogueke and Nwagwu (2007). This also agrees with the report of (Omafuvbe *et al.,* 2004) who reported an increase in acid value as fermentation progressed. Moat (1979) has shown that fermentations involving *Bacillus sp* usually result in the production of primary organic acids especially butyric acid. It is believed that the increase obtained in this study could be due to this.

Peroxide value is an indication of oxidative rancidity and in predicting shelf- life of oily foods. The peroxide values obtained during the fermentation are shown in Table 4.8. The peroxide values increased as the fermentation progressed in all the samples. From the table, the result suggested that peroxide value in the fermenting melon seeds began to vary significantly between one sample and another at 48 hours of fermentation.

At zero hour, sample 9 (fermented at 65%, 30°C and 50µm) recorded the highest value of 3.12 meq/kg while sample 13 (fermented at 75%, 35°C and 70µm) recorded the least peroxide value (2.64meq/kg). The 24th hour recorded sample 2 (fermented at 85%, 30°C and 70µm)as the sample with the highest peroxide value (3.37meq/kg) while sample 3 (fermented at 65%, 40°C and 70µm)had the least peroxide value of 2.72meq/kg. At 48 hours, sample 13 (fermented at 75%, 35°C and 70µm) was found to have the least value of 2.81meq/kg while sample 4 (fermented at 85%, 40°C and 70µm) had the highest value of 3.94meq/kg. Seventy-two (72h) hours of fermentation resulted in sample 2 (fermented at 85%, 30°C and 70µm) having the highest peroxide value of 4.16meq/kg while sample 13 (fermented at 75%, 35°C and 70µm) was found to have the least value (3.06meq/kg). Sample 6 (fermented at 85%, 35°C and 50µm) was observed to have the highest peroxide value (4.08meq/kg) at the 96th hour while sample 9 (fermented at 75%, 30°C and 50µm) had the lowest value of 2.86meq/kg. A high value may represent either the beginning of oxidation or advanced oxidation and values greater than 20meq/kg corresponds to very poor quality fats and oils (FAO, 2002). The low values for free fatty acid and peroxide values suggest that the tendency of the products to get rancid as a result of both oxidation (oxidative rancidity) and hydrolytic rancidity is low.

Table 4.9 presents the results obtained for amino nitrogen (AN) during the fermentation of melon seeds for ogiri production. Significant differences occurred between all the samples and at the period of the fermentation. As fermentation progressed, the amino nitrogen increased with sample 5 (fermented at 65%RH, 35°C, 50µm pore size) having the least values all through the fermentation periods from the zero hour to 96 hours. At zero hour, sample 8 (fermented at 85%RH, 35°C, 90µm pore size) had the highest AN value (4.06mgN/g) while sample 5 (fermented at 65%RH, 35°C, 50µm pore size) had the least value of AN (2.96mg/g). As fermentation progressed into the 24th hour, sample 8 (fermented at 85%RH, 35°C, 90µm pore size) also recorded the highest AN value (4.36mgN/g) and sample 5

(fermented at 65%RH, 35°C, 50µm pore size) recorded the least value of AN (3.45mgN/g). At 48 hours of fermentation it was observed that sample 8 (fermented at 85%RH, 35°C, 90µm pore size) had the highest AN value (5.65mgN/g) while samples 5 (fermented at 65%RH, 35°C, 50µm pore size) and 3 (fermented at 65%RH, 45°C, 70µm pore size) had the least values (4.42mgN/g) respectively. Seventy-two (72) hours of fermentation produced sample 6 (fermented at 85%RH, 35°C, 50µm pore size) as having the highest AN value of 5.77mgN/g while sample 5 (fermented at 65%RH, 35°C, 50µm pore size) had the least value of amino nitrogen (4.61mgN/g). Sample 9 (fermented at 65%RH, 30°C, 50µm pore size) was found to have the highest AN value (6.77mgN/g) while sample 5 (fermented at 65%RH, 35°C, 50µm pore size) had the least value (5.13mgN/g) at the 96 hours of fermentation. Amino nitrogen is a component of protein. The increase could be attributed to the high protein content of melon seeds which were acted upon by proteases during proteolysis to release amino acids and peptides. Deaminase activity however, results in increased production of ammonia which gives the product its characteristic aroma. However, accumulation of ammonia in fermented foods may become undesirable at high levels (Ogueke and Nwagwu, 2007). Asagbra *et al., (*2012) said that high protein content in fermented foods causes increased proteinase activity during fermentation which releases more amino acids and nitrogenous compounds which may probably give odour or smell depending on the fermentation period. However, Achi (2005) reported that free amino acid increases when fermented for longer period, may result in losses of lysine or other essential amino acids. The deaminase activity may have resulted in the increased amino nitrogen in this study.

Table 4.10 presents the pH values obtained during the fermentation of melon seeds. It was observed that the pH increased as fermentation progressed and the values obtained in all the samples differ significantly from each other.

At the zero hour, sample 4 (fermented at 85%, 40°C and 70µm pore size) produced the highest pH value (6.19) while sample 3 (fermented at 65%RH, 45°C, and 70µm

pore size) had the least value (5.82). At 24 hours of fermentation, samples 4 (fermented at 85%, 40°C and 70µm pore size) and 6 (fermented at 85%, 35°C and 50µm pore size) respectively had the highest pH values (6.25) while sample 3 (fermented at 65%RH, 45°C, and 70µm pore size) had the least pH value of 5.90. Similarly, sample 2 (fermented at 85%RH, 30°C, and 70µm pore size) had the highest pH value (7.18) at 48 hours fermentation while sample 1 (fermented at 65%RH, 30°C, and 70µm pore size) had the least pH value (6.13). At 72 hours of fermentation, sample 2 (fermented at 85%RH, 30°C, and 70µm pore size)also recorded the highest pH value (7.41) while sample 1 (fermented at 65%RH, 30°C, and 70µm pore size)had the least pH value (6.65). The 96th hour of fermentation produced sample 4 (fermented at 85% RH, 40°C and 70µm pore size) to have the highest pH value (8.16) while sample 1 (fermented at 65%RH, 30°C, and 70µm pore size)had the least pH value (7.16).

This trend towards alkaline medium has been reported by Achi (2005). He reported that pH increase is due to formation of ammonia from amino acids which may consequently encourage growth of spoilage organisms such as *lactobacilli* and *pediococci*. Achi (2005) also reported that increase in pH into the alkaline range may be physiologically important for tolerance and adaptation of fermenting microorganisms in the environment.

Other workers (Barimalaa *et al*., 1989; Achi, 1992; Barber and Achinewhu, 1992) also reported that amino acids produced from protein metabolism are responsible for the gradual pH increase and levelling off towards 7.16-8.16.

The overall results in this study showed that fermentation influenced pH value; tending towards the alkaline region which could be as a result of increased protease activities and the release of ammonia through deaminase activity following utilization of amino acids as carbon and energy source by *Bacillus subtilis* (Sarkar *et al,* 1993;Odunfa and Oyeyiola, 1985).

Tables 4.11 and 4.12 show the response surface analysis of the effect of the fermentation variables on amino nitrogen during the fermentation of melon seeds

for ogiri production. The R^2 (0.9956), adjusted R^2 (0.9877) and predicted R^2 (0.9076) values for the quadratic model were higher than 0.75 thus indicating that amino nitrogen can be predicted and probably optimized using the quadratic model. p – values less than 0.0500 are significant thus the model response was significant ($p < 0.05$). The effects of relative humidity and pore size of wrapping material were also significant. These variables accounted for 99.56% of the amino nitrogen variation during ogiri egusi fermentation. Response surface plots associated with the analysis are shown in Figures 4.1 to 4.3. The effects of relative humidity and pore size of wrapper (AC), and temperature and pore size of wrapper (BC) on amino nitrogen were significant ($p < 0.05$), thus they had significant effect on the amount of amino nitrogen produced during fermentation. The p – values were less than 0.0500.The suggested combination of variables for optimum fermentation of ogiri egusi is 75% relative humidity, temperature of 35°C and 70µm pore size of wrapping material

Since amino nitrogen can be used as an index of fermentation efficiency of proteinaceous food materials (Enujiugha, 2003), it could be used to monitor the progress of fermentation of melon seeds during ogiri egusi production.Usually the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared" is focused on (Table 4.11). Based on this the equation below in terms of coded factors can be used to predict the process as regards amino nitrogen production during fermentation of ogiri egusi.

The final equation in terms of actual factors is as follows:
Amino Nitrogen =-5.43023 + 0.16989 x Relative Humidity + 0.074371 x Temperature - 1.66684E-003 x Pore size - 9.13819E-005 x Relative Humidity xTemperature + 7.16940E-005 xRelative Humidity x Pore size + 5.45288E-004 x Temperature x Pore size - 1.10246E-003 x Relative Humidity^2 - 1.50119E-003 x Temperature^2 - 1.47656E-004 x Pore size^2 4.1

The final equation in terms of coded factors is shown below;

Amino Nitrogen $=+2.51 + 0.063 \times A + 3.021E\text{-}003 \times B + 0.042 \times C - 4.569E\text{-}003 \times A \times B + 0.014 \times A \times C + 0.055 \times B \times C - 0.11 \times A^2 - 0.038 \times B^2 - 0.059 \times C^2$ 4.2

Tables 4.13 and 4.14 show the daily amino nitrogen production during fermentation of melon and fluted pumpkin seeds respectively for ogiri production. From Table 4.13, fermentation got to its peak at 72 hours during the fermentation of fluted pumpkin seeds while fermentation got to its peak at 48 hours (Table 4.14) during the fermentation of melon seeds. Thus, use of starter culture and combination of the variables reduced fermentation time for the two products. It normally take 4-5 days for complete fermentation of the products. Sample 4 (fermented at 85% RH, 40°C and 70µm pore size) for fluted pumpkin had the highest AN value at 72h (2.45mgN/g) while sample 1 fermented at 65% RH, 30°C and 70µm pore size had the least value (1.53mgN/g). However, for melon seeds, sample 12 fermented at 85% RH, 40°C and 90µm had the highest AN value at 48 hour (1.44mgN/g) while sample 4 fermented at 85% RH, 40°C and 70µm had the least AN value (0.44mgN/g).

The response surface plots associated with the other parameters and the model summary statistics are shown in appendix I. From the analysis it was observed that these parameters could not fit into any of the models (linear, quadratic and cubic), thus they could not be predicted using any of the models since their adjusted R^2 and predicted R^2 were generally lower than 0.75.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

- Results obtained from the study have shown that fermenting melon and fluted pumpkin seeds for ogiri production under varying combination of fermentation affected the ogiri production.
- The variations in relative humidity, temperature and pore size increased the rate of fermentation for both melon and fluted pumpkin seeds.
- Free fatty acids, peroxide values, total titratable acidity, amino nitrogen and pH increased as fermentation progressed for both pumpkin and melon seeds in all the samples.
- Response surface analysis showed that amino nitrogen can be predicted using the quadratic model during fermentation, and can be used to monitor progress of fermentation of melon seeds. The combination of the variables; relative humidity and pore size of wrapping material had the greatest effect on amino nitrogen production during melon seeds fermentation. They account for 99.56% of the AN variations during fermentation of melon seeds.
- It was also observed that from the daily AN production that the fermentation got to their peaks at 48h and 72h for melon and fluted pumpkin seeds respectively. Sample 4 (fermented at 85% RH, 40°C and 70µm) for fluted pumpkin and sample 12 (fermented at 85% RH, 40°C and 90µm) for melon seeds had the highest daily AN values at 72h and 48h respectively.

5.2 RECOMMENDATIONS

- Since these variables are not the only conditions affecting the fermentation, it is recommended that further studies be conducted by adding more fermentation variables such as salt concentration to determine their effects on the fermentation.

- Since they are used as flavouring in soups, it is recommended that the amino acid profile is carried out so as to determine the level of glutamate in the samples.
- Based on the equations generated from the study, it is recommended that further fermentation trials be conducted using the equations generated from this study.

5.3 CONTRIBUTION TO KNOWLEDGE

Researches have been conducted on natural fermentation of fluted pumpkin and melon seeds for ogiri production. However, there is no information on the use of starter cultures in such fermentations and prediction of the process at different fermentation variables. The contributions of this work are in these areas.

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APPENDIX I

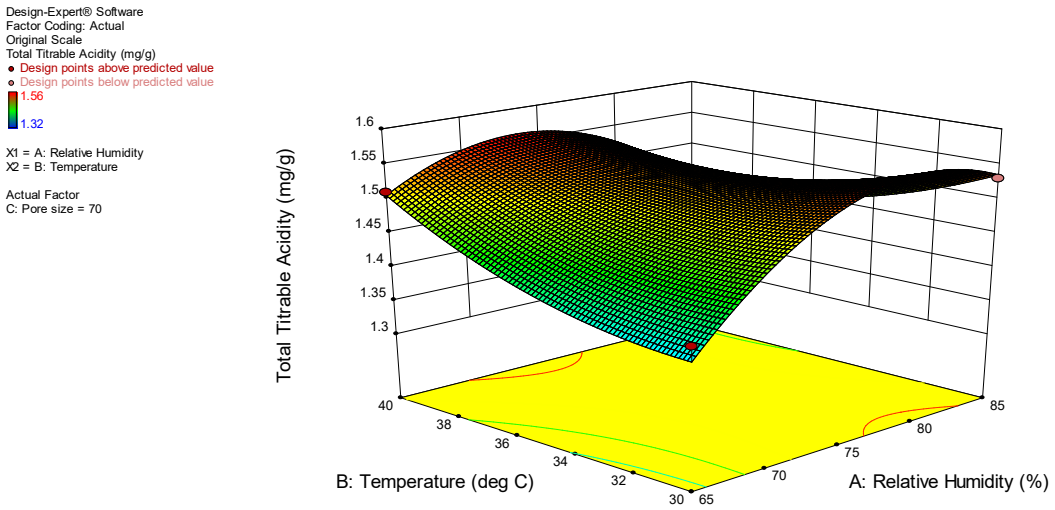

Fig 1: Effect of temperature and relative humidity on TTA during ogiri ugu production

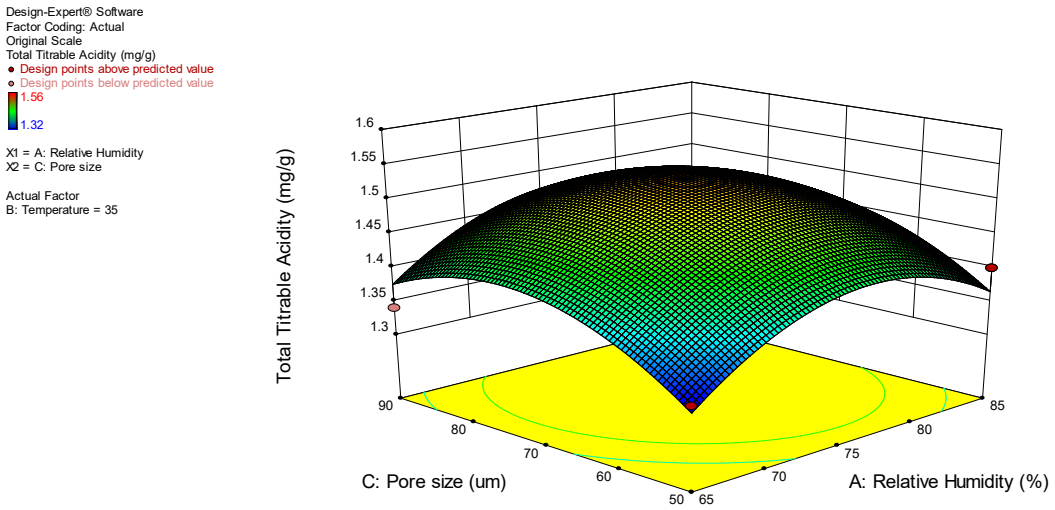

Fig 2: Effect of pore size and relative humidity on TTA during ogiri ugu production

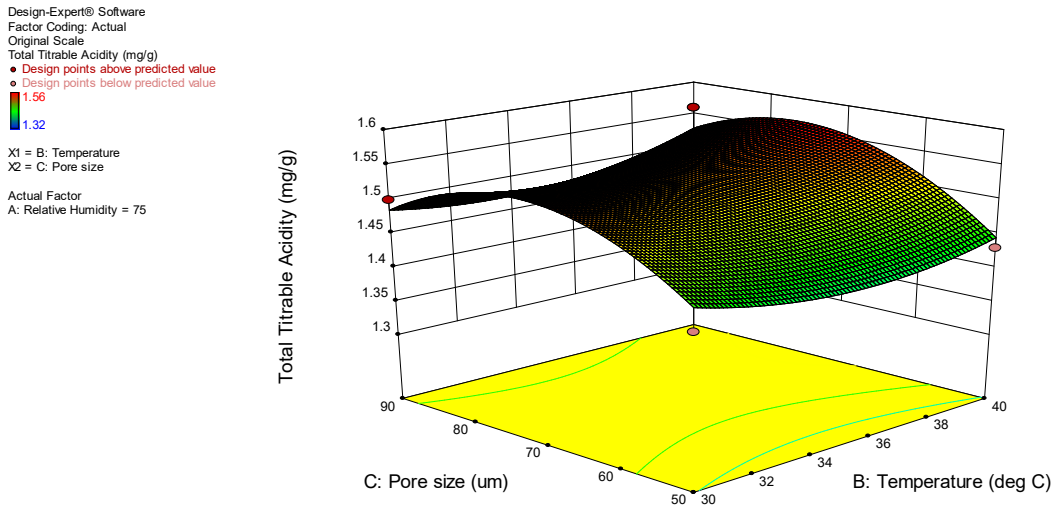

Fig 3: Effect of pore size and temperature on TTA during ogiri ugu production

Table 1: Model summary statistics for TTA

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.033	0.1677	-0.1098	-0.7450	0.020	
2FI	0.035	0.3545	-0.2910	-2.2127	0.038	
Quadratic	0.019	0.9070	0.6281			Suggested
Cubic						

"Model Summary Statistics": Focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared".

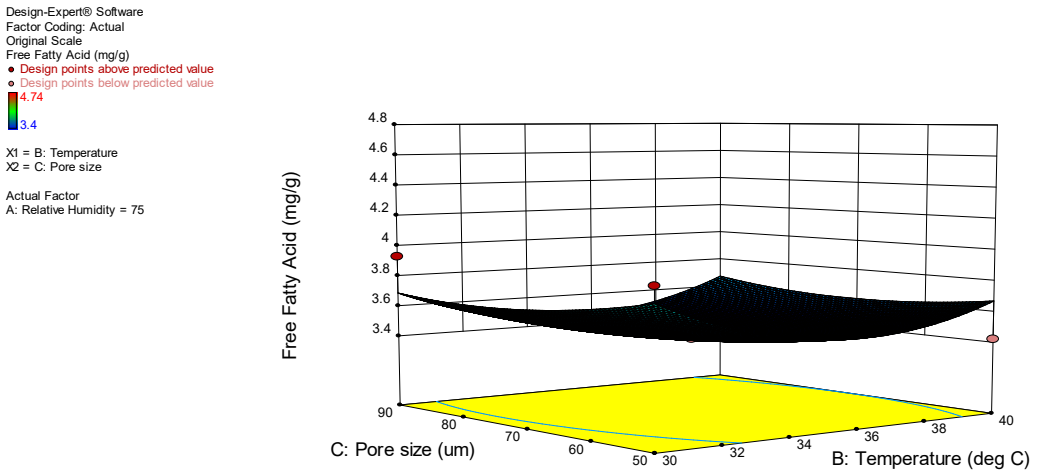

Fig 4: Effect of pore size and temperature on FFA during ogiri ugu production

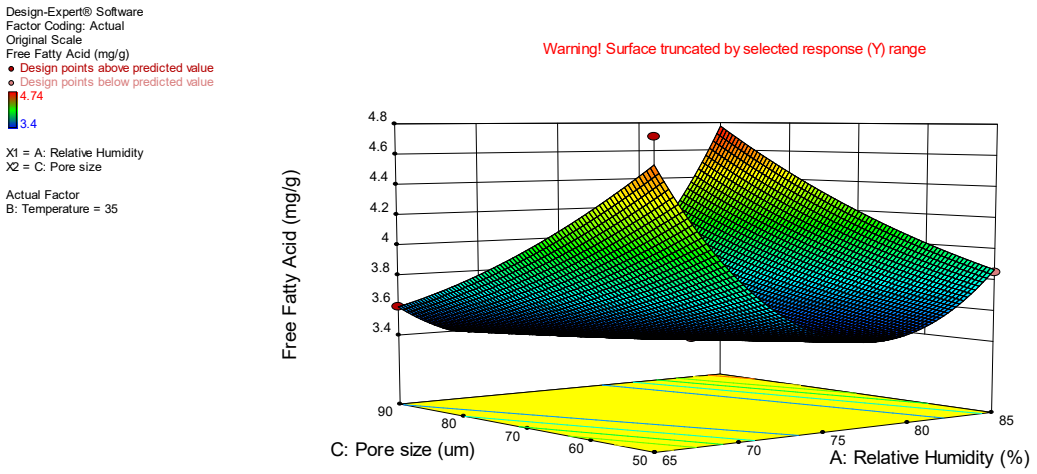

Fig 5: Effect of pore size and relative humidity on FFA during ogiri ugu production

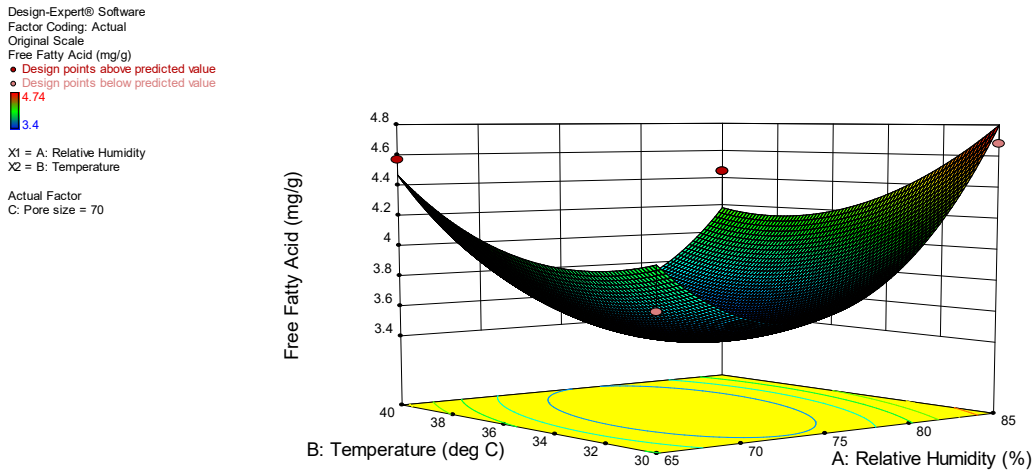

Fig 6: Effect of temperature and relative humidity on FFA during ogiri ugu production

Table 2: Model summary statistics for FFA

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.14	0.0421	-0.2772	-0.9712	0.37	
2FI	0.13	0.4193	-0.1614	-1.6330	0.49	
Quadratic	0.087	0.8773	0.5093			Suggested
Cubic						

"Model Summary Statistics": Focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared".

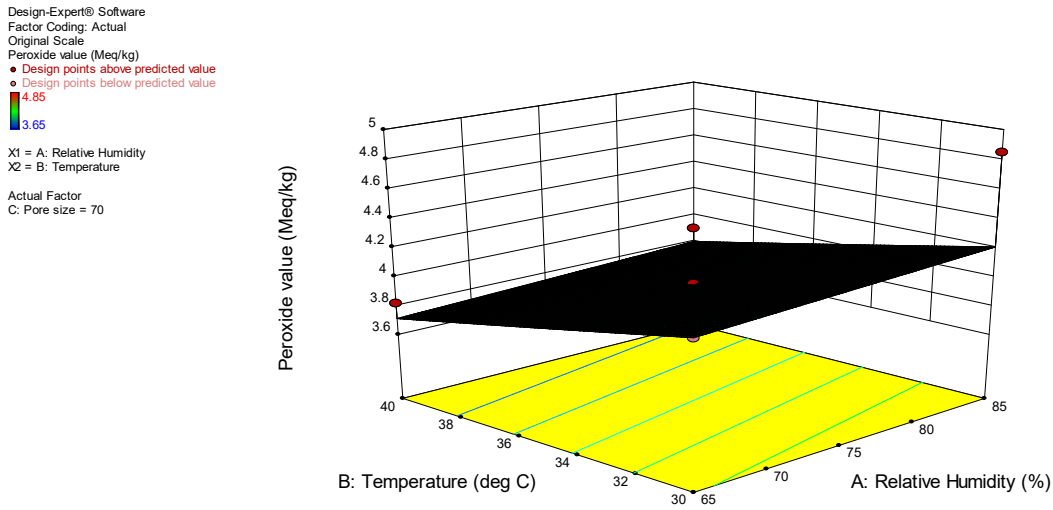

Fig 7: Effect of temperature and relative humidity on PV during ogiri ugu production

Table 3: Model summary statistics for PV

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.070	0.3535	0.1380	-0.4271	0.098	Suggested
2FI	0.079	0.4617	-0.0766	-2.0071	0.21	
Quadratic	0.083	0.7005	-0.1982			
Cubic						

"Model Summary Statistics": Focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared".

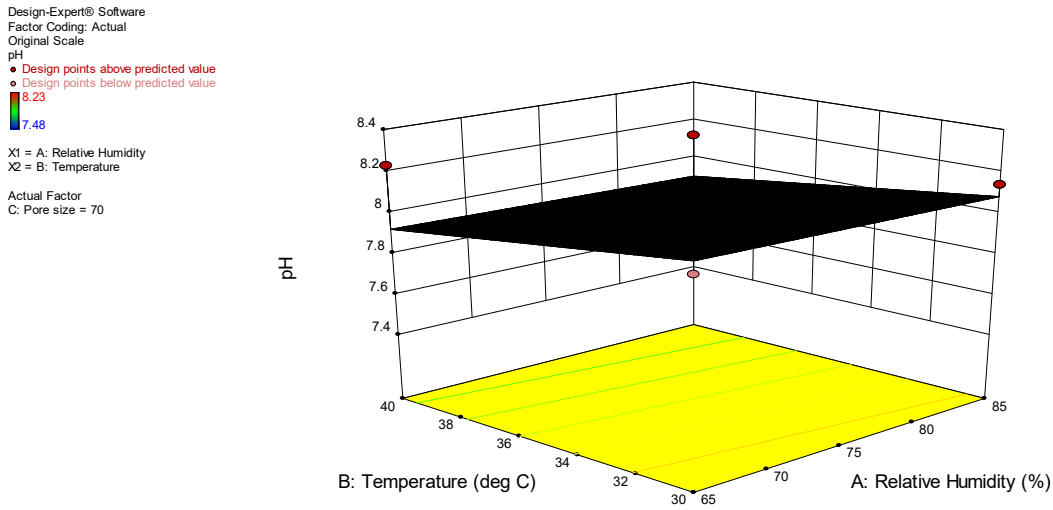

Fig 8: Effect of temperature and relative humidity on pH

Table 4: Model summary statistics for pH

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.035	0.2739	0.0319	-0.5234	0.023	Suggested
2FI	0.038	0.4256	-0.1489	-1.8705	0.044	
Quadratic	0.038	0.7193	-0.1229			
Cubic						

"Model Summary Statistics": Focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared".

Appendix II for fluted pumpkin

Statistical analysis generated on values obtained during fermentation

Free fatty acids (pumpkin)

0 hour

Source of variation	Sum squares	DF	Mean square	F statistic	p
Free fatty acids (pumpkin)	0.253	12	0.021	0.23	0.05
Residual	2.340	26	0.090		
Total	2.593	38			

LSD

Contrast	Difference	95% CI	
1 v 2	-0.090	-1.040	to 0.860
1 v 3	-0.080	-1.030	to 0.870
1 v 4	-0.080	-1.030	to 0.870
1 v 5	-0.080	-1.030	to 0.870
1 v 6	0.040	-0.910	to 0.990
1 v 7	0.110	-0.840	to 1.060
1 v 8	-0.150	-1.100	to 0.800
1 v 9	-0.080	-1.030	to 0.870
1 v 10	-0.050	-1.000	to 0.900
1 v 11	0.040	-0.910	to 0.990
1 v 12	0.080	-0.870	to 1.030
1 v 13	-0.150	-1.100	to 0.800
2 v 3	0.010	-0.940	to 0.960
2 v 4	0.010	-0.940	to 0.960
2 v 5	0.010	-0.940	to 0.960
2 v 6	0.130	-0.820	to 1.080
2 v 7	0.200	-0.750	to 1.150
2 v 8	-0.060	-1.010	to 0.890
2 v 9	0.010	-0.940	to 0.960
2 v 10	0.040	-0.910	to 0.990
2 v 11	0.130	-0.820	to 1.080
2 v 12	0.170	-0.780	to 1.120
2 v 13	-0.060	-1.010	to 0.890
3 v 4	0.000	-0.950	to 0.950
3 v 5	0.000	-0.950	to 0.950
3 v 6	0.120	-0.830	to 1.070
3 v 7	0.190	-0.760	to 1.140
3 v 8	-0.070	-1.020	to 0.880
3 v 9	0.000	-0.950	to 0.950
3 v 10	0.030	-0.920	to 0.980
3 v 11	0.120	-0.830	to 1.070
3 v 12	0.160	-0.790	to 1.110
3 v 13	-0.070	-1.020	to 0.880
4 v 5	0.000	-0.950	to 0.950
4 v 6	0.120	-0.830	to 1.070
4 v 7	0.190	-0.760	to 1.140
4 v 8	-0.070	-1.020	to 0.880
4 v 9	0.000	-0.950	to 0.950
4 v 10	0.030	-0.920	to 0.980
4 v 11	0.120	-0.830	to 1.070
4 v 12	0.160	-0.790	to 1.110
4 v 13	-0.070	-1.020	to 0.880
5 v 6	0.120	-0.830	to 1.070

5 v 7	0.190	-0.760	to 1.140
5 v 8	-0.070	-1.020	to 0.880
5 v 9	0.000	-0.950	to 0.950
5 v 10	0.030	-0.920	to 0.980
5 v 11	0.120	-0.830	to 1.070
5 v 12	0.160	-0.790	to 1.110
5 v 13	-0.070	-1.020	to 0.880
6 v 7	0.070	-0.880	to 1.020
6 v 8	-0.190	-1.140	to 0.760
6 v 9	-0.120	-1.070	to 0.830
6 v 10	-0.090	-1.040	to 0.860
6 v 11	0.000	-0.950	to 0.950
6 v 12	0.040	-0.910	to 0.990
6 v 13	-0.190	-1.140	to 0.760
7 v 8	-0.260	-1.210	to 0.690
7 v 9	-0.190	-1.140	to 0.760
7 v 10	-0.160	-1.110	to 0.790
7 v 11	-0.070	-1.020	to 0.880
7 v 12	-0.030	-0.980	to 0.920
7 v 13	-0.260	-1.210	to 0.690
8 v 9	0.070	-0.880	to 1.020
8 v 10	0.100	-0.850	to 1.050
8 v 11	0.190	-0.760	to 1.140
8 v 12	0.230	-0.720	to 1.180
8 v 13	0.000	-0.950	to 0.950
9 v 10	0.030	-0.920	to 0.980
9 v 11	0.120	-0.830	to 1.070
9 v 12	0.160	-0.790	to 1.110
9 v 13	-0.070	-1.020	to 0.880
10 v 11	0.090	-0.860	to 1.040
10 v 12	0.130	-0.820	to 1.080
10 v 13	-0.100	-1.050	to 0.850
11 v 12	0.040	-0.910	to 0.990
11 v 13	-0.190	-1.140	to 0.760
12 v 13	-0.230	-1.180	to 0.720

24 hour

Source of variation	Sum squares	DF	Mean square	F statistic	p
Free fatty acids (pumpkin)	3.863	12	0.322	3.58	0.05
Residual	2.340	26	0.090		
Total	6.203	38			

LSD

Contrast	Difference	95% CI	
1 v 2	-0.120	-1.070	to 0.830
1 v 3	-0.060	-1.010	to 0.890
1 v 4	-0.090	-1.040	to 0.860
1 v 5	-0.100	-1.050	to 0.850
1 v 6	0.020	-0.930	to 0.970
1 v 7	0.090	-0.860	to 1.040
1 v 8	-0.160	-1.110	to 0.790
1 v 9	-0.090	-1.040	to 0.860
1 v 10	0.300	-0.650	to 1.250
1 v 11	-0.530	-1.480	to 0.420
1 v 12	0.100	-0.850	to 1.050
1 v 13	-1.020	-1.970	to -0.070
2 v 3	0.060	-0.890	to 1.010
2 v 4	0.030	-0.920	to 0.980
2 v 5	0.020	-0.930	to 0.970
2 v 6	0.140	-0.810	to 1.090
2 v 7	0.210	-0.740	to 1.160
2 v 8	-0.040	-0.990	to 0.910
2 v 9	0.030	-0.920	to 0.980
2 v 10	0.420	-0.530	to 1.370
2 v 11	-0.410	-1.360	to 0.540
2 v 12	0.220	-0.730	to 1.170
2 v 13	-0.900	-1.850	to 0.050
3 v 4	-0.030	-0.980	to 0.920
3 v 5	-0.040	-0.990	to 0.910
3 v 6	0.080	-0.870	to 1.030
3 v 7	0.150	-0.800	to 1.100
3 v 8	-0.100	-1.050	to 0.850
3 v 9	-0.030	-0.980	to 0.920
3 v 10	0.360	-0.590	to 1.310
3 v 11	-0.470	-1.420	to 0.480
3 v 12	0.160	-0.790	to 1.110
3 v 13	-0.960	-1.910	to -0.010
4 v 5	-0.010	-0.960	to 0.940
4 v 6	0.110	-0.840	to 1.060
4 v 7	0.180	-0.770	to 1.130
4 v 8	-0.070	-1.020	to 0.880
4 v 9	0.000	-0.950	to 0.950
4 v 10	0.390	-0.560	to 1.340
4 v 11	-0.440	-1.390	to 0.510
4 v 12	0.190	-0.760	to 1.140
4 v 13	-0.930	-1.880	to 0.020
5 v 6	0.120	-0.830	to 1.070
5 v 7	0.190	-0.760	to 1.140
5 v 8	-0.060	-1.010	to 0.890
5 v 9	0.010	-0.940	to 0.960
5 v 10	0.400	-0.550	to 1.350
5 v 11	-0.430	-1.380	to 0.520

5 v 12	0.200	-0.750	to 1.150
5 v 13	-0.920	-1.870	to 0.030
6 v 7	0.070	-0.880	to 1.020
6 v 8	-0.180	-1.130	to 0.770
6 v 9	-0.110	-1.060	to 0.840
6 v 10	0.280	-0.670	to 1.230
6 v 11	-0.550	-1.500	to 0.400
6 v 12	0.080	-0.870	to 1.030
6 v 13	-1.040	-1.990	to -0.090
7 v 8	-0.250	-1.200	to 0.700
7 v 9	-0.180	-1.130	to 0.770
7 v 10	0.210	-0.740	to 1.160
7 v 11	-0.620	-1.570	to 0.330
7 v 12	0.010	-0.940	to 0.960
7 v 13	-1.110	-2.060	to -0.160
8 v 9	0.070	-0.880	to 1.020
8 v 10	0.460	-0.490	to 1.410
8 v 11	-0.370	-1.320	to 0.580
8 v 12	0.260	-0.690	to 1.210
8 v 13	-0.860	-1.810	to 0.090
9 v 10	0.390	-0.560	to 1.340
9 v 11	-0.440	-1.390	to 0.510
9 v 12	0.190	-0.760	to 1.140
9 v 13	-0.930	-1.880	to 0.020
10 v 11	-0.830	-1.780	to 0.120
10 v 12	-0.200	-1.150	to 0.750
10 v 13	-1.320	-2.270	to -0.370
11 v 12	0.630	-0.320	to 1.580
11 v 13	-0.490	-1.440	to 0.460
12 v 13	-1.120	-2.070	to -0.170

48 hour

Source of variation	Sum squares	DF	Mean square	F statistic	p
Free fatty acids (pumpkin)	3.030	12	0.253	2.81	0.05
Residual	2.340	26	0.090		
Total	5.370	38			

LSD

Contrast	Difference	95% CI	
1 v 2	-0.140	-1.090	to 0.810
1 v 3	-0.120	-1.070	to 0.830
1 v 4	-0.160	-1.110	to 0.790
1 v 5	-0.120	-1.070	to 0.830
1 v 6	-0.010	-0.960	to 0.940
1 v 7	0.100	-0.850	to 1.050
1 v 8	-0.230	-1.180	to 0.720
1 v 9	-0.755	-1.705	to 0.195
1 v 10	0.290	-0.660	to 1.240
1 v 11	-0.495	-1.445	to 0.455
1 v 12	0.290	-0.660	to 1.240
1 v 13	0.100	-0.850	to 1.050
2 v 3	0.020	-0.930	to 0.970
2 v 4	-0.020	-0.970	to 0.930
2 v 5	0.020	-0.930	to 0.970
2 v 6	0.130	-0.820	to 1.080
2 v 7	0.240	-0.710	to 1.190
2 v 8	-0.090	-1.040	to 0.860
2 v 9	-0.615	-1.565	to 0.335
2 v 10	0.430	-0.520	to 1.380
2 v 11	-0.355	-1.305	to 0.595
2 v 12	0.430	-0.520	to 1.380
2 v 13	0.240	-0.710	to 1.190
3 v 4	-0.040	-0.990	to 0.910
3 v 5	0.000	-0.950	to 0.950
3 v 6	0.110	-0.840	to 1.060
3 v 7	0.220	-0.730	to 1.170
3 v 8	-0.110	-1.060	to 0.840
3 v 9	-0.635	-1.585	to 0.315
3 v 10	0.410	-0.540	to 1.360
3 v 11	-0.375	-1.325	to 0.575
3 v 12	0.410	-0.540	to 1.360
3 v 13	0.220	-0.730	to 1.170
4 v 5	0.040	-0.910	to 0.990
4 v 6	0.150	-0.800	to 1.100
4 v 7	0.260	-0.690	to 1.210
4 v 8	-0.070	-1.020	to 0.880
4 v 9	-0.595	-1.545	to 0.355
4 v 10	0.450	-0.500	to 1.400
4 v 11	-0.335	-1.285	to 0.615
4 v 12	0.450	-0.500	to 1.400
4 v 13	0.260	-0.690	to 1.210
5 v 6	0.110	-0.840	to 1.060
5 v 7	0.220	-0.730	to 1.170
5 v 8	-0.110	-1.060	to 0.840
5 v 9	-0.635	-1.585	to 0.315
5 v 10	0.410	-0.540	to 1.360
5 v 11	-0.375	-1.325	to 0.575

5 v 12	0.410	-0.540	to 1.360
5 v 13	0.220	-0.730	to 1.170
6 v 7	0.110	-0.840	to 1.060
6 v 8	-0.220	-1.170	to 0.730
6 v 9	-0.745	-1.695	to 0.205
6 v 10	0.300	-0.650	to 1.250
6 v 11	-0.485	-1.435	to 0.465
6 v 12	0.300	-0.650	to 1.250
6 v 13	0.110	-0.840	to 1.060
7 v 8	-0.330	-1.280	to 0.620
7 v 9	-0.855	-1.805	to 0.095
7 v 10	0.190	-0.760	to 1.140
7 v 11	-0.595	-1.545	to 0.355
7 v 12	0.190	-0.760	to 1.140
7 v 13	0.000	-0.950	to 0.950
8 v 9	-0.525	-1.475	to 0.425
8 v 10	0.520	-0.430	to 1.470
8 v 11	-0.265	-1.215	to 0.685
8 v 12	0.520	-0.430	to 1.470
8 v 13	0.330	-0.620	to 1.280
9 v 10	1.045	0.095	to 1.995
9 v 11	0.260	-0.690	to 1.210
9 v 12	1.045	0.095	to 1.995
9 v 13	0.855	-0.095	to 1.805
10 v 11	-0.785	-1.735	to 0.165
10 v 12	0.000	-0.950	to 0.950
10 v 13	-0.190	-1.140	to 0.760
11 v 12	0.785	-0.165	to 1.735
11 v 13	0.595	-0.355	to 1.545
12 v 13	-0.190	-1.140	to 0.760

72 hour

Source of variation	Sum squares	DF	Mean square	F statistic	p
Free fatty acids (pumpkin)	7.601	12	0.633	7.04	0.05
Residual	2.340	26	0.090		
Total	9.941	38			

LSD

Contrast	Difference	95% CI	
1 v 2	-0.940	-1.890	to 0.010
1 v 3	-0.810	-1.760	to 0.140
1 v 4	-0.820	-1.770	to 0.130
1 v 5	-1.000	-1.950	to -0.050
1 v 6	0.080	-0.870	to 1.030
1 v 7	0.060	-0.890	to 1.010
1 v 8	-0.880	-1.830	to 0.070
1 v 9	-0.610	-1.560	to 0.340
1 v 10	-0.180	-1.130	to 0.770
1 v 11	-0.570	-1.520	to 0.380
1 v 12	0.180	-0.770	to 1.130
1 v 13	0.100	-0.850	to 1.050
2 v 3	0.130	-0.820	to 1.080
2 v 4	0.120	-0.830	to 1.070
2 v 5	-0.060	-1.010	to 0.890
2 v 6	1.020	0.070	to 1.970
2 v 7	1.000	0.050	to 1.950
2 v 8	0.060	-0.890	to 1.010
2 v 9	0.330	-0.620	to 1.280
2 v 10	0.760	-0.190	to 1.710
2 v 11	0.370	-0.580	to 1.320
2 v 12	1.120	0.170	to 2.070
2 v 13	1.040	0.090	to 1.990
3 v 4	-0.010	-0.960	to 0.940
3 v 5	-0.190	-1.140	to 0.760
3 v 6	0.890	-0.060	to 1.840
3 v 7	0.870	-0.080	to 1.820
3 v 8	-0.070	-1.020	to 0.880
3 v 9	0.200	-0.750	to 1.150
3 v 10	0.630	-0.320	to 1.580
3 v 11	0.240	-0.710	to 1.190
3 v 12	0.990	0.040	to 1.940
3 v 13	0.910	-0.040	to 1.860
4 v 5	-0.180	-1.130	to 0.770
4 v 6	0.900	-0.050	to 1.850
4 v 7	0.880	-0.070	to 1.830
4 v 8	-0.060	-1.010	to 0.890
4 v 9	0.210	-0.740	to 1.160
4 v 10	0.640	-0.310	to 1.590
4 v 11	0.250	-0.700	to 1.200
4 v 12	1.000	0.050	to 1.950
4 v 13	0.920	-0.030	to 1.870
5 v 6	1.080	0.130	to 2.030
5 v 7	1.060	0.110	to 2.010
5 v 8	0.120	-0.830	to 1.070
5 v 9	0.390	-0.560	to 1.340
5 v 10	0.820	-0.130	to 1.770
5 v 11	0.430	-0.520	to 1.380

5 v 12	1.180	0.230	to 2.130
5 v 13	1.100	0.150	to 2.050
6 v 7	-0.020	-0.970	to 0.930
6 v 8	-0.960	-1.910	to -0.010
6 v 9	-0.690	-1.640	to 0.260
6 v 10	-0.260	-1.210	to 0.690
6 v 11	-0.650	-1.600	to 0.300
6 v 12	0.100	-0.850	to 1.050
6 v 13	0.020	-0.930	to 0.970
7 v 8	-0.940	-1.890	to 0.010
7 v 9	-0.670	-1.620	to 0.280
7 v 10	-0.240	-1.190	to 0.710
7 v 11	-0.630	-1.580	to 0.320
7 v 12	0.120	-0.830	to 1.070
7 v 13	0.040	-0.910	to 0.990
8 v 9	0.270	-0.680	to 1.220
8 v 10	0.700	-0.250	to 1.650
8 v 11	0.310	-0.640	to 1.260
8 v 12	1.060	0.110	to 2.010
8 v 13	0.980	0.030	to 1.930
9 v 10	0.430	-0.520	to 1.380
9 v 11	0.040	-0.910	to 0.990
9 v 12	0.790	-0.160	to 1.740
9 v 13	0.710	-0.240	to 1.660
10 v 11	-0.390	-1.340	to 0.560
10 v 12	0.360	-0.590	to 1.310
10 v 13	0.280	-0.670	to 1.230
11 v 12	0.750	-0.200	to 1.700
11 v 13	0.670	-0.280	to 1.620
12 v 13	-0.080	-1.030	to 0.870

96 hour

Source of variation	Sum squares	DF	Mean square	F statistic	p
Free fatty acids (pumpkin)	9.122	12	0.760	8.45	0.05
Residual	2.340	26	0.090		
Total	11.462	38			

LSD

Contrast	Difference	95% CI	
1 v 2	-0.930	-1.880	to 0.020
1 v 3	-0.820	-1.770	to 0.130
1 v 4	-0.700	-1.650	to 0.250
1 v 5	-0.990	-1.940	to -0.040
1 v 6	-0.100	-1.050	to 0.850
1 v 7	0.160	-0.790	to 1.110
1 v 8	-0.850	-1.800	to 0.100
1 v 9	-0.150	-1.100	to 0.800
1 v 10	0.330	-0.620	to 1.280
1 v 11	-0.180	-1.130	to 0.770
1 v 12	0.190	-0.760	to 1.140
1 v 13	0.350	-0.600	to 1.300
2 v 3	0.110	-0.840	to 1.060
2 v 4	0.230	-0.720	to 1.180
2 v 5	-0.060	-1.010	to 0.890
2 v 6	0.830	-0.120	to 1.780
2 v 7	1.090	0.140	to 2.040
2 v 8	0.080	-0.870	to 1.030
2 v 9	0.780	-0.170	to 1.730
2 v 10	1.260	0.310	to 2.210
2 v 11	0.750	-0.200	to 1.700
2 v 12	1.120	0.170	to 2.070
2 v 13	1.280	0.330	to 2.230
3 v 4	0.120	-0.830	to 1.070
3 v 5	-0.170	-1.120	to 0.780
3 v 6	0.720	-0.230	to 1.670
3 v 7	0.980	0.030	to 1.930
3 v 8	-0.030	-0.980	to 0.920
3 v 9	0.670	-0.280	to 1.620
3 v 10	1.150	0.200	to 2.100
3 v 11	0.640	-0.310	to 1.590
3 v 12	1.010	0.060	to 1.960
3 v 13	1.170	0.220	to 2.120
4 v 5	-0.290	-1.240	to 0.660
4 v 6	0.600	-0.350	to 1.550
4 v 7	0.860	-0.090	to 1.810
4 v 8	-0.150	-1.100	to 0.800
4 v 9	0.550	-0.400	to 1.500
4 v 10	1.030	0.080	to 1.980
4 v 11	0.520	-0.430	to 1.470
4 v 12	0.890	-0.060	to 1.840
4 v 13	1.050	0.100	to 2.000
5 v 6	0.890	-0.060	to 1.840
5 v 7	1.150	0.200	to 2.100
5 v 8	0.140	-0.810	to 1.090
5 v 9	0.840	-0.110	to 1.790
5 v 10	1.320	0.370	to 2.270
5 v 11	0.810	-0.140	to 1.760
5 v 12	1.180	0.230	to 2.130

5 v 13	1.340	0.390	to 2.290
6 v 7	0.260	-0.690	to 1.210
6 v 8	-0.750	-1.700	to 0.200
6 v 9	-0.050	-1.000	to 0.900
6 v 10	0.430	-0.520	to 1.380
6 v 11	-0.080	-1.030	to 0.870
6 v 12	0.290	-0.660	to 1.240
6 v 13	0.450	-0.500	to 1.400
7 v 8	-1.010	-1.960	to -0.060
7 v 9	-0.310	-1.260	to 0.640
7 v 10	0.170	-0.780	to 1.120
7 v 11	-0.340	-1.290	to 0.610
7 v 12	0.030	-0.920	to 0.980
7 v 13	0.190	-0.760	to 1.140
8 v 9	0.700	-0.250	to 1.650
8 v 10	1.180	0.230	to 2.130
8 v 11	0.670	-0.280	to 1.620
8 v 12	1.040	0.090	to 1.990
8 v 13	1.200	0.250	to 2.150
9 v 10	0.480	-0.470	to 1.430
9 v 11	-0.030	-0.980	to 0.920
9 v 12	0.340	-0.610	to 1.290
9 v 13	0.500	-0.450	to 1.450
10 v 11	-0.510	-1.460	to 0.440
10 v 12	-0.140	-1.090	to 0.810
10 v 13	0.020	-0.930	to 0.970
11 v 12	0.370	-0.580	to 1.320
11 v 13	0.530	-0.420	to 1.480
12 v 13	0.160	-0.790	to 1.110

Anova tables for Free fatty Acids (Pumpkin)

SAMPLE 1

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	7.4652	4	1.8663	20.74	0.05
Residual	0.9000	10	0.0900		
Total	8.3652	14			

LSD

Contrast	Difference	95% CI
0 hour v 24 hour	-0.5000	-1.3773 to 0.3773
0 hour v 48 hour	-1.1800	-2.0573 to -0.3027
0 hour v 72 hour	-1.4800	-2.3573 to -0.6027
0 hour v 96 hour	-1.9900	-2.8673 to -1.1127
24 hour v 48 hour	-0.6800	-1.5573 to 0.1973
24 hour v 72 hour	-0.9800	-1.8573 to -0.1027
24 hour v 96hour	-1.4900	-2.3673 to -0.6127
48 hour v 72 hour	-0.3000	-1.1773 to 0.5773
48 hour v 96 hour	-0.8100	-1.6873 to 0.0673
72 hour v 96 hour	-0.5100	-1.3873 to 0.3673

SAMPLE 2

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	16.9630	4	4.2407	47.12	0.05
Residual	0.9000	10	0.0900		
Total	17.8630	14			

LSD

Contrast	Difference	95% CI
0 hour v 24 hour	-0.5300	-1.4073 to 0.3473
0 hour v 48 hour	-1.2300	-2.1073 to -0.3527
0 hour v 72 hour	-2.3300	-3.2073 to -1.4527
0 hour v 96 hour	-2.8300	-3.7073 to -1.9527
24 hour v 48 hour	-0.7000	-1.5773 to 0.1773
24 hour v 72 hour	-1.8000	-2.6773 to -0.9227
24 hour v 96hour	-2.3000	-3.1773 to -1.4227
48 hour v 72 hour	-1.1000	-1.9773 to -0.2227
48 hour v 96 hour	-1.6000	-2.4773 to -0.7227
72 hour v 96 hour	-0.5000	- to

	1.3773	0.3773

SAMPLE 3

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	15.7136	4	3.9284	43.65	0.05
Residual	0.9000	10	0.0900		
Total	16.6136	14			

LSD

Contrast	Difference	95% CI
0 hour v 24 hour	-0.4800	-1.3573 to 0.3973
0 hour v 48 hour	-1.2200	-2.0973 to -0.3427
0 hour v 72 hour	-2.2100	-3.0873 to -1.3327
0 hour v 96 hour	-2.7300	-3.6073 to -1.8527
24 hour v 48 hour	-0.7400	-1.6173 to 0.1373
24 hour v 72 hour	-1.7300	-2.6073 to -0.8527
24 hour v 96hour	-2.2500	-3.1273 to -1.3727
48 hour v 72 hour	-0.9900	-1.8673 to -0.1127
48 hour v 96 hour	-1.5100	-2.3873 to -0.6327
72 hour v 96 hour	-0.5200	-1.3973 to 0.3573

SAMPLE 4

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	14.6286	4	3.6572	40.64	0.05
Residual	0.9000	10	0.0900		
Total	15.5286	14			

LSD

Contrast	Difference	95% CI
0 hour v 24 hour	-0.5100	-1.3873 to 0.3673
0 hour v 48 hour	-1.2600	-2.1373 to -0.3827
0 hour v 72 hour	-2.2200	-3.0973 to -1.3427
0 hour v 96 hour	-2.6100	-3.4873 to -1.7327
24 hour v 48 hour	-0.7500	-1.6273 to 0.1273
24 hour v 72 hour	-1.7100	-2.5873 to -0.8327
24 hour v 96hour	-2.1000	-2.9773 to -1.2227
48 hour v 72 hour	-0.9600	-1.8373 to -0.0827
48 hour v 96 hour	-1.3500	-2.2273 to -0.4727
72 hour v 96 hour	-0.3900	-1.2673 to 0.4873

SAMPLE 5

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	18.0494	4	4.5124	50.14	0.05
Residual	0.9000	10	0.0900		
Total	18.9494	14			

LSD

Contrast	Difference	95% CI
0 hour v 24 hour	-0.5200	-1.3973 to 0.3573
0 hour v 48 hour	-1.2200	-2.0973 to -0.3427
0 hour v 72 hour	-2.4000	-3.2773 to -1.5227
0 hour v 96 hour	-2.9000	-3.7773 to -2.0227
24 hour v 48 hour	-0.7000	-1.5773 to 0.1773
24 hour v 72 hour	-1.8800	-2.7573 to -1.0027
24 hour v 96hour	-2.3800	-3.2573 to -1.5027
48 hour v 72 hour	-1.1800	-2.0573 to -0.3027
48 hour v 96 hour	-1.6800	-2.5573 to -0.8027
72 hour v 96 hour	-0.5000	-1.3773 to 0.3773

SAMPLE 6

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	8.2000	4	2.0500	22.78	0.05
Residual	0.9000	10	0.0900		
Total	9.1000	14			

LSD

Contrast	Difference	95% CI
0 hour v 24 hour	-0.5200	-1.3973 to 0.3573
0 hour v 48 hour	-1.2300	-2.1073 to -0.3527
0 hour v 72 hour	-1.4400	-2.3173 to -0.5627
0 hour v 96 hour	-2.1300	-3.0073 to -1.2527
24 hour v 48 hour	-0.7100	-1.5873 to 0.1673
24 hour v 72 hour	-0.9200	-1.7973 to -0.0427
24 hour v 96hour	-1.6100	-2.4873 to -0.7327
48 hour v 72 hour	-0.2100	-1.0873 to 0.6673
48 hour v 96 hour	-0.9000	-1.7773 to -0.0227
72 hour v 96 hour	-0.6900	-1.5673 to 0.1873

SAMPLE 7

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	7.2736	4	1.8184	20.20	0.05
Residual	0.9000	10	0.0900		
Total	8.1736	14			

LSD

Contrast	Difference	95% CI
0 hour v 24 hour	-0.5200	-1.3973 to 0.3573
0 hour v 48 hour	-1.1900	-2.0673 to -0.3127
0 hour v 72 hour	-1.5300	-2.4073 to -0.6527
0 hour v 96 hour	-1.9400	-2.8173 to -1.0627
24 hour v 48 hour	-0.6700	-1.5473 to 0.2073
24 hour v 72 hour	-1.0100	-1.8873 to -0.1327
24 hour v 96hour	-1.4200	-2.2973 to -0.5427
48 hour v 72 hour	-0.3400	-1.2173 to 0.5373
48 hour v 96 hour	-0.7500	-1.6273 to 0.1273
72 hour v 96 hour	-0.4100	-1.2873 to 0.4673

SAMPLE 8

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	15.2104	4	3.8026	42.25	0.05
Residual	0.9000	10	0.0900		
Total	16.1104	14			

LSD

Contrast	Difference	95% CI
0 hour v 24 hour	-0.5100	-1.3873 to 0.3673
0 hour v 48 hour	-1.2600	-2.1373 to -0.3827
0 hour v 72 hour	-2.2100	-3.0873 to -1.3327
0 hour v 96 hour	-2.6900	-3.5673 to -1.8127
24 hour v 48 hour	-0.7500	-1.6273 to 0.1273
24 hour v 72 hour	-1.7000	-2.5773 to -0.8227
24 hour v 96hour	-2.1800	-3.0573 to -1.3027
48 hour v 72 hour	-0.9500	-1.8273 to -0.0727
48 hour v 96 hour	-1.4300	-2.3073 to -0.5527
72 hour v 96 hour	-0.4800	-1.3573 to 0.3973

SAMPLE 9

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	11.1089	4	2.7772	30.86	0.05
Residual	0.9000	10	0.0900		
Total	12.0089	14			

LSD

Contrast	Difference	95% CI
0 hour v 24 hour	-0.5100	-1.3873 to 0.3673
0 hour v 48 hour	-1.8550	-2.7323 to -0.9777
0 hour v 72 hour	-2.0100	-2.8873 to -1.1327
0 hour v 96 hour	-2.0600	-2.9373 to -1.1827
24 hour v 48 hour	-1.3450	-2.2223 to -0.4677
24 hour v 72 hour	-1.5000	-2.3773 to -0.6227
24 hour v 96hour	-1.5500	-2.4273 to -0.6727
48 hour v 72 hour	-0.1550	-1.0323 to 0.7223
48 hour v 96 hour	-0.2050	-1.0823 to 0.6723
72 hour v 96 hour	-0.0500	-0.9273 to 0.8273

SAMPLE 10

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	7.1024	4	1.7756	19.73	<0.0001
Residual	0.9000	10	0.0900		
Total	8.0024	14			

LSD

Contrast	Difference	95% CI
0 hour v 24 hour	-0.1500	-1.0273 to 0.7273
0 hour v 48 hour	-0.8400	-1.7173 to 0.0373
0 hour v 72 hour	-1.6100	-2.4873 to -0.7327
0 hour v 96 hour	-1.6100	-2.4873 to -0.7327
24 hour v 48 hour	-0.6900	-1.5673 to 0.1873
24 hour v 72 hour	-1.4600	-2.3373 to -0.5827
24 hour v 96hour	-1.4600	-2.3373 to -0.5827
48 hour v 72 hour	-0.7700	-1.6473 to 0.1073
48 hour v 96 hour	-0.7700	-1.6473 to 0.1073
72 hour v 96 hour	0.0000	-0.8773 to 0.8773

SAMPLE 11

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	9.8966	4	2.4742	27.49	<0.0001
Residual	0.9000	10	0.0900		
Total	10.7966	14			

LSD

Contrast	Difference	95% CI
0 hour v 24 hour	-1.0700	-1.9473 to -0.1927
0 hour v 48 hour	-1.7150	-2.5923 to -0.8377
0 hour v 72 hour	-2.0900	-2.9673 to -1.2127
0 hour v 96 hour	-2.2100	-3.0873 to -1.3327
24 hour v 48 hour	-0.6450	-1.5223 to 0.2323
24 hour v 72 hour	-1.0200	-1.8973 to -0.1427
24 hour v 96hour	-1.1400	-2.0173 to -0.2627
48 hour v 72 hour	-0.3750	-1.2523 to 0.5023
48 hour v 96 hour	-0.4950	-1.3723 to 0.3823
72 hour v 96 hour	-0.1200	-0.9973 to 0.7573

SAMPLE 12

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	6.5198	4	1.6300	18.11	0.0001
Residual	0.9000	10	0.0900		
Total	7.4198	14			

LSD

Contrast	Difference	95% CI
0 hour v 24 hour	-0.4800	-1.3573 to 0.3973
0 hour v 48 hour	-0.9700	-1.8473 to -0.0927
0 hour v 72 hour	-1.3800	-2.2573 to -0.5027
0 hour v 96 hour	-1.8800	-2.7573 to -1.0027
24 hour v 48 hour	-0.4900	-1.3673 to 0.3873
24 hour v 72 hour	-0.9000	-1.7773 to -0.0227
24 hour v 96hour	-1.4000	-2.2773 to -0.5227
48 hour v 72 hour	-0.4100	-1.2873 to 0.4673
48 hour v 96 hour	-0.9100	-1.7873 to -0.0327
72 hour v 96 hour	-0.5000	-1.3773 to 0.3773

SAMPLE 13

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	4.3042	4	1.0760	11.96	0.05
Residual	0.9000	10	0.0900		
Total	5.2042	14			

LSD

Contrast	Difference	95% CI
0 hour v 24 hour	-1.3700	-2.2473 to -0.4927
0 hour v 48 hour	-0.9300	-1.8073 to -0.0527
0 hour v 72 hour	-1.2300	-2.1073 to -0.3527
0 hour v 96 hour	-1.4900	-2.3673 to -0.6127
24 hour v 48 hour	0.4400	-0.4373 to 1.3173
24 hour v 72 hour	0.1400	-0.7373 to 1.0173
24 hour v 96hour	-0.1200	-0.9973 to 0.7573
48 hour v 72 hour	-0.3000	-1.1773 to 0.5773
48 hour v 96 hour	-0.5600	-1.4373 to 0.3173
72 hour v 96 hour	-0.2600	-1.1373 to 0.6173

Total Titrable Acidity (pumpkin)

0 hour

Source of variation	Sum squares	DF	Mean square	F statistic	p
Total Titrable Acidity (pumpkin)	0.2660	12	0.0222	0.25	0.05
Residual	2.3400	26	0.0900		
Total	2.6060	38			

LSD

Contrast	Difference	95% CI
1 v 2	-0.1180	-1.0681 to 0.8321
1 v 3	0.0000	-0.9501 to 0.9501
1 v 4	0.0100	-0.9401 to 0.9601
1 v 5	0.0500	-0.9001 to 1.0001
1 v 6	0.0400	-0.9101 to 0.9901
1 v 7	-0.0100	-0.9601 to 0.9401
1 v 8	0.0200	-0.9301 to 0.9701
1 v 9	-0.0600	-1.0101 to 0.8901
1 v 10	0.1200	-0.8301 to 1.0701
1 v 11	0.0500	-0.9001 to 1.0001
1 v 12	0.0400	-0.9101 to 0.9901
1 v 13	-0.2200	-1.1701 to 0.7301
2 v 3	0.1180	-0.8321 to 1.0681
2 v 4	0.1280	-0.8221 to 1.0781
2 v 5	0.1680	-0.7821 to 1.1181
2 v 6	0.1580	-0.7921 to 1.1081
2 v 7	0.1080	-0.8421 to 1.0581
2 v 8	0.1380	-0.8121 to 1.0881
2 v 9	0.0580	-0.8921 to 1.0081
2 v 10	0.2380	-0.7121 to 1.1881
2 v 11	0.1680	-0.7821 to 1.1181

2 v 12	0.1580	-0.7921	to 1.1081
2 v 13	-0.1020	-1.0521	to 0.8481
3 v 4	0.0100	-0.9401	to 0.9601
3 v 5	0.0500	-0.9001	to 1.0001
3 v 6	0.0400	-0.9101	to 0.9901
3 v 7	-0.0100	-0.9601	to 0.9401
3 v 8	0.0200	-0.9301	to 0.9701
3 v 9	-0.0600	-1.0101	to 0.8901
3 v 10	0.1200	-0.8301	to 1.0701
3 v 11	0.0500	-0.9001	to 1.0001
3 v 12	0.0400	-0.9101	to 0.9901
3 v 13	-0.2200	-1.1701	to 0.7301
4 v 5	0.0400	-0.9101	to 0.9901
4 v 6	0.0300	-0.9201	to 0.9801
4 v 7	-0.0200	-0.9701	to 0.9301
4 v 8	0.0100	-0.9401	to 0.9601
4 v 9	-0.0700	-1.0201	to 0.8801
4 v 10	0.1100	-0.8401	to 1.0601
4 v 11	0.0400	-0.9101	to 0.9901
4 v 12	0.0300	-0.9201	to 0.9801
4 v 13	-0.2300	-1.1801	to 0.7201
5 v 6	-0.0100	-0.9601	to 0.9401
5 v 7	-0.0600	-1.0101	to 0.8901
5 v 8	-0.0300	-0.9801	to 0.9201
5 v 9	-0.1100	-1.0601	to 0.8401
5 v 10	0.0700	-0.8801	to 1.0201
5 v 11	0.0000	-0.9501	to 0.9501
5 v 12	-0.0100	-0.9601	to 0.9401
5 v 13	-0.2700	-1.2201	to 0.6801
6 v 7	-0.0500	-1.0001	to 0.9001
6 v 8	-0.0200	-0.9701	to 0.9301
6 v 9	-0.1000	-1.0501	to 0.8501
6 v 10	0.0800	-0.8701	to 1.0301
6 v 11	0.0100	-0.9401	to 0.9601
6 v 12	0.0000	-0.9501	to 0.9501
6 v 13	-0.2600	-1.2101	to 0.6901
7 v 8	0.0300	-0.9201	to 0.9801
7 v 9	-0.0500	-1.0001	to 0.9001
7 v 10	0.1300	-0.8201	to 1.0801
7 v 11	0.0600	-0.8901	to 1.0101
7 v 12	0.0500	-0.9001	to 1.0001
7 v 13	-0.2100	-1.1601	to 0.7401
8 v 9	-0.0800	-1.0301	to 0.8701
8 v 10	0.1000	-0.8501	to 1.0501
8 v 11	0.0300	-0.9201	to 0.9801
8 v 12	0.0200	-0.9301	to 0.9701
8 v 13	-0.2400	-1.1901	to 0.7101
9 v 10	0.1800	-0.7701	to 1.1301
9 v 11	0.1100	-0.8401	to 1.0601
9 v 12	0.1000	-0.8501	to 1.0501
9 v 13	-0.1600	-1.1101	to 0.7901
10 v 11	-0.0700	-1.0201	to 0.8801
10 v 12	-0.0800	-1.0301	to 0.8701
10 v 13	-0.3400	-1.2901	to 0.6101
11 v 12	-0.0100	-0.9601	to 0.9401
11 v 13	-0.2700	-1.2201	to 0.6801
12 v 13	-0.2600	-1.2101	to 0.6901

24 hour

Source of variation	Sum squares	DF	Mean square	F statistic	p
Total Titrable Acidity (pumpkin)	0.519	12	0.043	0.48	0.05
Residual	2.340	26	0.090		
Total	2.859	38			

LSd

Contrast	Difference	95% CI	
1 v 2	-0.110	-1.060	to 0.840
1 v 3	0.030	-0.920	to 0.980
1 v 4	0.110	-0.840	to 1.060
1 v 5	0.140	-0.810	to 1.090
1 v 6	0.030	-0.920	to 0.980
1 v 7	0.080	-0.870	to 1.030
1 v 8	0.000	-0.950	to 0.950
1 v 9	-0.010	-0.960	to 0.940
1 v 10	-0.070	-1.020	to 0.880
1 v 11	-0.180	-1.130	to 0.770
1 v 12	-0.020	-0.970	to 0.930
1 v 13	-0.300	-1.250	to 0.650
2 v 3	0.140	-0.810	to 1.090
2 v 4	0.220	-0.730	to 1.170
2 v 5	0.250	-0.700	to 1.200
2 v 6	0.140	-0.810	to 1.090
2 v 7	0.190	-0.760	to 1.140
2 v 8	0.110	-0.840	to 1.060
2 v 9	0.100	-0.850	to 1.050
2 v 10	0.040	-0.910	to 0.990
2 v 11	-0.070	-1.020	to 0.880
2 v 12	0.090	-0.860	to 1.040
2 v 13	-0.190	-1.140	to 0.760
3 v 4	0.080	-0.870	to 1.030
3 v 5	0.110	-0.840	to 1.060
3 v 6	0.000	-0.950	to 0.950
3 v 7	0.050	-0.900	to 1.000
3 v 8	-0.030	-0.980	to 0.920
3 v 9	-0.040	-0.990	to 0.910
3 v 10	-0.100	-1.050	to 0.850
3 v 11	-0.210	-1.160	to 0.740
3 v 12	-0.050	-1.000	to 0.900
3 v 13	-0.330	-1.280	to 0.620
4 v 5	0.030	-0.920	to 0.980
4 v 6	-0.080	-1.030	to 0.870
4 v 7	-0.030	-0.980	to 0.920
4 v 8	-0.110	-1.060	to 0.840
4 v 9	-0.120	-1.070	to 0.830
4 v 10	-0.180	-1.130	to 0.770
4 v 11	-0.290	-1.240	to 0.660
4 v 12	-0.130	-1.080	to 0.820
4 v 13	-0.410	-1.360	to 0.540
5 v 6	-0.110	-1.060	to 0.840
5 v 7	-0.060	-1.010	to 0.890
5 v 8	-0.140	-1.090	to 0.810
5 v 9	-0.150	-1.100	to 0.800
5 v 10	-0.210	-1.160	to 0.740

5 v 11	-0.320	-1.270	to 0.630
5 v 12	-0.160	-1.110	to 0.790
5 v 13	-0.440	-1.390	to 0.510
6 v 7	0.050	-0.900	to 1.000
6 v 8	-0.030	-0.980	to 0.920
6 v 9	-0.040	-0.990	to 0.910
6 v 10	-0.100	-1.050	to 0.850
6 v 11	-0.210	-1.160	to 0.740
6 v 12	-0.050	-1.000	to 0.900
6 v 13	-0.330	-1.280	to 0.620
7 v 8	-0.080	-1.030	to 0.870
7 v 9	-0.090	-1.040	to 0.860
7 v 10	-0.150	-1.100	to 0.800
7 v 11	-0.260	-1.210	to 0.690
7 v 12	-0.100	-1.050	to 0.850
7 v 13	-0.380	-1.330	to 0.570
8 v 9	-0.010	-0.960	to 0.940
8 v 10	-0.070	-1.020	to 0.880
8 v 11	-0.180	-1.130	to 0.770
8 v 12	-0.020	-0.970	to 0.930
8 v 13	-0.300	-1.250	to 0.650
9 v 10	-0.060	-1.010	to 0.890
9 v 11	-0.170	-1.120	to 0.780
9 v 12	-0.010	-0.960	to 0.940
9 v 13	-0.290	-1.240	to 0.660
10 v 11	-0.110	-1.060	to 0.840
10 v 12	0.050	-0.900	to 1.000
10 v 13	-0.230	-1.180	to 0.720
11 v 12	0.160	-0.790	to 1.110
11 v 13	-0.120	-1.070	to 0.830
12 v 13	-0.280	-1.230	to 0.670

48 hour

Source of variation	Sum squares	DF	Mean square	F statistic	p
Total Titrable Acidity (pumpkin)	0.2261	12	0.0188	0.21	0.05
Residual	2.3400	26	0.0900		
Total	2.5661	38			

LSD

Contrast	Difference	95% CI	
1 v 2	-0.1100	-1.0601	to 0.8401
1 v 3	-0.0850	-1.0351	to 0.8651
1 v 4	-0.0300	-0.9801	to 0.9201
1 v 5	0.0800	-0.8701	to 1.0301
1 v 6	0.0100	-0.9401	to 0.9601
1 v 7	0.0700	-0.8801	to 1.0201
1 v 8	0.0000	-0.9501	to 0.9501
1 v 9	0.0500	-0.9001	to 1.0001
1 v 10	0.0100	-0.9401	to 0.9601
1 v 11	-0.0500	-1.0001	to 0.9001
1 v 12	-0.1850	-1.1351	to 0.7651
1 v 13	0.0800	-0.8701	to 1.0301
2 v 3	0.0250	-0.9251	to 0.9751
2 v 4	0.0800	-0.8701	to 1.0301
2 v 5	0.1900	-0.7601	to 1.1401
2 v 6	0.1200	-0.8301	to 1.0701

2 v 7	0.1800	-0.7701	to 1.1301
2 v 8	0.1100	-0.8401	to 1.0601
2 v 9	0.1600	-0.7901	to 1.1101
2 v 10	0.1200	-0.8301	to 1.0701
2 v 11	0.0600	-0.8901	to 1.0101
2 v 12	-0.0750	-1.0251	to 0.8751
2 v 13	0.1900	-0.7601	to 1.1401
3 v 4	0.0550	-0.8951	to 1.0051
3 v 5	0.1650	-0.7851	to 1.1151
3 v 6	0.0950	-0.8551	to 1.0451
3 v 7	0.1550	-0.7951	to 1.1051
3 v 8	0.0850	-0.8651	to 1.0351
3 v 9	0.1350	-0.8151	to 1.0851
3 v 10	0.0950	-0.8551	to 1.0451
3 v 11	0.0350	-0.9151	to 0.9851
3 v 12	-0.1000	-1.0501	to 0.8501
3 v 13	0.1650	-0.7851	to 1.1151
4 v 5	0.1100	-0.8401	to 1.0601
4 v 6	0.0400	-0.9101	to 0.9901
4 v 7	0.1000	-0.8501	to 1.0501
4 v 8	0.0300	-0.9201	to 0.9801
4 v 9	0.0800	-0.8701	to 1.0301
4 v 10	0.0400	-0.9101	to 0.9901
4 v 11	-0.0200	-0.9701	to 0.9301
4 v 12	-0.1550	-1.1051	to 0.7951
4 v 13	0.1100	-0.8401	to 1.0601
5 v 6	-0.0700	-1.0201	to 0.8801
5 v 7	-0.0100	-0.9601	to 0.9401
5 v 8	-0.0800	-1.0301	to 0.8701
5 v 9	-0.0300	-0.9801	to 0.9201
5 v 10	-0.0700	-1.0201	to 0.8801
5 v 11	-0.1300	-1.0801	to 0.8201
5 v 12	-0.2650	-1.2151	to 0.6851
5 v 13	0.0000	-0.9501	to 0.9501
6 v 7	0.0600	-0.8901	to 1.0101
6 v 8	-0.0100	-0.9601	to 0.9401
6 v 9	0.0400	-0.9101	to 0.9901
6 v 10	0.0000	-0.9501	to 0.9501
6 v 11	-0.0600	-1.0101	to 0.8901
6 v 12	-0.1950	-1.1451	to 0.7551
6 v 13	0.0700	-0.8801	to 1.0201
7 v 8	-0.0700	-1.0201	to 0.8801
7 v 9	-0.0200	-0.9701	to 0.9301
7 v 10	-0.0600	-1.0101	to 0.8901
7 v 11	-0.1200	-1.0701	to 0.8301
7 v 12	-0.2550	-1.2051	to 0.6951
7 v 13	0.0100	-0.9401	to 0.9601
8 v 9	0.0500	-0.9001	to 1.0001
8 v 10	0.0100	-0.9401	to 0.9601
8 v 11	-0.0500	-1.0001	to 0.9001
8 v 12	-0.1850	-1.1351	to 0.7651
8 v 13	0.0800	-0.8701	to 1.0301
9 v 10	-0.0400	-0.9901	to 0.9101
9 v 11	-0.1000	-1.0501	to 0.8501
9 v 12	-0.2350	-1.1851	to 0.7151
9 v 13	0.0300	-0.9201	to 0.9801
10 v 11	-0.0600	-1.0101	to 0.8901

10 v 12	-0.1950	-1.1451	to 0.7551
10 v 13	0.0700	-0.8801	to 1.0201
11 v 12	-0.1350	-1.0851	to 0.8151
11 v 13	0.1300	-0.8201	to 1.0801
12 v 13	0.2650	-0.6851	to 1.2151

72 hours

Source of variation	Sum squares	DF	Mean square	F statistic	p
Total Titrable Acidity (pumpkin)	0.2188	12	0.0182	0.20	0.05
Residual	2.3400	26	0.0900		
Total	2.5588	38			

LSD

Contrast	Difference	95% CI	
1 v 2	-0.1200	-1.0701	to 0.8301
1 v 3	-0.1000	-1.0501	to 0.8501
1 v 4	0.0100	-0.9401	to 0.9601
1 v 5	0.0800	-0.8701	to 1.0301
1 v 6	0.0100	-0.9401	to 0.9601
1 v 7	0.0850	-0.8651	to 1.0351
1 v 8	-0.0450	-0.9951	to 0.9051
1 v 9	-0.0100	-0.9601	to 0.9401
1 v 10	-0.0400	-0.9901	to 0.9101
1 v 11	-0.1000	-1.0501	to 0.8501
1 v 12	-0.1700	-1.1201	to 0.7801
1 v 13	-0.1100	-1.0601	to 0.8401
2 v 3	0.0200	-0.9301	to 0.9701
2 v 4	0.1300	-0.8201	to 1.0801
2 v 5	0.2000	-0.7501	to 1.1501
2 v 6	0.1300	-0.8201	to 1.0801
2 v 7	0.2050	-0.7451	to 1.1551
2 v 8	0.0750	-0.8751	to 1.0251
2 v 9	0.1100	-0.8401	to 1.0601
2 v 10	0.0800	-0.8701	to 1.0301
2 v 11	0.0200	-0.9301	to 0.9701
2 v 12	-0.0500	-1.0001	to 0.9001
2 v 13	0.0100	-0.9401	to 0.9601
3 v 4	0.1100	-0.8401	to 1.0601
3 v 5	0.1800	-0.7701	to 1.1301
3 v 6	0.1100	-0.8401	to 1.0601
3 v 7	0.1850	-0.7651	to 1.1351
3 v 8	0.0550	-0.8951	to 1.0051
3 v 9	0.0900	-0.8601	to 1.0401
3 v 10	0.0600	-0.8901	to 1.0101
3 v 11	0.0000	-0.9501	to 0.9501
3 v 12	-0.0700	-1.0201	to 0.8801
3 v 13	-0.0100	-0.9601	to 0.9401
4 v 5	0.0700	-0.8801	to 1.0201
4 v 6	0.0000	-0.9501	to 0.9501
4 v 7	0.0750	-0.8751	to 1.0251
4 v 8	-0.0550	-1.0051	to 0.8951
4 v 9	-0.0200	-0.9701	to 0.9301
4 v 10	-0.0500	-1.0001	to 0.9001
4 v 11	-0.1100	-1.0601	to 0.8401
4 v 12	-0.1800	-1.1301	to 0.7701

4 v 13	-0.1200	-1.0701	to 0.8301
5 v 6	-0.0700	-1.0201	to 0.8801
5 v 7	0.0050	-0.9451	to 0.9551
5 v 8	-0.1250	-1.0751	to 0.8251
5 v 9	-0.0900	-1.0401	to 0.8601
5 v 10	-0.1200	-1.0701	to 0.8301
5 v 11	-0.1800	-1.1301	to 0.7701
5 v 12	-0.2500	-1.2001	to 0.7001
5 v 13	-0.1900	-1.1401	to 0.7601
6 v 7	0.0750	-0.8751	to 1.0251
6 v 8	-0.0550	-1.0051	to 0.8951
6 v 9	-0.0200	-0.9701	to 0.9301
6 v 10	-0.0500	-1.0001	to 0.9001
6 v 11	-0.1100	-1.0601	to 0.8401
6 v 12	-0.1800	-1.1301	to 0.7701
6 v 13	-0.1200	-1.0701	to 0.8301
7 v 8	-0.1300	-1.0801	to 0.8201
7 v 9	-0.0950	-1.0451	to 0.8551
7 v 10	-0.1250	-1.0751	to 0.8251
7 v 11	-0.1850	-1.1351	to 0.7651
7 v 12	-0.2550	-1.2051	to 0.6951
7 v 13	-0.1950	-1.1451	to 0.7551
8 v 9	0.0350	-0.9151	to 0.9851
8 v 10	0.0050	-0.9451	to 0.9551
8 v 11	-0.0550	-1.0051	to 0.8951
8 v 12	-0.1250	-1.0751	to 0.8251
8 v 13	-0.0650	-1.0151	to 0.8851
9 v 10	-0.0300	-0.9801	to 0.9201
9 v 11	-0.0900	-1.0401	to 0.8601
9 v 12	-0.1600	-1.1101	to 0.7901
9 v 13	-0.1000	-1.0501	to 0.8501
10 v 11	-0.0600	-1.0101	to 0.8901
10 v 12	-0.1300	-1.0801	to 0.8201
10 v 13	-0.0700	-1.0201	to 0.8801
11 v 12	-0.0700	-1.0201	to 0.8801
11 v 13	-0.0100	-0.9601	to 0.9401
12 v 13	0.0600	-0.8901	to 1.0101

96 hour

Source of variation	Sum squares	DF	Mean square	F statistic	p
Total Titrable Acidity (pumpkin)	0.2036	12	0.0170	0.19	0.05
Residual	2.3400	26	0.0900		
Total	2.5436	38			

LSD

Contrast	Difference	95% CI	
1 v 2	-0.1300	-1.0801	to 0.8201
1 v 3	-0.1100	-1.0601	to 0.8401
1 v 4	-0.0200	-0.9701	to 0.9301
1 v 5	0.0800	-0.8701	to 1.0301
1 v 6	0.0050	-0.9451	to 0.9551
1 v 7	0.0600	-0.8901	to 1.0101
1 v 8	0.0000	-0.9501	to 0.9501
1 v 9	-0.0200	-0.9701	to 0.9301
1 v 10	-0.0300	-0.9801	to 0.9201
1 v 11	-0.1000	-1.0501	to 0.8501

1 v 12	-0.1600	-1.1101	to 0.7901
1 v 13	-0.1200	-1.0701	to 0.8301
2 v 3	0.0200	-0.9301	to 0.9701
2 v 4	0.1100	-0.8401	to 1.0601
2 v 5	0.2100	-0.7401	to 1.1601
2 v 6	0.1350	-0.8151	to 1.0851
2 v 7	0.1900	-0.7601	to 1.1401
2 v 8	0.1300	-0.8201	to 1.0801
2 v 9	0.1100	-0.8401	to 1.0601
2 v 10	0.1000	-0.8501	to 1.0501
2 v 11	0.0300	-0.9201	to 0.9801
2 v 12	-0.0300	-0.9801	to 0.9201
2 v 13	0.0100	-0.9401	to 0.9601
3 v 4	0.0900	-0.8601	to 1.0401
3 v 5	0.1900	-0.7601	to 1.1401
3 v 6	0.1150	-0.8351	to 1.0651
3 v 7	0.1700	-0.7801	to 1.1201
3 v 8	0.1100	-0.8401	to 1.0601
3 v 9	0.0900	-0.8601	to 1.0401
3 v 10	0.0800	-0.8701	to 1.0301
3 v 11	0.0100	-0.9401	to 0.9601
3 v 12	-0.0500	-1.0001	to 0.9001
3 v 13	-0.0100	-0.9601	to 0.9401
4 v 5	0.1000	-0.8501	to 1.0501
4 v 6	0.0250	-0.9251	to 0.9751
4 v 7	0.0800	-0.8701	to 1.0301
4 v 8	0.0200	-0.9301	to 0.9701
4 v 9	0.0000	-0.9501	to 0.9501
4 v 10	-0.0100	-0.9601	to 0.9401
4 v 11	-0.0800	-1.0301	to 0.8701
4 v 12	-0.1400	-1.0901	to 0.8101
4 v 13	-0.1000	-1.0501	to 0.8501
5 v 6	-0.0750	-1.0251	to 0.8751
5 v 7	-0.0200	-0.9701	to 0.9301
5 v 8	-0.0800	-1.0301	to 0.8701
5 v 9	-0.1000	-1.0501	to 0.8501
5 v 10	-0.1100	-1.0601	to 0.8401
5 v 11	-0.1800	-1.1301	to 0.7701
5 v 12	-0.2400	-1.1901	to 0.7101
5 v 13	-0.2000	-1.1501	to 0.7501
6 v 7	0.0550	-0.8951	to 1.0051
6 v 8	-0.0050	-0.9551	to 0.9451
6 v 9	-0.0250	-0.9751	to 0.9251
6 v 10	-0.0350	-0.9851	to 0.9151
6 v 11	-0.1050	-1.0551	to 0.8451
6 v 12	-0.1650	-1.1151	to 0.7851
6 v 13	-0.1250	-1.0751	to 0.8251
7 v 8	-0.0600	-1.0101	to 0.8901
7 v 9	-0.0800	-1.0301	to 0.8701
7 v 10	-0.0900	-1.0401	to 0.8601
7 v 11	-0.1600	-1.1101	to 0.7901
7 v 12	-0.2200	-1.1701	to 0.7301
7 v 13	-0.1800	-1.1301	to 0.7701
8 v 9	-0.0200	-0.9701	to 0.9301
8 v 10	-0.0300	-0.9801	to 0.9201
8 v 11	-0.1000	-1.0501	to 0.8501
8 v 12	-0.1600	-1.1101	to 0.7901

8 v 13	-0.1200	-1.0701	to 0.8301
9 v 10	-0.0100	-0.9601	to 0.9401
9 v 11	-0.0800	-1.0301	to 0.8701
9 v 12	-0.1400	-1.0901	to 0.8101
9 v 13	-0.1000	-1.0501	to 0.8501
10 v 11	-0.0700	-1.0201	to 0.8801
10 v 12	-0.1300	-1.0801	to 0.8201
10 v 13	-0.0900	-1.0401	to 0.8601
11 v 12	-0.0600	-1.0101	to 0.8901
11 v 13	-0.0200	-0.9701	to 0.9301
12 v 13	0.0400	-0.9101	to 0.9901

Anova tables for Total Titratable Acidity (Pumpkin)

SAMPLE 1

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	0.6366	4	0.1592	1.77	0.05
Residual	0.9000	10	0.0900		
Total	1.5366	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.2200	-1.0973	to 0.6573
0 hour v 48 hour	-0.4100	-1.2873	to 0.4673
0 hour v 72 hour	-0.5100	-1.3873	to 0.3673
0 hour v 96 hour	-0.5600	-1.4373	to 0.3173
24 hour v 48 hour	-0.1900	-1.0673	to 0.6873
24 hour v 72 hour	-0.2900	-1.1673	to 0.5873
24 hour v 96hour	-0.3400	-1.2173	to 0.5373
48 hour v 72 hour	-0.1000	-0.9773	to 0.7773
48 hour v 96 hour	-0.1500	-1.0273	to 0.7273
72 hour v 96 hour	-0.0500	-0.9273	to 0.8273

SAMPLE 2

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	0.6577	4	0.1644	1.83	0.05
Residual	0.9000	10	0.0900		
Total	1.5577	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.2120	-1.0893	to 0.6653
0 hour v 48 hour	-0.4020	-1.2793	to 0.4753
0 hour v 72 hour	-0.5120	-1.3893	to 0.3653
0 hour v 96 hour	-0.5720	-1.4493	to 0.3053
24 hour v 48 hour	-0.1900	-1.0673	to 0.6873
24 hour v 72 hour	-0.3000	-1.1773	to 0.5773
24 hour v 96hour	-0.3600	-1.2373	to 0.5173
48 hour v 72 hour	-0.1100	-0.9873	to 0.7673
48 hour v 96 hour	-0.1700	-1.0473	to 0.7073
72 hour v 96 hour	-0.0600	-0.9373	to 0.8173

SAMPLE 3

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	0.9896	4	0.2474	2.75	0.05
Residual	0.9000	10	0.0900		
Total	1.8896	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.1900	-1.0673	to 0.6873
0 hour v 48 hour	-0.4950	-1.3723	to 0.3823
0 hour v 72 hour	-0.6100	-1.4873	to 0.2673
0 hour v 96 hour	-0.6700	-1.5473	to 0.2073
24 hour v 48 hour	-0.3050	-1.1823	to 0.5723
24 hour v 72 hour	-0.4200	-1.2973	to 0.4573
24 hour v 96hour	-0.4800	-1.3573	to 0.3973
48 hour v 72 hour	-0.1150	-0.9923	to 0.7623
48 hour v 96 hour	-0.1750	-1.0523	to 0.7023
72 hour v 96 hour	-0.0600	-0.9373	to 0.8173

SAMPLE 4

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	0.8020	4	0.2005	2.23	0.05
Residual	0.9000	10	0.0900		
Total	1.7020	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.1200	-0.9973	to 0.7573
0 hour v 48 hour	-0.4500	-1.3273	to 0.4273
0 hour v 72 hour	-0.5100	-1.3873	to 0.3673
0 hour v 96 hour	-0.5900	-1.4673	to 0.2873
24 hour v 48 hour	-0.3300	-1.2073	to 0.5473
24 hour v 72 hour	-0.3900	-1.2673	to 0.4873
24 hour v 96hour	-0.4700	-1.3473	to 0.4073
48 hour v 72 hour	-0.0600	-0.9373	to 0.8173
48 hour v 96 hour	-0.1400	-1.0173	to 0.7373
72 hour v 96 hour	-0.0800	-0.9573	to 0.7973

SAMPLE 5

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	0.6316	4	0.1579	1.75	0.05
Residual	0.9000	10	0.0900		
Total	1.5316	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.1300	-1.0073	to 0.7473
0 hour v 48 hour	-0.3800	-1.2573	to 0.4973
0 hour v 72 hour	-0.4800	-1.3573	to 0.3973
0 hour v 96 hour	-0.5300	-1.4073	to 0.3473
24 hour v 48 hour	-0.2500	-1.1273	to 0.6273
24 hour v 72 hour	-0.3500	-1.2273	to 0.5273
24 hour v 96hour	-0.4000	-1.2773	to 0.4773
48 hour v 72 hour	-0.1000	-0.9773	to 0.7773
48 hour v 96 hour	-0.1500	-1.0273	to 0.7273
72 hour v 96 hour	-0.0500	-0.9273	to 0.8273

SAMPLE 6

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	0.7216	4	0.1804	2.00	0.05
Residual	0.9000	10	0.0900		
Total	1.6216	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.2300	-1.1073	to 0.6473
0 hour v 48 hour	-0.4400	-1.3173	to 0.4373
0 hour v 72 hour	-0.5400	-1.4173	to 0.3373
0 hour v 96 hour	-0.5950	-1.4723	to 0.2823
24 hour v 48 hour	-0.2100	-1.0873	to 0.6673
24 hour v 72 hour	-0.3100	-1.1873	to 0.5673
24 hour v 96hour	-0.3650	-1.2423	to 0.5123
48 hour v 72 hour	-0.1000	-0.9773	to 0.7773
48 hour v 96 hour	-0.1550	-1.0323	to 0.7223
72 hour v 96 hour	-0.0550	-0.9323	to 0.8223

SAMPLE 7

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	0.4964	4	0.1241	1.38	0.05
Residual	0.9000	10	0.0900		
Total	1.3964	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.1300	-1.0073	to 0.7473
0 hour v 48 hour	-0.3300	-1.2073	to 0.5473
0 hour v 72 hour	-0.4150	-1.2923	to 0.4623
0 hour v 96 hour	-0.4900	-1.3673	to 0.3873
24 hour v 48 hour	-0.2000	-1.0773	to 0.6773
24 hour v 72 hour	-0.2850	-1.1623	to 0.5923
24 hour v 96hour	-0.3600	-1.2373	to 0.5173
48 hour v 72 hour	-0.0850	-0.9623	to 0.7923
48 hour v 96 hour	-0.1600	-1.0373	to 0.7173
72 hour v 96 hour	-0.0750	-0.9523	to 0.8023

SAMPLE 8

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	0.7302	4	0.1826	2.03	0.05
Residual	0.9000	10	0.0900		
Total	1.6302	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.2400	-1.1173	to 0.6373
0 hour v 48 hour	-0.4300	-1.3073	to 0.4473
0 hour v 72 hour	-0.5750	-1.4523	to 0.3023
0 hour v 96 hour	-0.5800	-1.4573	to 0.2973
24 hour v 48 hour	-0.1900	-1.0673	to 0.6873

24 hour v 72 hour	-0.3350	-1.2123	to 0.5423
24 hour v 96hour	-0.3400	-1.2173	to 0.5373
48 hour v 72 hour	-0.1450	-1.0223	to 0.7323
48 hour v 96 hour	-0.1500	-1.0273	to 0.7273
72 hour v 96 hour	-0.0050	-0.8823	to 0.8723

SAMPLE 9

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	0.5412	4	0.1353	1.50	0.05
Residual	0.9000	10	0.0900		
Total	1.4412	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.1700	-1.0473	to 0.7073
0 hour v 48 hour	-0.3000	-1.1773	to 0.5773
0 hour v 72 hour	-0.4600	-1.3373	to 0.4173
0 hour v 96 hour	-0.5200	-1.3973	to 0.3573
24 hour v 48 hour	-0.1300	-1.0073	to 0.7473
24 hour v 72 hour	-0.2900	-1.1673	to 0.5873
24 hour v 96hour	-0.3500	-1.2273	to 0.5273
48 hour v 72 hour	-0.1600	-1.0373	to 0.7173
48 hour v 96 hour	-0.2200	-1.0973	to 0.6573
72 hour v 96 hour	-0.0600	-0.9373	to 0.8173

SAMPLE 10

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	0.9728	4	0.2432	2.70	0.05
Residual	0.9000	10	0.0900		
Total	1.8728	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.4100	-1.2873	to 0.4673
0 hour v 48 hour	-0.5200	-1.3973	to 0.3573
0 hour v 72 hour	-0.6700	-1.5473	to 0.2073
0 hour v 96 hour	-0.7100	-1.5873	to 0.1673
24 hour v 48 hour	-0.1100	-0.9873	to 0.7673
24 hour v 72 hour	-0.2600	-1.1373	to 0.6173
24 hour v 96hour	-0.3000	-1.1773	to 0.5773
48 hour v 72 hour	-0.1500	-1.0273	to 0.7273
48 hour v 96 hour	-0.1900	-1.0673	to 0.6873
72 hour v 96 hour	-0.0400	-0.9173	to 0.8373

SAMPLE 11

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	0.9496	4	0.2374	2.64	0.05
Residual	0.9000	10	0.0900		
Total	1.8496	14			

LSD

Contrast	Difference	95% CI

0 hour v 24 hour	-0.4500	-1.3273	to 0.4273
0 hour v 48 hour	-0.5100	-1.3873	to 0.3673
0 hour v 72 hour	-0.6600	-1.5373	to 0.2173
0 hour v 96 hour	-0.7100	-1.5873	to 0.1673
24 hour v 48 hour	-0.0600	-0.9373	to 0.8173
24 hour v 72 hour	-0.2100	-1.0873	to 0.6673
24 hour v 96hour	-0.2600	-1.1373	to 0.6173
48 hour v 72 hour	-0.1500	-1.0273	to 0.7273
48 hour v 96 hour	-0.2000	-1.0773	to 0.6773
72 hour v 96 hour	-0.0500	-0.9273	to 0.8273

SAMPLE 12

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	1.2913	4	0.3228	3.59	0.05
Residual	0.9000	10	0.0900		
Total	2.1913	14			

LSD

Contrast	Difference	95% CI	
Day 1 v Day 2	-0.2800	-0.8258	to 0.2658
0 hour v 24 hour	-0.6350	-1.1808	to -0.0892
0 hour v 48 hour	-0.7200	-1.2658	to -0.1742
0 hour v 72 hour	-0.7600	-1.3058	to -0.2142
0 hour v 96 hour	-0.3550	-0.9008	to 0.1908
24 hour v 48 hour	-0.4400	-0.9858	to 0.1058
24 hour v 72 hour	-0.4800	-1.0258	to 0.0658
24 hour v 96hour	-0.0850	-0.6308	to 0.4608
48 hour v 72 hour	-0.1250	-0.6708	to 0.4208
48 hour v 96 hour	-0.0400	-0.5858	to 0.5058
72 hour v 96 hour			

SAMPLE 13

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	0.4534	4	0.1133	1.26	0.05
Residual	0.9000	10	0.0900		
Total	1.3534	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.3000	-1.1773	to 0.5773
0 hour v 48 hour	-0.1100	-0.9873	to 0.7673
0 hour v 72 hour	-0.4000	-1.2773	to 0.4773
0 hour v 96 hour	-0.4600	-1.3373	to 0.4173
24 hour v 48 hour	0.1900	-0.6873	to 1.0673
24 hour v 72 hour	-0.1000	-0.9773	to 0.7773
24 hour v 96hour	-0.1600	-1.0373	to 0.7173
48 hour v 72 hour	-0.2900	-1.1673	to 0.5873
48 hour v 96 hour	-0.3500	-1.2273	to 0.5273
72 hour v 96 hour	-0.0600	-0.9373	to 0.8173

Amino Nitrogen (Pumpkin)

0 hour

Source of variation	Sum squares	DF	Mean square	F statistic	p
Amino Nitrogen (Pumpkin)	5.1104	12	0.4259	4.73	0.05
Residual	2.3400	26	0.0900		
Total	7.4504	38			

LSD

Contrast	Difference	95% CI	
1 v 2	0.2650	-0.6851	to 1.2151
1 v 3	0.1350	-0.8151	to 1.0851
1 v 4	-0.6250	-1.5751	to 0.3251
1 v 5	0.3050	-0.6451	to 1.2551
1 v 6	-0.6050	-1.5551	to 0.3451
1 v 7	0.0550	-0.8951	to 1.0051
1 v 8	-0.9150	-1.8651	to 0.0351
1 v 9	-0.4050	-1.3551	to 0.5451
1 v 10	-0.0700	-1.0201	to 0.8801
1 v 11	-0.4950	-1.4451	to 0.4551
1 v 12	-0.1950	-1.1451	to 0.7551
1 v 13	-0.0850	-1.0351	to 0.8651
2 v 3	-0.1300	-1.0801	to 0.8201
2 v 4	-0.8900	-1.8401	to 0.0601
2 v 5	0.0400	-0.9101	to 0.9901
2 v 6	-0.8700	-1.8201	to 0.0801
2 v 7	-0.2100	-1.1601	to 0.7401
2 v 8	-1.1800	-2.1301	to -0.2299
2 v 9	-0.6700	-1.6201	to 0.2801
2 v 10	-0.3350	-1.2851	to 0.6151
2 v 11	-0.7600	-1.7101	to 0.1901
2 v 12	-0.4600	-1.4101	to 0.4901
2 v 13	-0.3500	-1.3001	to 0.6001
3 v 4	-0.7600	-1.7101	to 0.1901
3 v 5	0.1700	-0.7801	to 1.1201
3 v 6	-0.7400	-1.6901	to 0.2101
3 v 7	-0.0800	-1.0301	to 0.8701
3 v 8	-1.0500	-2.0001	to -0.0999
3 v 9	-0.5400	-1.4901	to 0.4101
3 v 10	-0.2050	-1.1551	to 0.7451
3 v 11	-0.6300	-1.5801	to 0.3201
3 v 12	-0.3300	-1.2801	to 0.6201
3 v 13	-0.2200	-1.1701	to 0.7301
4 v 5	0.9300	-0.0201	to 1.8801
4 v 6	0.0200	-0.9301	to 0.9701
4 v 7	0.6800	-0.2701	to 1.6301
4 v 8	-0.2900	-1.2401	to 0.6601
4 v 9	0.2200	-0.7301	to 1.1701
4 v 10	0.5550	-0.3951	to 1.5051
4 v 11	0.1300	-0.8201	to 1.0801
4 v 12	0.4300	-0.5201	to 1.3801
4 v 13	0.5400	-0.4101	to 1.4901
5 v 6	-0.9100	-1.8601	to 0.0401
5 v 7	-0.2500	-1.2001	to 0.7001
5 v 8	-1.2200	-2.1701	to -0.2699
5 v 9	-0.7100	-1.6601	to 0.2401
5 v 10	-0.3750	-1.3251	to 0.5751

5 v 11	-0.8000	-1.7501	to 0.1501
5 v 12	-0.5000	-1.4501	to 0.4501
5 v 13	-0.3900	-1.3401	to 0.5601
6 v 7	0.6600	-0.2901	to 1.6101
6 v 8	-0.3100	-1.2601	to 0.6401
6 v 9	0.2000	-0.7501	to 1.1501
6 v 10	0.5350	-0.4151	to 1.4851
6 v 11	0.1100	-0.8401	to 1.0601
6 v 12	0.4100	-0.5401	to 1.3601
6 v 13	0.5200	-0.4301	to 1.4701
7 v 8	-0.9700	-1.9201	to -0.0199
7 v 9	-0.4600	-1.4101	to 0.4901
7 v 10	-0.1250	-1.0751	to 0.8251
7 v 11	-0.5500	-1.5001	to 0.4001
7 v 12	-0.2500	-1.2001	to 0.7001
7 v 13	-0.1400	-1.0901	to 0.8101
8 v 9	0.5100	-0.4401	to 1.4601
8 v 10	0.8450	-0.1051	to 1.7951
8 v 11	0.4200	-0.5301	to 1.3701
8 v 12	0.7200	-0.2301	to 1.6701
8 v 13	0.8300	-0.1201	to 1.7801
9 v 10	0.3350	-0.6151	to 1.2851
9 v 11	-0.0900	-1.0401	to 0.8601
9 v 12	0.2100	-0.7401	to 1.1601
9 v 13	0.3200	-0.6301	to 1.2701
10 v 11	-0.4250	-1.3751	to 0.5251
10 v 12	-0.1250	-1.0751	to 0.8251
10 v 13	-0.0150	-0.9651	to 0.9351
11 v 12	0.3000	-0.6501	to 1.2501
11 v 13	0.4100	-0.5401	to 1.3601
12 v 13	0.1100	-0.8401	to 1.0601

24 hour

Source of variation	Sum squares	DF	Mean square	F statistic	p
Amino Nitrogen (Pumpkin)	7.1507	12	0.5959	6.62	0.05
Residual	2.3400	26	0.0900		
Total	9.4907	38			

LSD

Contrast	Difference	95% CI	
1 v 2	0.2650	-0.6851	to 1.2151
1 v 3	0.1200	-0.8301	to 1.0701
1 v 4	-0.5900	-1.5401	to 0.3601
1 v 5	0.3000	-0.6501	to 1.2501
1 v 6	-0.5700	-1.5201	to 0.3801
1 v 7	0.0300	-0.9201	to 0.9801
1 v 8	-0.8100	-1.7601	to 0.1401
1 v 9	-0.3700	-1.3201	to 0.5801
1 v 10	-0.1500	-1.1001	to 0.8001
1 v 11	-0.5200	-1.4701	to 0.4301
1 v 12	-0.1200	-1.0701	to 0.8301
1 v 13	-1.2000	-2.1501	to -0.2499
2 v 3	-0.1450	-1.0951	to 0.8051
2 v 4	-0.8550	-1.8051	to 0.0951
2 v 5	0.0350	-0.9151	to 0.9851
2 v 6	-0.8350	-1.7851	to 0.1151

2 v 7	-0.2350	-1.1851	to 0.7151
2 v 8	-1.0750	-2.0251	to -0.1249
2 v 9	-0.6350	-1.5851	to 0.3151
2 v 10	-0.4150	-1.3651	to 0.5351
2 v 11	-0.7850	-1.7351	to 0.1651
2 v 12	-0.3850	-1.3351	to 0.5651
2 v 13	-1.4650	-2.4151	to -0.5149
3 v 4	-0.7100	-1.6601	to 0.2401
3 v 5	0.1800	-0.7701	to 1.1301
3 v 6	-0.6900	-1.6401	to 0.2601
3 v 7	-0.0900	-1.0401	to 0.8601
3 v 8	-0.9300	-1.8801	to 0.0201
3 v 9	-0.4900	-1.4401	to 0.4601
3 v 10	-0.2700	-1.2201	to 0.6801
3 v 11	-0.6400	-1.5901	to 0.3101
3 v 12	-0.2400	-1.1901	to 0.7101
3 v 13	-1.3200	-2.2701	to -0.3699
4 v 5	0.8900	-0.0601	to 1.8401
4 v 6	0.0200	-0.9301	to 0.9701
4 v 7	0.6200	-0.3301	to 1.5701
4 v 8	-0.2200	-1.1701	to 0.7301
4 v 9	0.2200	-0.7301	to 1.1701
4 v 10	0.4400	-0.5101	to 1.3901
4 v 11	0.0700	-0.8801	to 1.0201
4 v 12	0.4700	-0.4801	to 1.4201
4 v 13	-0.6100	-1.5601	to 0.3401
5 v 6	-0.8700	-1.8201	to 0.0801
5 v 7	-0.2700	-1.2201	to 0.6801
5 v 8	-1.1100	-2.0601	to -0.1599
5 v 9	-0.6700	-1.6201	to 0.2801
5 v 10	-0.4500	-1.4001	to 0.5001
5 v 11	-0.8200	-1.7701	to 0.1301
5 v 12	-0.4200	-1.3701	to 0.5301
5 v 13	-1.5000	-2.4501	to -0.5499
6 v 7	0.6000	-0.3501	to 1.5501
6 v 8	-0.2400	-1.1901	to 0.7101
6 v 9	0.2000	-0.7501	to 1.1501
6 v 10	0.4200	-0.5301	to 1.3701
6 v 11	0.0500	-0.9001	to 1.0001
6 v 12	0.4500	-0.5001	to 1.4001
6 v 13	-0.6300	-1.5801	to 0.3201
7 v 8	-0.8400	-1.7901	to 0.1101
7 v 9	-0.4000	-1.3501	to 0.5501
7 v 10	-0.1800	-1.1301	to 0.7701
7 v 11	-0.5500	-1.5001	to 0.4001
7 v 12	-0.1500	-1.1001	to 0.8001
7 v 13	-1.2300	-2.1801	to -0.2799
8 v 9	0.4400	-0.5101	to 1.3901
8 v 10	0.6600	-0.2901	to 1.6101
8 v 11	0.2900	-0.6601	to 1.2401
8 v 12	0.6900	-0.2601	to 1.6401
8 v 13	-0.3900	-1.3401	to 0.5601
9 v 10	0.2200	-0.7301	to 1.1701
9 v 11	-0.1500	-1.1001	to 0.8001
9 v 12	0.2500	-0.7001	to 1.2001
9 v 13	-0.8300	-1.7801	to 0.1201
10 v 11	-0.3700	-1.3201	to 0.5801

10 v 12	0.0300	-0.9201	to 0.9801
10 v 13	-1.0500	-2.0001	to -0.0999
11 v 12	0.4000	-0.5501	to 1.3501
11 v 13	-0.6800	-1.6301	to 0.2701
12 v 13	-1.0800	-2.0301	to -0.1299

48 hour

Source of variation	Sum squares	DF	Mean square	F statistic	p
Amino Nitrogen (Pumpkin)	5.9286	12	0.4941	5.49	0.05
Residual	2.3400	26	0.0900		
Total	8.2686	38			

LSD

Contrast	Difference	95% CI	
1 v 2	-0.0300	-0.9801	to 0.9201
1 v 3	0.0100	-0.9401	to 0.9601
1 v 4	0.2100	-0.7401	to 1.1601
1 v 5	0.2000	-0.7501	to 1.1501
1 v 6	-0.7000	-1.6501	to 0.2501
1 v 7	-0.0300	-0.9801	to 0.9201
1 v 8	-0.9150	-1.8651	to 0.0351
1 v 9	-0.3800	-1.3301	to 0.5701
1 v 10	-0.2600	-1.2101	to 0.6901
1 v 11	-0.6900	-1.6401	to 0.2601
1 v 12	-0.5000	-1.4501	to 0.4501
1 v 13	-0.9300	-1.8801	to 0.0201
2 v 3	0.0400	-0.9101	to 0.9901
2 v 4	0.2400	-0.7101	to 1.1901
2 v 5	0.2300	-0.7201	to 1.1801
2 v 6	-0.6700	-1.6201	to 0.2801
2 v 7	0.0000	-0.9501	to 0.9501
2 v 8	-0.8850	-1.8351	to 0.0651
2 v 9	-0.3500	-1.3001	to 0.6001
2 v 10	-0.2300	-1.1801	to 0.7201
2 v 11	-0.6600	-1.6101	to 0.2901
2 v 12	-0.4700	-1.4201	to 0.4801
2 v 13	-0.9000	-1.8501	to 0.0501
3 v 4	0.2000	-0.7501	to 1.1501
3 v 5	0.1900	-0.7601	to 1.1401
3 v 6	-0.7100	-1.6601	to 0.2401
3 v 7	-0.0400	-0.9901	to 0.9101
3 v 8	-0.9250	-1.8751	to 0.0251
3 v 9	-0.3900	-1.3401	to 0.5601
3 v 10	-0.2700	-1.2201	to 0.6801
3 v 11	-0.7000	-1.6501	to 0.2501
3 v 12	-0.5100	-1.4601	to 0.4401
3 v 13	-0.9400	-1.8901	to 0.0101
4 v 5	-0.0100	-0.9601	to 0.9401
4 v 6	-0.9100	-1.8601	to 0.0401
4 v 7	-0.2400	-1.1901	to 0.7101
4 v 8	-1.1250	-2.0751	to -0.1749
4 v 9	-0.5900	-1.5401	to 0.3601
4 v 10	-0.4700	-1.4201	to 0.4801
4 v 11	-0.9000	-1.8501	to 0.0501
4 v 12	-0.7100	-1.6601	to 0.2401
4 v 13	-1.1400	-2.0901	to -0.1899

5 v 6	-0.9000	-1.8501	to 0.0501
5 v 7	-0.2300	-1.1801	to 0.7201
5 v 8	-1.1150	-2.0651	to -0.1649
5 v 9	-0.5800	-1.5301	to 0.3701
5 v 10	-0.4600	-1.4101	to 0.4901
5 v 11	-0.8900	-1.8401	to 0.0601
5 v 12	-0.7000	-1.6501	to 0.2501
5 v 13	-1.1300	-2.0801	to -0.1799
6 v 7	0.6700	-0.2801	to 1.6201
6 v 8	-0.2150	-1.1651	to 0.7351
6 v 9	0.3200	-0.6301	to 1.2701
6 v 10	0.4400	-0.5101	to 1.3901
6 v 11	0.0100	-0.9401	to 0.9601
6 v 12	0.2000	-0.7501	to 1.1501
6 v 13	-0.2300	-1.1801	to 0.7201
7 v 8	-0.8850	-1.8351	to 0.0651
7 v 9	-0.3500	-1.3001	to 0.6001
7 v 10	-0.2300	-1.1801	to 0.7201
7 v 11	-0.6600	-1.6101	to 0.2901
7 v 12	-0.4700	-1.4201	to 0.4801
7 v 13	-0.9000	-1.8501	to 0.0501
8 v 9	0.5350	-0.4151	to 1.4851
8 v 10	0.6550	-0.2951	to 1.6051
8 v 11	0.2250	-0.7251	to 1.1751
8 v 12	0.4150	-0.5351	to 1.3651
8 v 13	-0.0150	-0.9651	to 0.9351
9 v 10	0.1200	-0.8301	to 1.0701
9 v 11	-0.3100	-1.2601	to 0.6401
9 v 12	-0.1200	-1.0701	to 0.8301
9 v 13	-0.5500	-1.5001	to 0.4001
10 v 11	-0.4300	-1.3801	to 0.5201
10 v 12	-0.2400	-1.1901	to 0.7101
10 v 13	-0.6700	-1.6201	to 0.2801
11 v 12	0.1900	-0.7601	to 1.1401
11 v 13	-0.2400	-1.1901	to 0.7101
12 v 13	-0.4300	-1.3801	to 0.5201

72 hour

Source of variation	Sum squares	DF	Mean square	F statistic	p
Amino Nitrogen (Pumpkin)	6.499	12	0.542	6.02	0.05
Residual	2.340	26	0.090		
Total	8.839	38			

LSD

Contrast	Difference	95% CI	
1 v 2	-0.350	-1.300	to 0.600
1 v 3	-0.480	-1.430	to 0.470
1 v 4	-0.710	-1.660	to 0.240
1 v 5	-0.350	-1.300	to 0.600
1 v 6	-1.360	-2.310	to -0.410
1 v 7	-0.500	-1.450	to 0.450
1 v 8	-1.370	-2.320	to -0.420
1 v 9	-0.730	-1.680	to 0.220
1 v 10	-0.950	-1.900	to 0.000
1 v 11	-1.130	-2.080	to -0.180

1 v 12	-1.150	-2.100	to -0.200
1 v 13	-0.980	-1.930	to -0.030
2 v 3	-0.130	-1.080	to 0.820
2 v 4	-0.360	-1.310	to 0.590
2 v 5	0.000	-0.950	to 0.950
2 v 6	-1.010	-1.960	to -0.060
2 v 7	-0.150	-1.100	to 0.800
2 v 8	-1.020	-1.970	to -0.070
2 v 9	-0.380	-1.330	to 0.570
2 v 10	-0.600	-1.550	to 0.350
2 v 11	-0.780	-1.730	to 0.170
2 v 12	-0.800	-1.750	to 0.150
2 v 13	-0.630	-1.580	to 0.320
3 v 4	-0.230	-1.180	to 0.720
3 v 5	0.130	-0.820	to 1.080
3 v 6	-0.880	-1.830	to 0.070
3 v 7	-0.020	-0.970	to 0.930
3 v 8	-0.890	-1.840	to 0.060
3 v 9	-0.250	-1.200	to 0.700
3 v 10	-0.470	-1.420	to 0.480
3 v 11	-0.650	-1.600	to 0.300
3 v 12	-0.670	-1.620	to 0.280
3 v 13	-0.500	-1.450	to 0.450
4 v 5	0.360	-0.590	to 1.310
4 v 6	-0.650	-1.600	to 0.300
4 v 7	0.210	-0.740	to 1.160
4 v 8	-0.660	-1.610	to 0.290
4 v 9	-0.020	-0.970	to 0.930
4 v 10	-0.240	-1.190	to 0.710
4 v 11	-0.420	-1.370	to 0.530
4 v 12	-0.440	-1.390	to 0.510
4 v 13	-0.270	-1.220	to 0.680
5 v 6	-1.010	-1.960	to -0.060
5 v 7	-0.150	-1.100	to 0.800
5 v 8	-1.020	-1.970	to -0.070
5 v 9	-0.380	-1.330	to 0.570
5 v 10	-0.600	-1.550	to 0.350
5 v 11	-0.780	-1.730	to 0.170
5 v 12	-0.800	-1.750	to 0.150
5 v 13	-0.630	-1.580	to 0.320
6 v 7	0.860	-0.090	to 1.810
6 v 8	-0.010	-0.960	to 0.940
6 v 9	0.630	-0.320	to 1.580
6 v 10	0.410	-0.540	to 1.360
6 v 11	0.230	-0.720	to 1.180
6 v 12	0.210	-0.740	to 1.160
6 v 13	0.380	-0.570	to 1.330
7 v 8	-0.870	-1.820	to 0.080
7 v 9	-0.230	-1.180	to 0.720
7 v 10	-0.450	-1.400	to 0.500
7 v 11	-0.630	-1.580	to 0.320
7 v 12	-0.650	-1.600	to 0.300
7 v 13	-0.480	-1.430	to 0.470
8 v 9	0.640	-0.310	to 1.590
8 v 10	0.420	-0.530	to 1.370
8 v 11	0.240	-0.710	to 1.190
8 v 12	0.220	-0.730	to 1.170

8 v 13	0.390	-0.560	to 1.340
9 v 10	-0.220	-1.170	to 0.730
9 v 11	-0.400	-1.350	to 0.550
9 v 12	-0.420	-1.370	to 0.530
9 v 13	-0.250	-1.200	to 0.700
10 v 11	-0.180	-1.130	to 0.770
10 v 12	-0.200	-1.150	to 0.750
10 v 13	-0.030	-0.980	to 0.920
11 v 12	-0.020	-0.970	to 0.930
11 v 13	0.150	-0.800	to 1.100
12 v 13	0.170	-0.780	to 1.120

96 hour

Source of variation	Sum squares	DF	Mean square	F statistic	p
Amino Nitrogen (Pumpkin)	3.6597	12	0.3050	3.39	0.05
Residual	2.3400	26	0.0900		
Total	5.9997	38			

LSD

Contrast	Difference	95% CI	
1 v 2	-0.3450	-1.2951	to 0.6051
1 v 3	-0.2100	-1.1601	to 0.7401
1 v 4	-0.7200	-1.6701	to 0.2301
1 v 5	-0.5500	-1.5001	to 0.4001
1 v 6	-0.6300	-1.5801	to 0.3201
1 v 7	0.4500	-0.5001	to 1.4001
1 v 8	-0.4400	-1.3901	to 0.5101
1 v 9	-0.5800	-1.5301	to 0.3701
1 v 10	-0.2400	-1.1901	to 0.7101
1 v 11	-0.6600	-1.6101	to 0.2901
1 v 12	-0.3100	-1.2601	to 0.6401
1 v 13	-0.2400	-1.1901	to 0.7101
2 v 3	0.1350	-0.8151	to 1.0851
2 v 4	-0.3750	-1.3251	to 0.5751
2 v 5	-0.2050	-1.1551	to 0.7451
2 v 6	-0.2850	-1.2351	to 0.6651
2 v 7	0.7950	-0.1551	to 1.7451
2 v 8	-0.0950	-1.0451	to 0.8551
2 v 9	-0.2350	-1.1851	to 0.7151
2 v 10	0.1050	-0.8451	to 1.0551
2 v 11	-0.3150	-1.2651	to 0.6351
2 v 12	0.0350	-0.9151	to 0.9851
2 v 13	0.1050	-0.8451	to 1.0551
3 v 4	-0.5100	-1.4601	to 0.4401
3 v 5	-0.3400	-1.2901	to 0.6101
3 v 6	-0.4200	-1.3701	to 0.5301
3 v 7	0.6600	-0.2901	to 1.6101
3 v 8	-0.2300	-1.1801	to 0.7201
3 v 9	-0.3700	-1.3201	to 0.5801
3 v 10	-0.0300	-0.9801	to 0.9201
3 v 11	-0.4500	-1.4001	to 0.5001
3 v 12	-0.1000	-1.0501	to 0.8501
3 v 13	-0.0300	-0.9801	to 0.9201
4 v 5	0.1700	-0.7801	to 1.1201
4 v 6	0.0900	-0.8601	to 1.0401
4 v 7	1.1700	0.2199	to 2.1201

4 v 8	0.2800	-0.6701	to 1.2301
4 v 9	0.1400	-0.8101	to 1.0901
4 v 10	0.4800	-0.4701	to 1.4301
4 v 11	0.0600	-0.8901	to 1.0101
4 v 12	0.4100	-0.5401	to 1.3601
4 v 13	0.4800	-0.4701	to 1.4301
5 v 6	-0.0800	-1.0301	to 0.8701
5 v 7	1.0000	0.0499	to 1.9501
5 v 8	0.1100	-0.8401	to 1.0601
5 v 9	-0.0300	-0.9801	to 0.9201
5 v 10	0.3100	-0.6401	to 1.2601
5 v 11	-0.1100	-1.0601	to 0.8401
5 v 12	0.2400	-0.7101	to 1.1901
5 v 13	0.3100	-0.6401	to 1.2601
6 v 7	1.0800	0.1299	to 2.0301
6 v 8	0.1900	-0.7601	to 1.1401
6 v 9	0.0500	-0.9001	to 1.0001
6 v 10	0.3900	-0.5601	to 1.3401
6 v 11	-0.0300	-0.9801	to 0.9201
6 v 12	0.3200	-0.6301	to 1.2701
6 v 13	0.3900	-0.5601	to 1.3401
7 v 8	-0.8900	-1.8401	to 0.0601
7 v 9	-1.0300	-1.9801	to -0.0799
7 v 10	-0.6900	-1.6401	to 0.2601
7 v 11	-1.1100	-2.0601	to -0.1599
7 v 12	-0.7600	-1.7101	to 0.1901
7 v 13	-0.6900	-1.6401	to 0.2601
8 v 9	-0.1400	-1.0901	to 0.8101
8 v 10	0.2000	-0.7501	to 1.1501
8 v 11	-0.2200	-1.1701	to 0.7301
8 v 12	0.1300	-0.8201	to 1.0801
8 v 13	0.2000	-0.7501	to 1.1501
9 v 10	0.3400	-0.6101	to 1.2901
9 v 11	-0.0800	-1.0301	to 0.8701
9 v 12	0.2700	-0.6801	to 1.2201
9 v 13	0.3400	-0.6101	to 1.2901
10 v 11	-0.4200	-1.3701	to 0.5301
10 v 12	-0.0700	-1.0201	to 0.8801
10 v 13	0.0000	-0.9501	to 0.9501
11 v 12	0.3500	-0.6001	to 1.3001
11 v 13	0.4200	-0.5301	to 1.3701
12 v 13	0.0700	-0.8801	to 1.0201

Anova tables for Amino Nitrogen (Pumpkin)

SAMPLE 1

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	32.8516	4	8.2129	91.25	0.05
Residual	0.9000	10	0.0900		
Total	33.7516	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.2150	-1.0923	to 0.6623
0 hour v 48 hour	-1.1650	-2.0423	to -0.2877
0 hour v 72 hour	-2.6950	-3.5723	to -1.8177
0 hour v 96 hour	-3.8550	-4.7323	to -2.9777
24 hour v 48 hour	-0.9500	-1.8273	to -0.0727
24 hour v 72 hour	-2.4800	-3.3573	to -1.6027
24 hour v 96hour	-3.6400	-4.5173	to -2.7627
48 hour v 72 hour	-1.5300	-2.4073	to -0.6527
48 hour v 96 hour	-2.6900	-3.5673	to -1.8127
72 hour v 96 hour	-1.1600	-2.0373	to -0.2827

SAMPLE 2

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	45.6290	4	11.4072	126.75	0.05
Residual	0.9000	10	0.0900		
Total	46.5290	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.2150	-1.0923	to 0.6623
0 hour v 48 hour	-1.4600	-2.3373	to -0.5827
0 hour v 72 hour	-3.3100	-4.1873	to -2.4327
0 hour v 96 hour	-4.4650	-5.3423	to -3.5877
24 hour v 48 hour	-1.2450	-2.1223	to -0.3677
24 hour v 72 hour	-3.0950	-3.9723	to -2.2177
24 hour v 96hour	-4.2500	-5.1273	to -3.3727
48 hour v 72 hour	-1.8500	-2.7273	to -0.9727
48 hour v 96 hour	-3.0050	-3.8823	to -2.1277
72 hour v 96 hour	-1.1550	-2.0323	to -0.2777

SAMPLE 3

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	42.0148	4	10.5037	116.71	0.05
Residual	0.9000	10	0.0900		
Total	42.9148	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.2300	-1.1073	to 0.6473
0 hour v 48 hour	-1.2900	-2.1673	to -0.4127
0 hour v 72 hour	-3.3100	-4.1873	to -2.4327
0 hour v 96 hour	-4.2000	-5.0773	to -3.3227
24 hour v 48 hour	-1.0600	-1.9373	to -0.1827
24 hour v 72 hour	-3.0800	-3.9573	to -2.2027
24 hour v 96hour	-3.9700	-4.8473	to -3.0927
48 hour v 72 hour	-2.0200	-2.8973	to -1.1427
48 hour v 96 hour	-2.9100	-3.7873	to -2.0327
72 hour v 96 hour	-0.8900	-1.7673	to -0.0127

SAMPLE 4

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	38.9660	4	9.7415	108.24	0.05
Residual	0.9000	10	0.0900		
Total	39.8660	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.1800	-1.0573	to 0.6973
0 hour v 48 hour	-0.3300	-1.2073	to 0.5473
0 hour v 72 hour	-2.7800	-3.6573	to -1.9027
0 hour v 96 hour	-3.9500	-4.8273	to -3.0727
24 hour v 48 hour	-0.1500	-1.0273	to 0.7273
24 hour v 72 hour	-2.6000	-3.4773	to -1.7227
24 hour v 96hour	-3.7700	-4.6473	to -2.8927
48 hour v 72 hour	-2.4500	-3.3273	to -1.5727
48 hour v 96 hour	-3.6200	-4.4973	to -2.7427
72 hour v 96 hour	-1.1700	-2.0473	to -0.2927

SAMPLE 5

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	50.4822	4	12.6206	140.23	0.05
Residual	0.9000	10	0.0900		
Total	51.3822	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.2200	-1.0973	to 0.6573
0 hour v 48 hour	-1.2700	-2.1473	to -0.3927
0 hour v 72 hour	-3.3500	-4.2273	to -2.4727
0 hour v 96 hour	-4.7100	-5.5873	to -3.8327
24 hour v 48 hour	-1.0500	-1.9273	to -0.1727
24 hour v 72 hour	-3.1300	-4.0073	to -2.2527
24 hour v 96hour	-4.4900	-5.3673	to -3.6127
48 hour v 72 hour	-2.0800	-2.9573	to -1.2027
48 hour v 96 hour	-3.4400	-4.3173	to -2.5627
72 hour v 96 hour	-1.3600	-2.2373	to -0.4827

SAMPLE 6

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	39.5830	4	9.8957	109.95	0.05
Residual	0.9000	10	0.0900		
Total	40.4830	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.1800	-1.0573	to 0.6973
0 hour v 48 hour	-1.2600	-2.1373	to -0.3827
0 hour v 72 hour	-3.4500	-4.3273	to -2.5727
0 hour v 96 hour	-3.8800	-4.7573	to -3.0027
24 hour v 48 hour	-1.0800	-1.9573	to -0.2027
24 hour v 72 hour	-3.2700	-4.1473	to -2.3927
24 hour v 96hour	-3.7000	-4.5773	to -2.8227
48 hour v 72 hour	-2.1900	-3.0673	to -1.3127
48 hour v 96 hour	-2.6200	-3.4973	to -1.7427
72 hour v 96 hour	-0.4300	-1.3073	to 0.4473

SAMPLE 7

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	32.1186	4	8.0297	89.22	0.05
Residual	0.9000	10	0.0900		
Total	33.0186	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.2400	-1.1173	to 0.6373
0 hour v 48 hour	-1.2500	-2.1273	to -0.3727
0 hour v 72 hour	-3.2500	-4.1273	to -2.3727
0 hour v 96 hour	-3.4600	-4.3373	to -2.5827
24 hour v 48 hour	-1.0100	-1.8873	to -0.1327
24 hour v 72 hour	-3.0100	-3.8873	to -2.1327
24 hour v 96hour	-3.2200	-4.0973	to -2.3427
48 hour v 72 hour	-2.0000	-2.8773	to -1.1227
48 hour v 96 hour	-2.2100	-3.0873	to -1.3327
72 hour v 96 hour	-0.2100	-1.0873	to 0.6673

SAMPLE 8

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	31.5979	4	7.8995	87.77	0.05
Residual	0.9000	10	0.0900		
Total	32.4979	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.1100	-0.9873	to 0.7673
0 hour v 48 hour	-1.1650	-2.0423	to -0.2877
0 hour v 72 hour	-3.1500	-4.0273	to -2.2727
0 hour v 96 hour	-3.3800	-4.2573	to -2.5027
24 hour v 48 hour	-1.0550	-1.9323	to -0.1777
24 hour v 72 hour	-3.0400	-3.9173	to -2.1627
24 hour v 96hour	-3.2700	-4.1473	to -2.3927
48 hour v 72 hour	-1.9850	-2.8623	to -1.1077
48 hour v 96 hour	-2.2150	-3.0923	to -1.3377

72 hour v 96 hour	-0.2300	-1.1073	to 0.6473

SAMPLE 9

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	38.0458	4	9.5114	105.68	0.05
Residual	0.9000	10	0.0900		
Total	38.9458	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.1800	-1.0573	to 0.6973
0 hour v 48 hour	-1.1400	-2.0173	to -0.2627
0 hour v 72 hour	-3.0200	-3.8973	to -2.1427
0 hour v 96 hour	-4.0300	-4.9073	to -3.1527
24 hour v 48 hour	-0.9600	-1.8373	to -0.0827
24 hour v 72 hour	-2.8400	-3.7173	to -1.9627
24 hour v 96hour	-3.8500	-4.7273	to -2.9727
48 hour v 72 hour	-1.8800	-2.7573	to -1.0027
48 hour v 96 hour	-2.8900	-3.7673	to -2.0127
72 hour v 96 hour	-1.0100	-1.8873	to -0.1327

SAMPLE 10

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	41.3754	4	10.3439	114.93	0.05
Residual	0.9000	10	0.0900		
Total	42.2754	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.2950	-1.1723	to 0.5823
0 hour v 48 hour	-1.3550	-2.2323	to -0.4777
0 hour v 72 hour	-3.5750	-4.4523	to -2.6977
0 hour v 96 hour	-4.0250	-4.9023	to -3.1477
24 hour v 48 hour	-1.0600	-1.9373	to -0.1827
24 hour v 72 hour	-3.2800	-4.1573	to -2.4027
24 hour v 96hour	-3.7300	-4.6073	to -2.8527
48 hour v 72 hour	-2.2200	-3.0973	to -1.3427
48 hour v 96 hour	-2.6700	-3.5473	to -1.7927
72 hour v 96 hour	-0.4500	-1.3273	to 0.4273

SAMPLE 11

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	39.4080	4	9.8520	109.47	0.05
Residual	0.9000	10	0.0900		
Total	40.3080	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.2400	-1.1173	to 0.6373
0 hour v 48 hour	-1.3600	-2.2373	to -0.4827
0 hour v 72 hour	-3.3300	-4.2073	to -2.4527
0 hour v 96 hour	-4.0200	-4.8973	to -3.1427
24 hour v 48 hour	-1.1200	-1.9973	to -0.2427
24 hour v 72 hour	-3.0900	-3.9673	to -2.2127
24 hour v 96hour	-3.7800	-4.6573	to -2.9027
48 hour v 72 hour	-1.9700	-2.8473	to -1.0927

48 hour v 96 hour	-2.6600	-3.5373	to -1.7827
72 hour v 96 hour	-0.6900	-1.5673	to 0.1873

SAMPLE 12

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	42.6760	4	10.6690	118.54	0.05
Residual	0.9000	10	0.0900		
Total	43.5760	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-0.1400	-1.0173	to 0.7373
0 hour v 48 hour	-1.4700	-2.3473	to -0.5927
0 hour v 72 hour	-3.6500	-4.5273	to -2.7727
0 hour v 96 hour	-3.9700	-4.8473	to -3.0927
24 hour v 48 hour	-1.3300	-2.2073	to -0.4527
24 hour v 72 hour	-3.5100	-4.3873	to -2.6327
24 hour v 96hour	-3.8300	-4.7073	to -2.9527
48 hour v 72 hour	-2.1800	-3.0573	to -1.3027
48 hour v 96 hour	-2.5000	-3.3773	to -1.6227
72 hour v 96 hour	-0.3200	-1.1973	to 0.5573

SAMPLE 13

Source of variation	Sum squares	DF	Mean square	F statistic	p
Groups	32.5214	4	8.1304	90.34	0.05
Residual	0.9000	10	0.0900		
Total	33.4214	14			

LSD

Contrast	Difference	95% CI	
0 hour v 24 hour	-1.3300	-2.2073	to -0.4527
0 hour v 48 hour	-2.0100	-2.8873	to -1.1327
0 hour v 72 hour	-3.5900	-4.4673	to -2.7127
0 hour v 96 hour	-4.0100	-4.8873	to -3.1327
24 hour v 48 hour	-0.6800	-1.5573	to 0.1973
24 hour v 72 hour	-2.2600	-3.1373	to -1.3827
24 hour v 96hour	-2.6800	-3.5573	to -1.8027
48 hour v 72 hour	-1.5800	-2.4573	to -0.7027
48 hour v 96 hour	-2.0000	-2.8773	to -1.1227
72 hour v 96 hour	-0.4200	-1.2973	to 0.4573

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