ELECTRONIC NOSE EVALUATION OF GRAPE MATURITY

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ABSTRACT

Grape maturity is a critical attribute impacting potential wine quality. Maturity evaluation is difficult due to the many interrelated factors that impact physicochemical changes and limitations in the understanding of these factors. Current methods of assessing grape maturity are destructive, expensive, time consuming, subjective, and do not always strongly correlated to potential wine quality. This study evaluated the applicability of a conducting polymer-based electronic nose to monitor grape maturity by analyzing headspace volatiles. In the first part of the study, system and experimental parameters affecting the electronic nose operation were investigated to optimize detection of wine grape aroma. In the second part, the ability of an electronic nose to classify Cabernet Sauvignon (*Vitis vinifera* L.) grapes based on maturity was investigated. Maturity of samples collected at different weeks post-bloom was evaluated by measuring berry weight, pH, Brix, titratable acidity, total phenols, color intensity, hue, total anthocyanins, and total and phenol-free glycosides. Results were compared, using discriminant and canonical discriminant analysis, with analysis of headspace volatiles via the hand-held electronic nose. The electronic nose was able to determine the difference between the sample groups. Field measurements demonstrated the potential for the electronic nose as a rapid, non-destructive tool for evaluating grape maturity.

DEDICATION

My father, Ibrahim M. Athamneh, and mother, Ameena M. Al-Yaseen

My brothers Safwan, Khaled, and Mohammad

My beloved sister Hana

… To whom I owe everything I have achieved in my life.

ATTRIBUTION

Author Ahmad I. Athamneh is the major contributor and writer of the manuscripts in chapter three and chapter four of this thesis. Co-authors Dr. Parameswarakumar Mallikarjunan, Ph.D., Food Engineering, University of Guelph, Canada 1993, Committee Chair, and Prof. Bruce W. Zoecklein, Ph.D., Food Science and Technology, Virginia Tech, 1995, Committee member, provided advise, supervision, funding, and laboratory support.

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CHAPTER ONE:

INTRODUCTION

1.1 BACKGROUND

Wine consumption in the United States has been rising slowly and consistently over the last 11 years. Last year, consumers in the United States purchased 627 million gallons, valued at USD 26.6 billion retail, with a 5 percent growth from the year 2004 (USDA 2006). Wine sales were up 12 percent in value and 5 percent in quantity, which reflects increased demand for quality product. Wine priced at USD 7 per bottle and above showed good growth. These numbers are in agreement with Heien and Martin (2003) observation that more people are drinking less, but better, wine. Consumers have upgraded their taste and ever more demanded wine of better quality.

The United States' share of the global wine market stood at 5 percent in 2005, down 17 percent from the year before. Wine exports fell to USD 659 from USD 795.5 due to increased global competition highlighted by the USDA (2006) report. The greatest competition comes from the European Union and, to a lesser degree, Australia. Italy, France, Spain, Portugal and Germany had 64 percent of the global wine export market. In addition to the decrease in exports, the year 2005 witnessed a record high wine imports to the United States of USD 3.79 billion.

Under such circumstances, winemakers find themselves in a position where they cannot compromise on quality. Obviously, this has a sudden impact on grape growers, since winemakers are only willing to pay good prices for good quality grapes. For example, the price of a ton of grapes may range form USD 65, just enough to cover picking cost, to USD 3,700 (Heien and Martin 2003). Therefore, the objective of grape growers is clear: to deliver maximum quality crop at minimum production cost.

Determining the optimum level of maturity is key to achieving full economic value of the grape crop. The quality of the grapes at harvest determines the maximum quality potential of wine. For most wine styles, it is important for grape growers to maximize the level of aromas in the grapes. High levels of aroma development are only possible if certain prerequisites are met, including optimum nutrients, water, light, vine balance (exposed leaf area to fruit-weight ratio), and maturity. Each grape variety has a certain spectrum of aromas that exist in the fruit. Often, five to 20 aromas are sufficient to characterize a particular grape variety. The combination of aromas changes during the ripening process. In general, grassy aromas predominate early, and floral, fruity or spicy aromas evolve later in the ripening process. This evolution is not directly related to sugar accumulations, particularly in a warm climate such as Virginia's. However, changes in aroma follow the general course of berry development.

Currently, growers and winemakers evaluate maturity by sensory evaluation and some physicochemical measurements on fruit or juice. While a helpful maturity gauge, sensory evaluation of grape juice aroma is confounded by a host of variables, including sample size, processing technique, and subjectivity. The physicochemical indices, including

levels of sugar, pH and acidity level, are objective measurements but do not necessarily strongly correlate to grape aroma. In addition, sensory analysis and physicochemical maturity measurements are destructive, expensive, and often time consuming.

The electronic nose is a relatively new technology utilized in a variety of applications in the food industry. It is designed to mimic the human olfactory system, and intended to aid in decision-making when volatile compounds correlate strongly with certain sample attributes. This technology has been suggested as a non-destructive tool for maturity assessment of various fruits including apples, bananas, mandarins, nectarines, peaches and pears. To our knowledge, there have been no studies of the usefulness of the electronic nose in the study of wine grape maturity.

1.2 SIGNIFICANCE

Because of the difficulties associated with current methods, there is a need for a simple, reliable, and objective technique for evaluation of fruit maturity. The successful implementation of electronic nose will not only reduce the cost of maturity evaluation, but will also help determining optimum maturity based on the quality of grape volatiles responsible for varietal aroma. To that end, this research evaluated the capability of a conducting polymer-based electronic nose system to monitor Cabernet Sauvignon (*Vitis vinifera* L.) fruit maturity by analyzing headspace volatiles.

1.3 HYPOTHESIS

A conducting polymer-based electronic nose system can discriminate grape samples based on levels of maturity, and, therefore, can be used as a simple, non-destructive and objective tool for maturity evaluation.

1.4 OBJECTIVES

The objectives of this research are to:

1) optimize the electronic nose system for optimum detection of grape volatiles;

2) use conventional maturity indices to evaluate maturity levels for samples harvested at different dates;

3) test the capability of the electronic nose to discriminate Cabernet Sauvignon (*Vitis vinifera* L.) fruit samples of different maturities in the laboratory; and

4) test the capability of the electronic nose system to non-destructively discriminate Cabernet Sauvignon (*Vitis vinifera* L.) fruit samples of different maturities in the vineyard

1.5 THESIS OUTLINE

This thesis consists of four chapters. Following the introduction in chapter one, chapter two sets the stage for this research by reviewing related published work on the subjects. The chapter provides a background on the physiology of grape berry development and the factors that define maturity. It also reviews currently used maturity indices, including

physicochemical measurement of grape composite and evaluation of aroma potential. Chapter two ends by a survey of the studies published in the past six year on the applications of electronic nose in the food industry. Chapter three is a manuscript written for the Journal of Food Science. The manuscript reports the study conducted to optimize the electronic nose for optimum detection of wine-grape aroma. Chapter four is a manuscript submitted to the American Journal of Enology and Viticulture. This manuscript reports the study conducted to evaluate the capacity of the Cyranose 320 electronic nose system to monitor Cabernet Sauvignon (*Vitis vinifera* L.) fruit maturity.

CHAPTER TWO:

LITERATURE REVIEW

2.1 GRAPE MATURITY

Determining the best time to harvest is one of the most critical decisions faced by grape growers and winemakers. The quality of the grapes at harvest determines the maximum potential quality of the wine. Underripe grapes are low in sugar, high in acidity, and have 'green' flavors and aromas or harsh tannins. Overripe grapes may also have off or uncharacteristic aromas and too low acidity. It is safe to say that, if fruit maturity evaluations are not performed properly, subsequent winemaking step to help assure quality may be of limited value (Zoecklein 2001).

Unfortunately, grape maturity evaluation is difficult due to the involvement of many interrelated factors impacting physicochemical changes in berries (Coombe 1992, Robinson and Davies 2000), and the limitations in the understanding of these factor (Coombe 1992, Zoecklein et al. 1999a, Watson 2003). Adding to the difficulty is the fact that there are no universal standards that define optimum maturity. In fact, maturity is most often a subjective judgment, primarily a function of the intended use for the grapes.

2.1.1 FRUIT DEVELOPMENT

The Grape berry exhibits two distinct phases of growth (Figure 1) separated by a lag period (Coombe 1992). After flowering and fruit set, the first phase, or stage I, is characterized by the rapid increase in size of the pericarp (flesh and skin) and seeds, and the accumulation and storage of organic acids, mainly tartaric and malic acid, in mesocarp (flesh). During this stage, the berry is green and hard, and accumulates little sugar.

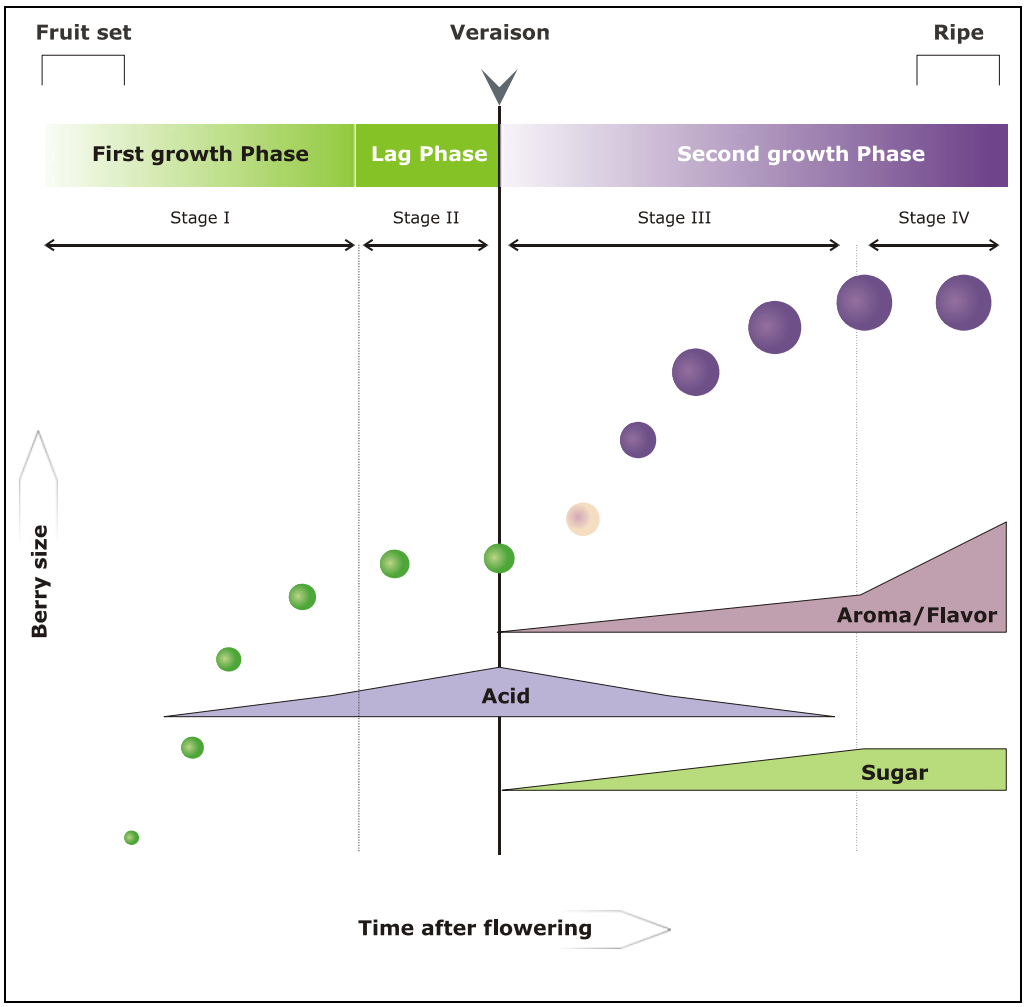

Figure 1. Schematic illustration of grape berry development.

The first phase is followed by a lag period, or stage II, in which cell expansion slows down while seed maturation is completed. The boundaries between stage II and stage III is called veraison which signals the beginning of the second growth phase. Stage III is characterized by softening of the berry, rapid color change, further increase in berry volume, accumulation of sugar, synthesis of anthocyanins, degradation of chlorophyll, metabolism of malic acid as the major carbon source for respiration, and accumulation of aroma and flavor components.

Coombe and McCarthy (1997) suggested a distinct fourth stage that takes place during the advanced stages of fruit ripening. Stage IV, or engustment as they termed it, is characterized by rapid increase in the accumulation of aroma and flavor components, with little to no sugar accumulation. This characterization of the berry ripening process had significant implications on the understanding of maturity evaluation. It essentially meant that sugar level, the traditionally most commonly used index of maturity, was no longer trustworthy.

2.1.2 VARIETAL AROMA

Each grape variety has its unique varietal character. For example, Chardonnay has the characteristics of the fruitiness of apples, pears, and lemons, as compared to the perfumed floral character of Riesling, the spiciness of Shiraz, and the cassis and cedar characteristics in Cabernet Sauvignon. Varietal aroma becomes increasingly defined and distinct as berries mature.

It could be argued that one of the most important maturity parameters is varietal aroma and its intensity. Even though aroma substances in the wine arise from several factors

involved in the winemaking process, one of the fundamental sources of aroma that distinguishes quality wine is the varietal character originated from the grapes (Jordan and Croser 1983). This is the primary reason way many producers evaluate aroma as a maturity gauge.

2.1.3 SECONDARY METABOLITES

Varietal aroma is the product of grape-derived secondary metabolites (Gunata et al. 1985, Hardie et al. 1996) present in grapes as free volatiles or non-volatile bound sugar conjugates. Free volatiles, which may contribute directly to odor, are a divers group of potent aroma compounds such as monoterpenes, norisoprenoids, volatile phenols and methoxypyrazines (Zoecklein et al. 1999a). Collectively, these compounds impart the varietal character of the grapes. For example, monoterpenes contribute floral and fruity aromas while methoxypyrazines contribute vegetative and herbaceous aromas.

The non-volatile sugar conjugates, or glycosides, include a wide range of components representing, in part, the potential aroma of a grape variety. During winemaking, free volatiles can be liberated, through enzyme or acid hydrolysis of the glycosidic bond, and potentially enhancing wine quality (Williams et al. 1982, Gunata et al. 1990, Francis et al. 1992). Therefore, analysis of grape glycosides gives an estimation of the total pool of secondary metabolites, which include important aroma precursors (Abbott et al. 1993). Indeed, it has been demonstrated that there is a positive correlation between the concentration of bound secondary metabolites and ultimate wine quality (Abbott et al. 1991, Francis et al. 1992, Sefton 1998).

2.1.4 STRATEGIES TO ENHANCE MATURITY

Advances in berry ripening research, and the increased understanding of the origins of aroma potential directed attention back to the vineyard where grapes are originally made (Lund and Bohlmann 2006). There has been a tremendous interest in studying factors that influence the concentration of aroma components and their precursors in fruits, including the natural environment (Jackson and Lombard 1993), vineyard management practices (Hardie and Martin 1990, Long 1997, Zoecklein et al. 1998b), and vine genotype (Martin and Bohlmann 2004). Along a parallel line, research also focused on the ways and means by which aroma aroma potential can be utilized to enhance wine quality (Williams et al. 1982, Gunata et al. 1985, 1986, Gunata et al. 1990, Zoecklein et al. 1998a, Zoecklein et al. 1999b, Fernandez-Gonzalez et al. 2003, D'Incecco et al. 2004, Palomo et al. 2006).

2.2 GRAPE MATURITY EVALUATION

Harvest decision is often based on a combination of factors including vineyard history, sensory evaluation, and measurements of some physicochemical indices such as weight, color, sugar content, pH, and titratable acidity (Jordan and Croser 1983, Zoecklein et al. 1999a, Bisson 2001, Watson 2003, Allen 2004, Hellman 2004). There is no single factor or index that can be used independently as a reliable measure of maturity (Jordan and Croser 1983, Zoecklein et al. 1999a), and there is no single set of numbers that defines maturity for a grape variety under all circumstances and for all purposes. Maturity is often a subjective judgment defined by individuals for a particular use.

The following is a review of the main maturity indices that may be used to aid harvest decision. These are primarily physicochemical measurements (or estimations) of the

grape composite. Some indices are easily measured by grape growers or winemakers, including berry weight, soluble solids, pH and titratable acidity, and therefore considered to be a standard viticultural practice. While other indices (such as analysis of glycosides, anthocyanins, and phenols) require more elaborate procedures, expensive equipment, and advanced experience, and, thus, inadequate for practical use.

2.2.1 SAMPLING CONSIDERATION

The grape berry is an independent biochemical unit, which has the capacity to synthesize primary metabolites essential for survival (such as sugar, amino acids, minerals, and micronutrients), and all other berry components, including aroma and flavor compounds (Coombe 1992, Robinson and Davies 2000). This essentially means that there is a potential for a large variation in maturity between berries within the same cluster, and therefore within the vines and the vineyard. The fact that each berry is potentially different from others has a very serious implication on sampling for maturity evaluation (Rankine et al. 1962, Zoecklein et al. 1999a).

2.2.2 PHYSICOCHEMICAL MATURITY INDICES

Berry weight is often measured when evaluating maturity. Many grape-derived secondary metabolites, including aroma/flavor and phenolic compounds, are located in the skin. Therefore, the change in berry size, estimated by weight, should be considered when evaluating maturity. Additionally, if monitored periodically using a representative sample, weight can provide a useful index of the hydration state of the berries. Hydration, or dehydration, affects the concentration of different substances in berries. Thus berry weight can be used to, correct or, better evaluate measurements of other maturity indices.

Soluble solids content, mainly sugars, is the most widely used index for maturity evaluation. Usually measured in °Brix (g/100 mL or % soluble solids), soluble solids can be easily determined in the laboratory and in the field, and indicates the potential alcohol yield of fermentation. Degree Brix cab be strongly correlated to wine quality in cold to cool regions, but in warm regions the correlation is much less robust. Additionally, studies showed weak correlation between sugar levels and the accumulation of secondary metabolites, and thus ultimate wine quality (Coombe 1992). Furthermore, °Brix measurement is a ratio (wt/wt) of sugar to water, and thus may change as a result of the hydration status of the berry (Zoecklein et al. 1999a). For instance, °Brix may show no change, but in fact there may be a significant change in berry weight (decrease or increase) due to hydration or dehydration. Accordingly, Zoecklein et al. (1999a) suggested using the 'sugar per berry' index, which utilizes the normal °Brix measurement but takes into account berry weight. Sugar per berry provides a more realistic assessment of maturation.

Although evaluation of 'sugar ripeness' remains a standard viticultural practice, it has been increasingly recognized that there is a need for backing up sugar measurements with observations of acid, color, aroma and flavor development (Amerine and Winkler 1940, Coombe et al. 1980, Jordan and Croser 1983, Zoecklein 2001, Watson 2003, Allen 2004) (Du Plessis 1984), (Bisson 2001).

Earlier, Amerine and Winkler (1940) suggested using °Brix/acid (usually measured as titratable acidity) ratio as the basis for determining the best dates for harvest. However, Coombe et al. (1980) pointed to the defects of the °Brix/acid ratio, primarily the fact that

titratable acidity is an untrustworthy indicator of acidity in grapes. Accordingly they suggested a maturity index that combines °Brix and pH as an alternative, and found that °Brix × pH^2 gave acceptable criteria for indicating maturity. Sinton et al. (1978) found that °Brix × pH was the most practical indicator of aroma intensity, but there was no significant correlation between °Brix × pH and the overall wine sensory scores.

Titratable acidity and **pH** are important maturity parameters due to their effect on fermentation, by influencing oxidation-reduction reactions, taste balance, microbial and chemical stability, and on color and flavor of wines. Titratable acidity estimates acid content in the grape juice, primarily tartaric and malic acids. pH is another measure of acidity and is generally inversely correlated with titratable acidity, but high levels of potassium in the grape juice can elevate pH levels for a given titratable acidity value (Allen 2004). Both indices are easily measured in the laboratory, but they are highly influenced by sample preparation methods (Zoecklein et al. 1999a, Watson 2003). It has been reported that significant variations in the analysis will occur if sample preparation methods are not standardized (Zoecklein et al. 1999a). Additionally, there are no universal standards as to what are the desired values for titratable acidity and pH (Amerine and Winkler 1940, Coombe et al. 1980, Jordan and Croser 1983).

Grape phenols are secondary metabolites that include many compounds with different chemical and sensory properties. Phenolic molecules can be broadly categorized into flavonoid and non-flavonoid phenols. Grape phenols have a significant influence on wine structure including volume, tannin intensity, astringency, bitterness and dryness. Increases in total phenolic compounds have been associated with maturity. Total phenols

tend to increase during berry ripening in both red and white varieties (Singleton 1966). Phenols can be estimated by spectrometric analysis or determined using HPLC (Zoecklein et al. 1999a). However, when evaluating grape phenols, it is qualitative, not quantitative, factors that are most significant (Zoecklein 2001). Therefore, measurements of phenols may not indicate the actual quality of the grapes, in particular the degree of maturation. Additionally, the behavior of phenolic compounds varies, decreasing or increasing, depending upon the part of the berry studied. During maturation, phenolic compounds in the skins increase and those of the seeds slightly decrease (Zoecklein 2001).

Anthocyanins are important flavonoid phenolic compounds found predominantly in the skin of the fruit (Robinson and Davies 2000). They are especially important in red wines as they are the primary coloring compounds in the juice. Anthocyanins can be estimated by spectrometric analysis (Zoecklein et al. 1999a) or determined using HPLC (Wulf and Nagel 1978). Anthocyanins levels have been associated with maturity (Gonzalez-San Jose 1990). The concentration of anthocyanins increases during maturation, and reaches maximum when berries are fully ripe (Du Plessis 1984). However, the biochemical pathways for the production of anthocyanins and aroma/flavor compounds in berries are different (Zoecklein et al. 1999a, Robinson and Davies 2000). Therefore, levels of anthocyanins do not necessarily correlate to aroma/flavor potential.

2.2.3 EVALUATION OF AROMA POTENTIAL

During the advanced stages of fruit maturity, the accumulation of the pool of free aroma components and their precursors (secondary metabolites) increases rapidly (Coombe and

McCarthy 1997). In this stage, minimum to no change occurs to levels of sugar and some other primary metabolites (Coombe 1992, Coombe and McCarthy 1997). For instance, any change to sugar levels in this stage is attributed to dehydration, not a physiological change (Zoecklein 2001). This essentially means that maturity indices, such as °Brix, acidity and pH, that measure primary metabolites, say little about aroma potential, and, accordingly, ultimate wine quality. Therefore, it is important to consider secondary metabolites in any maturity evaluation plan in order to achieve a more objective measure of potential aroma components in the fruit.

Currently, the most widely used technique of evaluating aroma potential is human sensory analysis, which is made possible due to the relationship between grape aroma and maturity. Winemakers subjectively evaluate grape aroma to determine the point along the maturity continuum that best fits the type and style of the wine they want to make. However, for sensory analysis to be successful, it must be conducted by experienced individuals who have gained experience of maturity patterns in their vineyards, and use sensory information in conjunction with measurements of sugar, pH and acidity (Jordan and Croser 1983). Unfortunately, most winemakers are not in such position.

There are a number of analytical procedures that can be used to estimate aroma potential, and, thus, may provide reliable and objective measure of fruit maturity. These include analysis of free and potentially volatile terpenes (Dimitriadis and Williams 1984), total and phenol-free glycosides (Abbott et al. 1993, Zoecklein et al. 2000) and chromatographic methods (Salles et al. 1990, Voirin et al. 1992a, Voirin et al. 1992b,

Ebeler 2001, Sanchez-Palomo et al. 2005). However, these analyses are either restricted to high-terpene varieties, expensive and/or time consuming.

2.3 THE ELECTRONIC NOSE

The history of the electronic nose is brief. The idea of the electronic nose as an 'intelligent' chemical array sensor system was first proposed in 1982 at the University of Warwick in the UK by Persaud and Dodd (1982) (Gardner and Bartlett 1994, Schaller et al. 1998). Gardner and Bartlett (1994) define the electronic nose as "an instrument, which comprises an array of electronic chemical sensors with partial specificity and an appropriate pattern-recognition system, capable of recognising simple or complex odours." Regardless of the technology is use, all electronic noses operate according to the same concept: a mechanism of pattern recognition utilizes the response of a group of sensors, each having greater or lesser affinity to particular types or classes or volatile compounds, to form a digital *smellprint* that characterizes a specific aroma.

The different aspects of the electronic nose technology, including sensors, signal preparation and pattern-recognition techniques, have been discussed by many authors (Gardner and Bartlett 1994, Schaller et al. 1998, Strike et al. 1999, Taurino et al. 2003, Arshak et al. 2004, Deisingh et al. 2004, David et al. 2005, Skov and Bro 2005). The most widely used sensor technologies includes metal oxide semiconductor (MOS), conducting polymer (CP), quartz microbalance sensors (QMB), surface acoustic wave (SAW), and metal oxide semiconductor field effect transistors (MOSFET) . There are a number of commercial electronic nose systems available in the market (Van Deventer 2001, Strike et al. 1999), and most often these are designed as multipurpose instruments

that can be used for a variety of application and products. However, the trend is increasingly moving toward creating a custom system for one specific application (Schaller et al. 1998, Shevade et al. 2003).

2.3.1 APPLICATIONS TO FOOD INDUSTRY

The electronic nose has been suggested for a wide range of applications in various fields including quality and process control, security screening, military, medical diagnosis, and space program, but most of the studies have been focusing on the applicability of the electronic nose as a quality tool in the food industry. Schaller et al. (1998) reviewed (to date) the applications of different electronic nose systems to different foods including, meat, grains, coffee, beer, mushrooms, cheese, sugar, fish, blueberry, orange juice, cola, alcoholic beverages, and packaging. They concluded that the electronic nose can be regarded as an interesting tool for a quick 'yes or no' quality test, and could occasionally replace sensory analysis, and even perform better, in cases where non-odorous or irritant gases need to be detected.

For the last few years, more and more electronic nose applications have been suggested to food industry (Table 1). Considerable work has been carried out on hazel nuts, live oil, cheese, honey, tomato, poultry meat, milk, yogurt, wine, salmon, tuna, tea, truffle, coffee, cod roe, bread, peppers, cod fish, ginseng, ice cream, pectin gel, barley grain, peanut, beef, shrimp, and packaging material. Applications include spoilage detection, freshness assessment, process monitoring, shelf-life investigation, authenticity assessment, and maturity evaluation.

Table 1. Applications of different electronic nose systems to different foods.

Product	Application	Sensor Technology	Instrumental system/ Manufacturer	Reference
Apple	Maturity	CP	Cyranose 320, Cyrano Sciences, Pasadena, CA	Pathange et al. (2006)
Apple	Maturity	QMB	LibraNose, Technobiochip, Italy	Saevels et al. (2003)
barley grain	Mycotoxin contamination	MOSFET	VCM 422, S-SENCE, Linköping University, Linköping, Sweden	Olsson et al. (2002)
Beef	Microbial quality	CP	Cyranose 320, Cyrano Sciences, Pasadena, CA	Balasubramanian et al. (2004)
Bread	spoilage moulds	CP	BH114, Bloodhound Sensors Ltd, Leeds, UK	Keshri et al. (2002)
Cod Roe	Flavor profile	No information	FreshSense, IFL and Bodvaki-Maritech, Kópavogur, Iceland	Jonsdottir et al. (2004)
Coffee	Classification based on sensory parameters	MOS	Pico1, laboratory- made	Pardo and Sberveglieri (2002)
Crescenza Cheese	Shelf Life	MOS, MOSFET	model 3320, Applied Sensor Laboratory Emission Analyser; Applied Sensor Co., Linkoping, Sweden),	Benedetti et al. (2005)
Dairy products	Review article			Ampuero and Bosset (2003)
Egg	Freshness assessment	MOS	Laboratory-made	Ritaban et al. (2003)
Emmental Cheese	ripening	QMB	Laboratory-made	Bargon et al. (2003)
Fish	Freshness	MOS	Laboratory-made	O'Connell et al.(2001)
Fruit and grape wine	Discrimination based on type of fruit	(MOS)	FOX 3000; Alpha MOS; France	McKellar et al. (2005)
Fuji Apples	Maturity	QMB	LibraNose, Technobiochip, Italy	Echeverria et al. (2004)
Ginseng	Change in aroma profile during preperaton	(MOS)	FOX 3000; Alpha MOS; France	Lee et al. (2005)
Ground red peppers	capsaicin, dihydrocapsaicin, and total capsaicinoids levels	CP	e-NOSE 4000, EEV Inc., Amsford, NJ	Korel et al. (2002)

Product	Application	Sensor Technology	Instrumental system/ Manufacturer	Reference
Hazel Nuts	Varietal aroma	CP	eNOSE 4000, EEV, Inc., NJ, USA	Alasalvar et al. (2004)
Honey	Classification of samples of different geographical and botanical origin	MOS, MOSFET	model 3320 Applied Sensor Lab Emission Analyser; Applied Sensor Co., Linkoping, Sweden	Benedetti et al.(2004)
Mandarin	Maturity	MOS	PEN2, WMA Airsense Analysentechnik GmbH, Schwerin, Germany	Gomez et al. (2006)
Many fruits	Maturity	MOS	Laboratory-made	Brezmes et al. (2005)
Milk	Spoilage	CP	model BH-114: Bloodhound Sensors Ltd., Leeds, UK	Magan et al. (2001)
Milk	Classification of samples from different dairies	MOS	Laboratory-made	Brudzewski et al. (2004)
Milk	Rancidity	MOS	IME-CNR laboratory in Lecce	Capone et al. (2001)
Milk	Microbial quality	CP	e-NOSE 4000, EEV Inc., Amsford, NJ	Korel and Balaban (2002)
Oak barrels	Monitoring of toasting homogeneity	MOS	Fox 4000, Alpha MOS, France	Chatonnet and Dubourdieu (1999)
Olive Oil	Rancid defect	CP	AromaScan plc, Crewe, U.K.	Aparicio et al. (2000)
Olive Oil	Dicriination of different types of oil	CP	Laboratory-made	Stella et al. (2000)
Oranges/Apples	Defects in post-harvest fruits	TSMR	University of Rome Tor Vergata and Technobiochip	Di Natale et al. (2001)
Packaging	Odor of retained solvents	CO, MOS, QMB	Cyranose 320, Cyrano Sciences, Pasadena, CA Fox 3000, Alpha MOS, France HKR Sensorsystems, Munich, Germany	Deventer and Mallikarjunan (2002)
Peanut	Off-flavor detection	CP	A-32S, AromaScan Inc., Hollis, NH	Osborn et al. (2001)
Peanuts	Flavor fade	QMB	HKR Sensorsystems, Munich, Germany	Williams et al. (2006)
Pectin gel	changes in flavor release with aging	MOS	Laboratory-made	Monge et al. (2004)

Product	Application	Sensor Technology	Instrumental system/ Manufacturer	Reference
Pink salmon	Detecting spoilage	CP	Cyranose 320, Cyrano Sciences, Pasadena, CA	Chantarachoti et al. (2006)
Pink lady Apple	maturity	MOS	Laboratory-made	Brezmes et al. (2001)
Poultry meat	Microbial quality	MOS	Fox 3000, Alpha MOS America, Inc, Hillsborough, NJ, USA	Dorothy D H Boothe (2002)
Raw and Cooked cod fish	Quality degradation during storage	CP	e-NOSE 4000, EEV Inc., Amsford, NJ	Korel et al. (2001)
Salmon Fillets	Microbial quality	CP	Aromascan, AromaScan Inc., Hollis, NH	Du et al. (2002)
Shrimp	Monitoring quality changes under different cooling conditions	No information	FreshSense, IFL and Bodvaki-Maritech, Kópavogur, Iceland	Zeng et al. (2005)
Strawberry ice cream	Discrimination based on fat content	IMCELL, MOS	MGD-1, Environics Oy, Kuopio, Finland	Miettinen et al. (2002)
Tea	flavours of teas manufactured under different processing conditions	MOS	Laboratory-made	Dutta et al. (2003)
Tomato	Various quality levels	QMB	enQbe, University of Rome 'Tor Vergata', Italy)	Berna et al. (2005)
Truffle	Aging	MOS	Pico1, laboratory- made	Falasconi et al. (2005)
Various dairy products	Classification based on type of product	MOS	Fox 4000 Alpha MOS, France	Collier et al. (2003)
Wine	Discrimination based on sensory quality	MOS	Laboratory-made	Penza and Cassano (2004a)
Wine	Discrimination based on sensory quality	TSMR	University of Rome Tor Vergata and Technobiochip	Di Natale et al. (2004)
Wine	Off-flavor detection	MOS	Fox 4000, Alpha MOS, France	Ragazzo-Sanchez et al. (2005)
Wine	Discrimination based on quality	MOS	Laboratory-made	Garcia et al. (2006)
Wine	Discrimination based on variety and origin	MOS	Laboratory-made	Santos et al. (2004)
Yellowfin Tuna	Microbial quality	CP	Aromascan, AromaScan Inc., Hollis, NH	Du et al. (2001)
Yogurt	Fermentation control	MOSFET, MOS	No information	Cimander et al. (2002)

In the wine industry, the electronic nose has been suggested as a tool to monitor toasting homogeneity of oak barrels (Chatonnet and Dubourdieu 1999), and for wine discrimination (Di Natale et al. 1996, Rong et al. 2000, Penza and Cassano 2004b, a, Ragazzo-Sanchez et al. 2005, Garcia et al. 2006). Santos et al. (2004) demonstrated that electronic nose evaluation of Madrid wines was consistent with GC/MS analyses.

The electronic nose has been also suggested as a non-destructive tool for maturity assessment of various fruits including apples (Brezmes et al. 2001, Saevels et al. 2003, Echeverria et al. 2004, Pathange et al. 2006), bananas (Llobet et al. 1999), mandarins (Gomez et al. 2006), and nectarines, peaches and pears (Brezmes et al. 2005).

The electronic nose has been proven as an effective discrimination tool when volatile compounds correlate with certain sample attributes. However, the vast majority of studies were carried out in the controlled environment of the laboratories and yet to be tested in real-life industrial settings. Before it can be treated as a reliable industrial instrument, more work on the feasibility and reproducibility is still needed.

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CHAPTER THREE:

OPTIMIZATION OF ELECTRONIC NOSE SYSTEM PARAMETERS FOR DETECTION OF WINE GRAPE AROMA

(Manuscript submitted to Journal of Food Science)

Optimization of Electronic Nose System Parameters for Detection of Wine Grape Aroma

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Short version of title: **E-nose optimization for grape aroma…**

Choice of journal section: **Food Engineering and Physical Properties**

ABSTRACT:

A conducting polymer-based electronic nose system was optimized for detection of wine grape aroma. Response surface methodology was used to study the effect of systems pump speed (60, 120, and 180 cc/min) and sample incubation time (15, 30, and 45 min) on sensors' response. The effect of sample temperature, in the range of 15 to 27 °C, was also studied. Optimum sensors response was obtained at 120 cc/min pump speed and 37 min incubation time. Sample temperature had a significant effect on the response of individual sensors, but had no effect on the discriminant function of the electronic nose. Results suggest a potential for the electronic nose to discriminate grape samples and, due to its portability and insensitivity to sample temperature, the electronic nose may be useful for field operation.

Key words: electronic nose, optimization, grape aroma, Cabernet Sauvignon, grape maturity

Introduction

Maturity is a critical attribute of grape quality and potential wine quality. Underripe grapes are low in sugar, high in acidity, and have 'green' flavors and aromas or harsh tannins. Overripe grapes may also have off or uncharacteristic aromas and too-low acidity. If maturity evaluations were not performed properly, subsequent winemaking measures to help assure quality will be of limited value (Jordan and Croser 1983; Zoecklein 2001).

Current methods of assessing grape maturity are time consuming, destructive, subjective, and do not always strongly correlate with grape varietal aroma, a decisive factor in determining potential wine quality. The difficulty stems from the many interrelated physicochemical changes that occur in berries during maturation (Coombe 1992; Robinson and Davies 2000), and the limitations of current used methods in evaluating these changes (Coombe 1992; Zoecklein and others 1999; Watson 2003). Berry weight, sugar content (°Brix), pH, titratable acidity, malic acid, and color are common maturity indices used individually or in combination (Zoecklein and others 1999). These physicochemical measurements are influenced by sampling variation and processing methodologies (Rankine and others 1962; Zoecklein and others 1999; Watson 2003). Additionally, sugar levels, acidity, pH, and color do not always strongly correlate with available grape-derived secondary metabolites (Zoecklein 2001) that are responsible for varietal aroma (Hardie and others 1996).

Components responsible for varietal aroma exist as free volatiles, contributing directly to odor, and non-volatile sugar conjugates, representing aroma precursors that can

potentially be released during winemaking (Gunata and others 1985). The pool of free aroma components and their precursors increases rapidly in the advanced stages of fruit maturity (Coombe and McCarthy 1997). A number of procedures can be used to estimate aroma potential, including analysis of total and phenol-free glycosides (Abbott and others 1993; Zoecklein and others 2000) and chromatographic procedures (Salles and others 1990; Ebeler 2001; Sanchez-Palomo and others 2005). These analyses are expensive and time consuming. Sensory analysis of grape aroma is commonly used to assess maturity, due to the relationship between grape maturity and varietal aroma. However, the problem with sensory analysis is subjectivity (Jordan and Croser 1983). Therefore, there is a need for a simple, reliable, objective, and non-destructive technique that can help determine optimum grape maturity.

Recent research suggests that electronic nose technology may provide an objective complement to the subjective human nose in the agrofood industry (Di Natale and others 1997; Schaller and others 1998). A relatively new technology, the electronic nose has been suggested for a variety of applications including quality control, process monitoring, freshness evaluation, and authenticity assessment (Schaller and others 1998). Additionally, it has been suggested as a non-destructive tool for maturity assessment of apples (Brezmes and others 2001; Saevels and others 2003; Echeverria and others 2004; Pathange and others 2006), bananas (Llobet and others 1999), mandarins (Gomez and others 2006), nectarines, peaches and pears (Brezmes and others 2005). In each case, the electronic nose system was able to discriminate fruit samples into respective maturity groups based on analysis of headspace volatiles. The amount of research already available suggests that the electronic nose has the potential of becoming a reliable

instrument for evaluating fruit maturity. Yet, to our knowledge, there has been no study of the usefulness of the electronic nose for grape maturity evaluation, despite various difficulties in current methods and the need for efficient alternatives.

This work was the first stage of a project aimed at determining the applicability of the electronic nose as a rapid, non-destructive, and objective tool for assessing grape maturity. The specific objective was to optimize a hand-held, conducting polymer-based, electronic nose to obtain maximum sensors' response to Cabernet Sauvignon (*Vitis vinifera* L.) fruit aroma. Cabernet Sauvignon is one of the most important wine grape varieties, constantly rated among top five varieties grown globally and in the United States.

Materials and Methods

The electronic nose

The Cyranose® 320 (Cyrano Sciences, Pasadena, CA, USA) is a standalone instrument that provides the capabilities of both measurement and data processing. It is a handheld electronic nose system equipped with 32 conducting polymer-based sensors, each sensor having greater or lesser affinity to particular types or classes of volatile compounds. The response of all sensors constitutes an observation vector, or a digital *smellprint*, that characterizes a specific aroma. When exposed to an unknown sample, the electronic nose compares the new smellprint with those stored in its memory, and situates it within the closest set of stored smellprints.

The measurements of the Cyranose 320 are based on the change in sensors' resistance when exposed to a sample headspace. Typically, the response of the sensors will look like that in Figure 1. The resistance of the sensors, being R_o during the baseline gas flow, increases during sample exposure to a steady state value, R_{max}. The sensor response is recorded as $(R_{max}-R_o)/R_o$. The Cyranose 320 software package, PCnose (Cyrano Sciences, Pasadena, CA, USA), was used to adjust settings, access datasets and monitor sensors' response.

Experimental design and statistical analysis

Statistical analysis was conducted using Design Expert software (Stat-Ease, Inc., Minneapolis, MN, USA). Response Surface Methodology (RSM) is a powerful tool for process optimization, as it characterizes the relationship between the response and the set of quantitative factors of interest. The response can be described, over the applicable range of the factors in interest, by fitting a model referred to as *response surface*, where the response is graphed as a surface in 3D space, and can be explored to determine important characteristics such as optimum operating conditions. There are different types of RSM designs, including 3-level factorial design, central composite design (CCD), Box-Behnken design, and D-optimal design. Criteria for choosing the appropriate design are well described in the literature (Mason and others 2003).

Three-level factorial design was employed to examine the effect of sample incubation time (15, 30, and 45 min) and the electronic nose pump speed (60, 120, and 180 cc/min) on sensors' response, by fitting a response surface for each of the 32 sensors. The layout of the three-level factorial design is given in Table 1, using coded and actual values.

Complete randomized design, with 5 replications, was used to investigate the effect of sample temperature, in the range of 15 to 27 °C, on individual sensor response and on the overall performance of the electronic nose.

Sample preparation

Samples of Cabernet Sauvignon (*Vitis vinifera* L.) were randomly collected from Virginia Tech's Alson H. Smith, Jr., Agricultural Research and Extension Center at Winchester, Virginia, USA. All samples were stored at 4 °C and processed within one week of sampling. Twenty-five berries were left at room temperature for 30 min, then incubated in mason jars for the required time at the required temperature in a water bath, followed by insertion of the electronic nose needle through a rubber septum (Figure 2). A vent was inserted through the septum at the time of measurement to avoid vacuum buildup, which would disrupt the flow of the headspace gas into the electronic nose. For the RSM experiment, all samples were incubated at 21 °C. For the temperature effect experiment, all samples were incubated for 30 min.

Results and Discussion

Several initial runs were conducted to determine the appropriate electronic nose time settings for baseline, sample, and purge (Table 2). Forty seconds sample draw time was found to be sufficient for the sensors to reach a steady state maximum value (Figure 3).

Both incubation time and pump speed, in the range studied, had significant effect (α=0.05) on sensors' response. Regression calculations on the data generated from the 3-level factorial experiment suggested that the response of 28 sensors were best fitted using

quadratic polynomial functions. The remaining 4 sensors (6, 8, 23, and 31) were best fitted using linear functions of the two factors. Unlike other sensors, the response of these 4 sensors increased linearly as incubation time and pump speed increased. This can be attributed to relatively poor sensitivity to grape volatiles, in other words, these 4 sensors are sensitive to chemical compounds that are not present in grape headspace at amounts sufficient to produce equilibrium under examined conditions.

The variation in the mode of response for different sensors is due to the specific sensitivity of individual sensors to different chemical compounds. Information about the specific sensitivity of each sensor is required to explain variations in response behavior. Unfortunately such information is not available from the manufacturer.

To determine the optimum values of the examined parameters, response surfaces were generated for the 32 sensors. The relationship between the examined parameters and the response of sensor 17, for instance, is illustrated in Figure 4. Similar response surfaces were obtained for the rest of the sensors. The maximum response occurred between 120 and 180 cc/min pump speed and 30 and 45 min incubation time. The optimum point, considering the response of all 32 sensors, was found to be 151.57 cc/min pump speed and 37.16 min incubation time. These numerical solutions were obtained using the Design Expert optimization tool. Given that the electronic nose pump speed can only be set to low (60 cc/min), medium (120 cc/min) or high (180 cc/min), the results suggest that using medium or high pump speed may produce similar results, in terms of optimum response levels. But the electronic nose consumes less energy when set to medium pump speed. This is an important consideration when field work is involved where access to a power supply is not always immediate. Therefore, for future studies, setting the electronic

nose to medium pump speed (120 cc/min) and using 37 min incubation time are recommended to obtain optimum sensors' response.

The effect of sample temperature on response was not included in the response surface analysis for two reasons. First, it is expected that increasing sample temperature produces a higher concentration of volatiles in the headspace and, thus, causes higher sensor response. Second, if the electronic nose is to be used outside the laboratory where temperature is not controlled, including temperature in the optimization processes is meaningless and can, in fact, be damaging. What is critical to know, however, is to what extent the variation in sample temperature affects the ability of the electronic nose to discriminate samples.

The effect of sample temperature on sensors response was significant ($P<0.0001$) for all 32 sensors, as expected. Response increased as sample temperature increased (Figure 5). However, variation in sample temperature had no effect on the discriminant function of the electronic nose. This can be seen in Figure 6, which shows the canonical projection plot for samples incubated at 18, 21, 24, and 27 °C. The graph shows no clear structure or separation between samples incubated at different temperatures. The statistical analyses, such as canonical and discriminate analysis, used by the electronic nose to evaluate the data are based on distance between observation vectors rather than their magnitudes.

Conclusions

The results from this study are important for further studies looking into use of the Cyranose 230 electronic nose system to discriminate grape samples based on levels of

maturity. Optimum settings were determined and the effect of sample temperature was considered. The electronic nose time settings shown in Table 2, medium pump speed, and 37 min incubation time are recommended to obtain optimum sensors response in future studies. Sample temperature had significant effect on the response of individual sensors, but had no effect on the overall discriminant function of the electronic nose. The results from this study demonstrate the potential ability of the electronic nose to discriminate grape samples. Given its portability and insensitivity to sample temperature, the electronic nose has the capacity to be used for field operation.

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Tables

Table 1. Layout of the experimental design for the response surface analysis showing factor-level combinations in coded and uncoded formats.

Exp. no.	Coded Factors		Uncoded Factors	
	Pump Speed	Incubation time	Pump Speed (cc/min)	Incubation time (min)
1	-1	-1	60	15
2	0	-1	120	15
3	1	-1	180	15
4	-1	0	60	30
5	0	0	120	30
6	1	0	180	30
7	-1	1	60	45
8	0	1	120	45
9	1	1	180	45
10	0	0	120	30

Table 2. Electronic nose time settings.

Setting		Time (s)
Baseline	purge	30
Sample	draw 1	40
	draw 2	0
Purge	Snout removal	0
	1st sample gas purge	0
	1st air intake purge	10
	2nd sample gas purge	90
	2nd Air intake purge	0

Figures

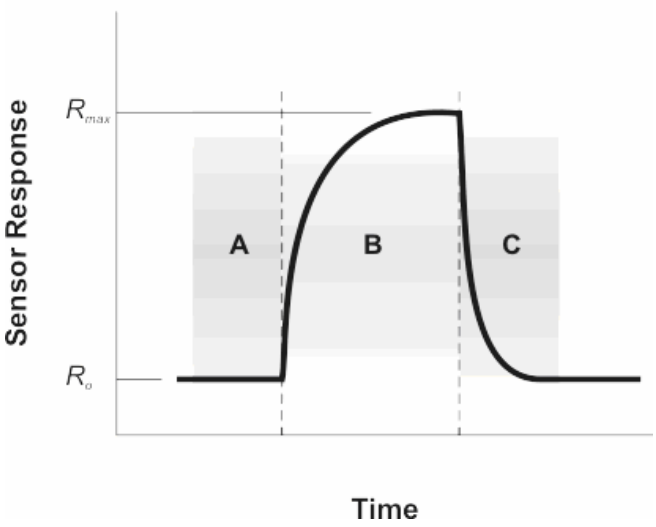

Figure 1. Profile of typical sensor response during (A) baseline purge, (B) sample purge, and (C) sensor refresh.

Figure 2. The Cyranose 320 analyzing the headspace of a grape sample.

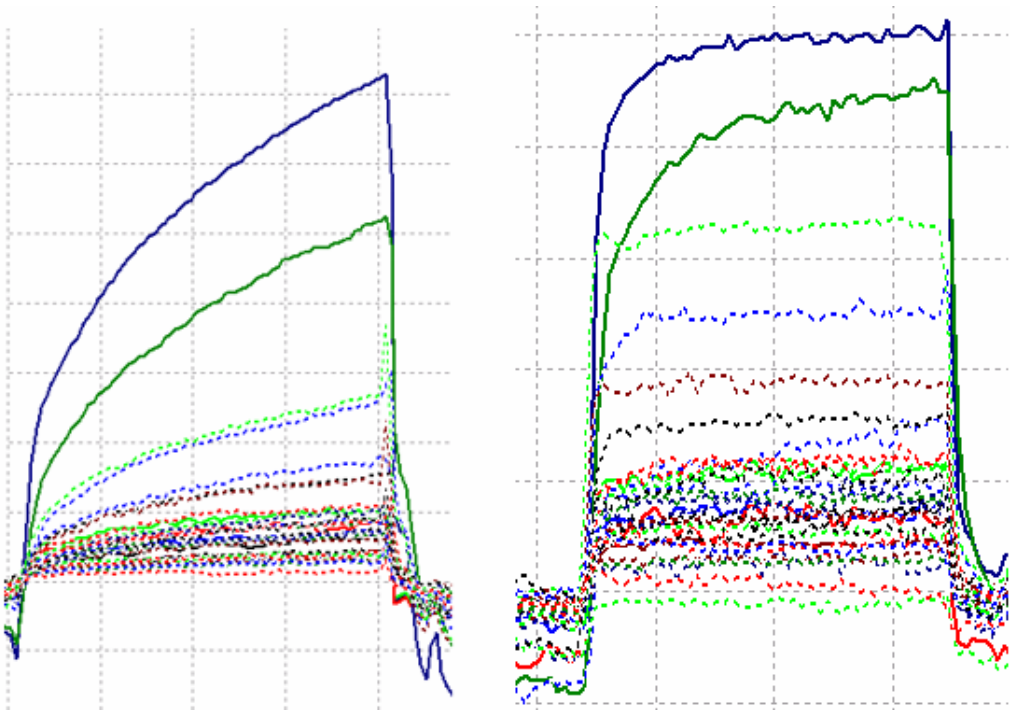

Figure 3. Sensors response with (A) insufficient and (B) sufficient sample draw time.

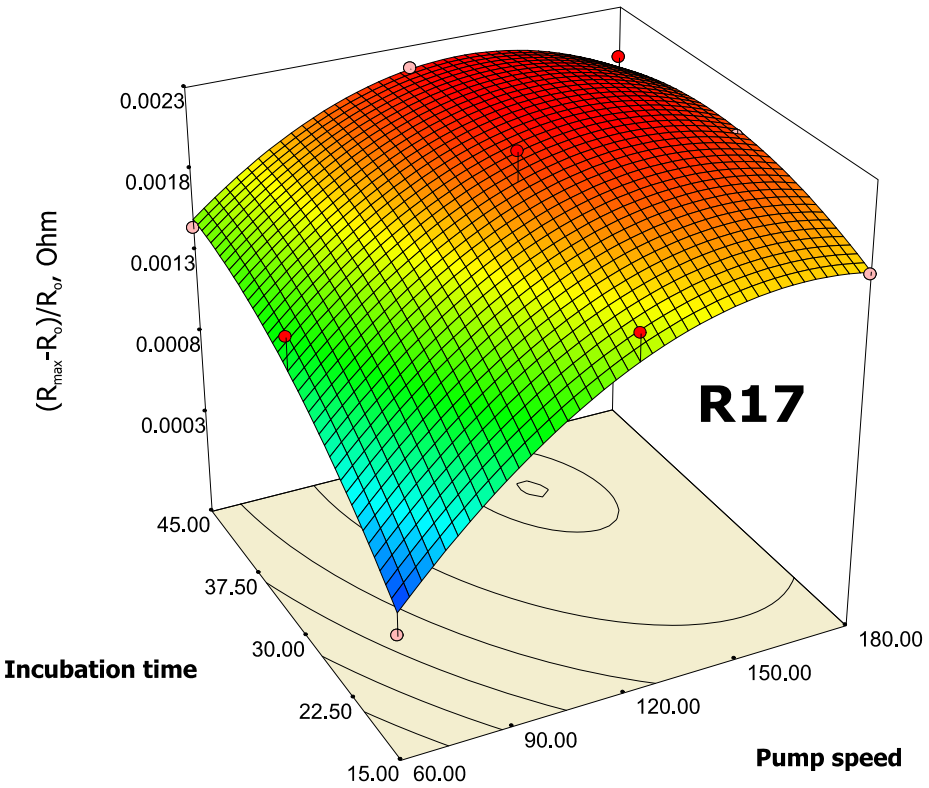

Figure 4. Response surface characterizing the response of sensor 17 as a function of sample incubation time and pump speed.

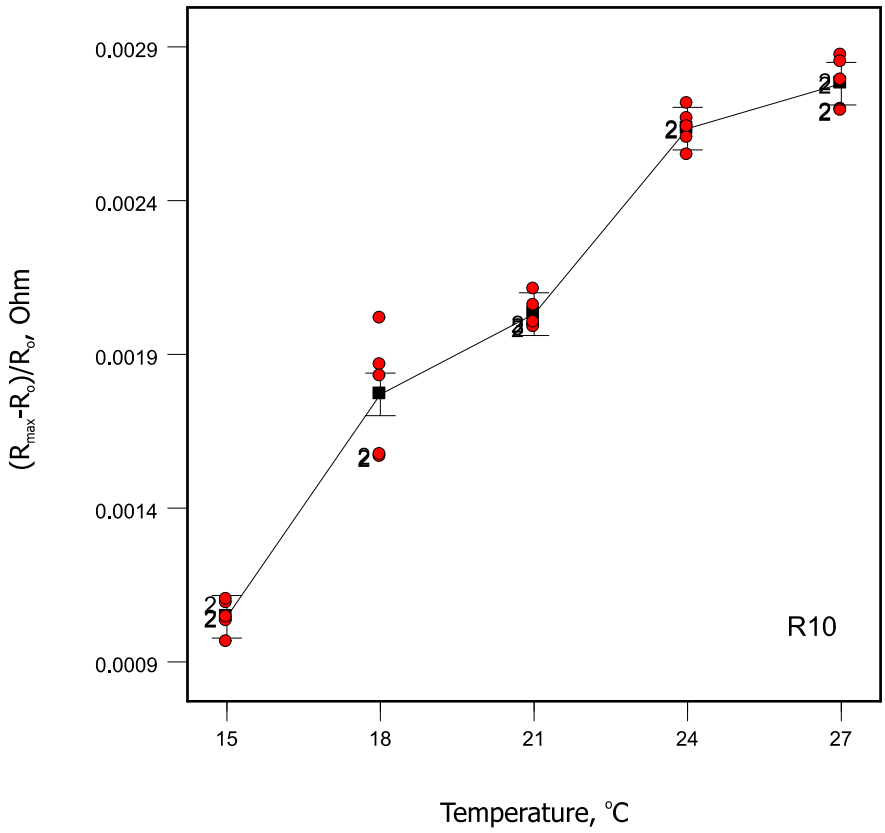

Figure 5. Effect of temperature on sensor response.

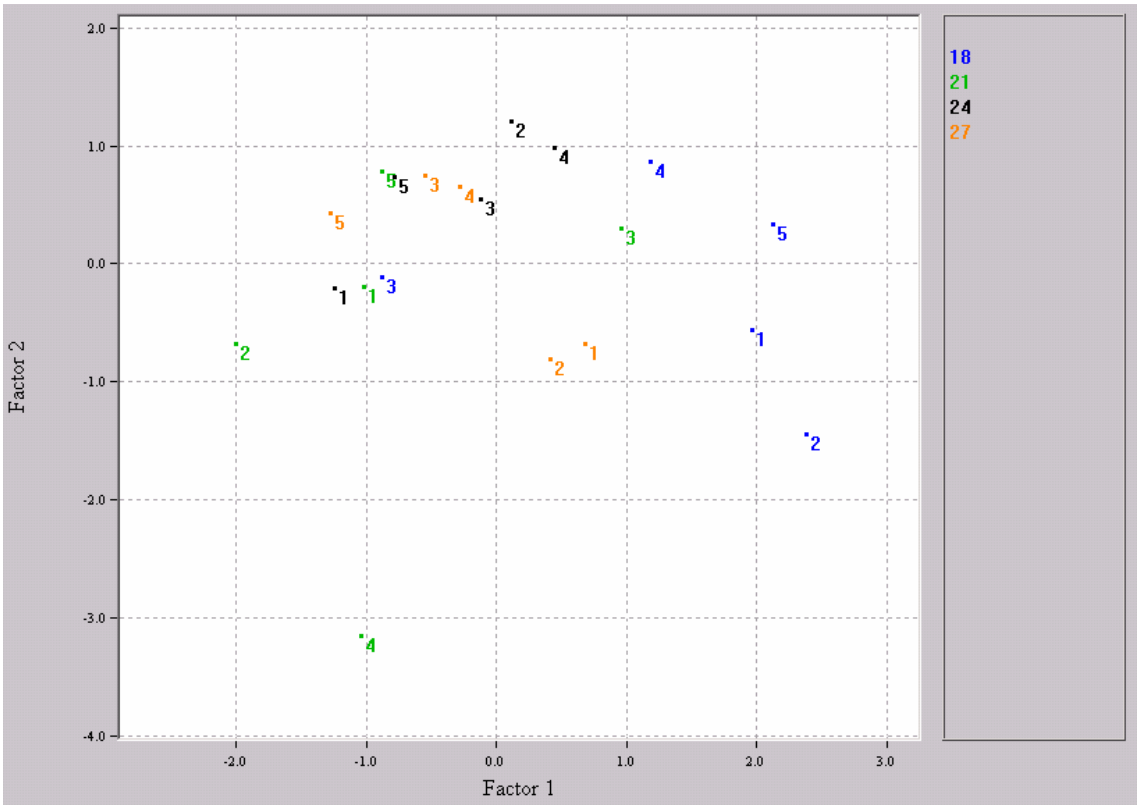

Figure 6. Canonical projection plot showing no separation between samples incubated at different temperature.

CHAPTER FOUR:

ELECTRONIC NOSE EVALUATION OF CABERNET SAUVIGNON FRUIT MATURITY

(Manuscript submitted to American Journal of Enology and Viticulture)

Electronic Nose Evaluation of Cabernet Sauvignon Fruit Maturity

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Abstract: The ability of an electronic nose to classify Cabernet Sauvignon (*Vitis vinifera* L.) grapes based on maturity was investigated. Maturity of samples collected 18, 19, and 20 weeks post-bloom was evaluated by measuring berry weight, pH, Brix, titratable acidity, total phenols, color intensity, hue, total anthocyanins, and total and phenol-free glycosides. Results were compared, using discriminant and canonical discriminant analysis, with analysis of headspace volatiles via a hand-held electronic nose. The electronic nose was able to determine the difference between the three sample groups. Field measurements demonstrated the potential for the electronic nose as a rapid, non-destructive tool for evaluating grape maturity.

Key words: grape maturity, Cabernet Sauvignon, electronic nose, grape volatiles, grape aroma

Introduction

Grape maturity is a critical attribute impacting potential wine quality. Maturity evaluation is difficult due to the many interrelated factors that impact physicochemical changes (Coombe 1992, Robinson and Davies 2000) and limitations in the understanding of these factors (Coombe 1992, Watson 2003). Currently, grape maturity evaluation often includes some measurement of physical and chemical properties. Berry weight, sugar content (Brix), pH, titratable acidity, malic acid, and color are common indices used individually or in combination. These assays may be influenced by sample and process variations (Rankine et al. 1962, Zoecklein et al. 1999). Additionally, specific levels of sugar, acidity, pH, and color are not always strongly correlated to potential wine quality (Hardie et al. 1996).

Wine varietal character is the product of grape-derived volatile compounds (Gunata et al. 1985, Hardie et al. 1996). Free volatiles may contribute directly to odor, while some non-volatile conjugates represent aroma precursors that may be released during winemaking and aging (Gunata et al. 1985, 1990). Procedures used to estimate aroma potential include analysis of free and potentially volatile terpenes (Dimitriadis and Williams 1984), total and phenol-free glycosides (Abbott et al. 1993, Zoecklein et al. 2000) and chromatographic methods (Salles et al. 1990, Ebeler 2001, Sanchez-Palomo et al. 2005). However, these analyses are restricted to high-terpene varieties, expensive and/or time consuming. The pool of free aroma components and their precursors increases rapidly in the advanced stages of fruit maturity, referred to as engustment by Coombe and McCarthy (1997). For that reason, many producers sensorially and subjectively evaluate juice aroma as a maturity gauge (Jordan and Croser 1983). Because

of the difficulties associated with current methods, there is a need for a simple, reliable, and objective technique for evaluation of fruit maturity.

The electronic nose is a relatively new technology utilized in a variety of applications in the medical field (Gardner et al. 2000) and food industries (Di Natale et al. 1997, Schaller et al. 1998). It is a basic simulation of the human olfactory system (Gardner and Bartlett 1999), intended to aid in decision-making when volatile compounds correlate strongly with certain sample attributes. In the wine industry, the electronic nose has been suggested as a tool to monitor toasting homogeneity of oak barrels (Chatonnet and Dubourdieu 1999), and for wine discrimination (Di Natale et al. 1996, Rong et al. 2000, Penza and Cassano 2004, Ragazzo-Sanchez et al. 2005, Garcia et al. 2006). Santos et al. (2004) demonstrated that electronic nose evaluation of Madrid wines was consistent with GC/MS analyses. This technology has been used as a non-destructive tool for maturity assessment of apples (Pathange et al. 2006), bananas (Llobet et al. 1999), mandarins (Gomez et al. 2006), and nectarines, peaches and pears (Brezmes et al. 2005). This study evaluated the capacity of a conducting polymer-based electronic nose to monitor Cabernet Sauvignon (*Vitis vinifera* L.) fruit maturity by analyzing headspace volatiles.

Materials and Methods

Vineyard site and fruit sampling. Cabernet Sauvignon (*Vitis vinifera* L.) was grown on an open lyre divided canopy training system in Winchester, Virginia, USA (39°12'N), which has a macroclimate typified as warm, humid and continental. Mean monthly precipitation from April through October is 76 mm, with 1890 accumulated heat units and a mean relative humidity in September of 75%. Vines were grafted to C-3309

rootstock, planted in 1998, and spaced 2.1 m apart in 3.6 m north-south oriented rows. Soil is a Fredrick-Poplimento loam, with an effective rooting depth greater than 100 cm. Vines were not irrigated, and were subject to pest management and other general cultural practices routinely used in the region.

Within a 0.5 ha plot, 15 vines were randomly selected for fruit maturity evaluation. Samples of 25 berries were randomly collected from both sides of each vine canopy, as described by Jordan and Croser (1983), at 18, 19, and 20 weeks post-bloom, for a total of 15 replicates per sampling week, and stored at -80°C. At the time of commercial harvest (20 weeks post-bloom), clusters per shoot, clusters per vine, cluster weight, shoots per vine, and fruit weight per vine were determined.

Laboratory analysis. Berries were thawed for 2 hr at ambient temperature, weighed, homogenized (after removing seeds) in a Waring (New Hartford, CT) commercial laboratory blender with 2 µL Pec5L pectic enzyme (Scott Laboratory, Petaluma, CA), centrifuged at 1800 × *g* for 3 min, and the supernatant was filtered through a 0.45 µm syringe filter (Whatman, Clifton, NJ). Analysis of pH, Brix, titratable acidity, color intensity (absorbance at 520 nm + absorbance at 420 nm), hue (absorbance at 420 nm/absorbance at 520 nm), and estimates of total phenols (absorbance at 280nm - 4) and total anthocyanins were determined as described by Zoecklein et al. (1999). Total glycoside concentration was determined as described by Iland et al. (1996). Phenol-free glycosides were estimated as described by Zoecklein et al. (2000). The above indices were measured on each of the 15 sampling replicates at 18, 19 and 20 weeks post-bloom.

Electronic nose. The Cyranose 320 (Cyrano Sciences, Pasadena, CA) is a hand-held electronic nose system with 32 polymer-based sensors. Electronic nose measurements were conducted prior to berry maceration for chemical analysis. Twenty berries, stored at -80°C, were thawed at ambient temperature for 2 hr, and incubated in mason jars for 30 min at 21°C in a water bath. At the time of measurement, the electronic nose sampling needle was inserted through a rubber septum (Figure 1a), with a vent to avoid vacuum buildup. Sample incubation time and temperature, and electronic nose settings (Table 1), were chosen based upon a previous study to identify the optimum parameters (Athamneh et al. 2006).

Electronic nose measurements were conducted in the field at 18, 19 and 20 weeks post-bloom. Sixteen randomly-selected clusters were wrapped in 43.2 x 38.1 cm polyethylene bags (Inteplast, Livingston, NJ) for 45 min, followed by electronic nose headspace analysis (Figure 1b). Field measurements took place between 0800 and 1200 hr, and cluster temperatures were determined prior to analysis.

Statistical analysis. All statistical analysis was performed with SAS (version 9.1; SAS Institute, Cary, NC). The GLM procedure was used for analysis of variance. The CANDISC procedure was used to conduct canonical discriminate analysis to visually summarize the separation between the three harvest groups. Discriminant analysis was performed using the DISCRIM procedure, with non-parametric method and k=3 nearest neighbors, to validate the classification of individual samples into the three maturity groups.

Results and Discussion

At commercial harvest, no variations in yield components were noted among sampling replications (data not shown). Brix, pH, hue, and phenol-free glycosides were found to be significantly different among the three sampling dates (Figures 2a and b). Differences were confirmed by multivariate analysis, showing significant differences among sampling dates (Wilks' Lambda at $p<0.0001$). The differences in phenol-free glycosides (Figure 2b) were not mirrored in differences among berry weights. Zoecklein et al. (1998, 2000) reported that increases in phenol-free glycosides may reflect increases in the pool of potential aroma and flavor compounds. It is likely that this higher concentration in the later sampling dates reflected an increased production of free volatiles, or engustment as suggested by Coombe and McCarthy (1997).

The canonical discriminate analysis plot of all physicochemical analyses data from 18, 19 and 20 weeks post-bloom (Figure 3a) showed clustering according to sampling week. The separation indicates the similarity within a particular group, and the difference between the three groups, as expected.

The canonical discriminate analysis plot of the electronic nose measurements performed in the laboratory and vineyard (Figures 3b and c, respectively) showed that the electronic nose produced the same separation with one measurement, as compared to the separation based on the 11 physicochemical indices, clustering samples according to sampling week, indicating the ability to differentiate among the three maturity groups. Discriminant analysis validated this grouping of samples.

The discriminant analysis cross-validation summary of physicochemical and electronic nose data measured in the laboratory and the vineyard for 18, 19, and 20 weeks post-bloom (Tables 2a, b, and c) showed that most samples were correctly classified in their respective sampling weeks. The similarities in the discriminant analysis results further demonstrated the similarities between physicochemical analyses and results obtained from the electronic nose, both in the laboratory and in the vineyard.

Conclusions

A conducting polymer-based electronic nose (Cyranose 320) was used to differentiate levels of Cabernet Sauvignon maturity based on the evaluation of grape volatiles. The electronic nose evaluation was compared with 11 maturity indices, many routinely used by the wine industry. The system was able to differentiate between three maturity groups with one non-destructive measurement. The success of this approach in maturity evaluation is likely due to the vast number of chemical species which contribute to grape varietal character, most of which are generally not considered in standard chemical analysis.

This research demonstrates the potential for this relatively new technology to be used as a rapid and objective tool for evaluating grape maturity, which may contribute to maximizing wine quality with minimum cost. Future research should include correlations between fruit volatiles, wine volatiles and wine sensory response.

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Table 1. Electronic nose settings used for field and laboratory evaluation of Cabernet Sauvignon grape samples.

Action	Setting	Time (sec)
Baseline	purge	30
Sample	draw 1	40
	draw 2	0
Purge	Snout removal	0
	1st sample gas purge	0
	1st air intake purge	10
	2nd sample gas purge	90
	2nd air intake purge	0

Table 2. Cross-validation of the discriminant analysis of the (a) physicochemical data, (b) electronic nose data measured in the laboratory, and (c) electronic nose data measured in the vineyard for Cabernet Sauvignon grapes sampled 18, 19, and 20 weeks post-bloom. Cells indicate number and percentage of samples collected for a particular week (rows), and week in which discriminant analysis indicated they should be categorized (columns).

Classified as

Sampling date: Weeks post-bloom	18	19	20
18	14 93.33%	0 0.0	1 6.67%
19	1 6.67%	12 82.0%	2 13.33%
20	0 0.0	0 0.0	15 100.0%

(a)

Classified as

Sampling date: Weeks post-bloom	18	19	20
18	15 100.0%	0 0.0	0 0.0
19	0 0.0	15 100.0%	0 0.0
20	0 0.0	0 0.0	15 100.0%

(b)

Classified as

Sampling date: Weeks post-bloom	18	19	20
18	16 100.0%	0 0.0	0 0.0
19	0 0.0	16 100.0%	0 0.0
20	0 0.0	1 6.25%	15 93.75%

(c)

(a) (b)

Figure 1. Cyranose 320 in use in the analysis of Cabernet Sauvignon grape cluster headspace volatiles (a) in its stand in the laboratory, and (b) in the vineyard.

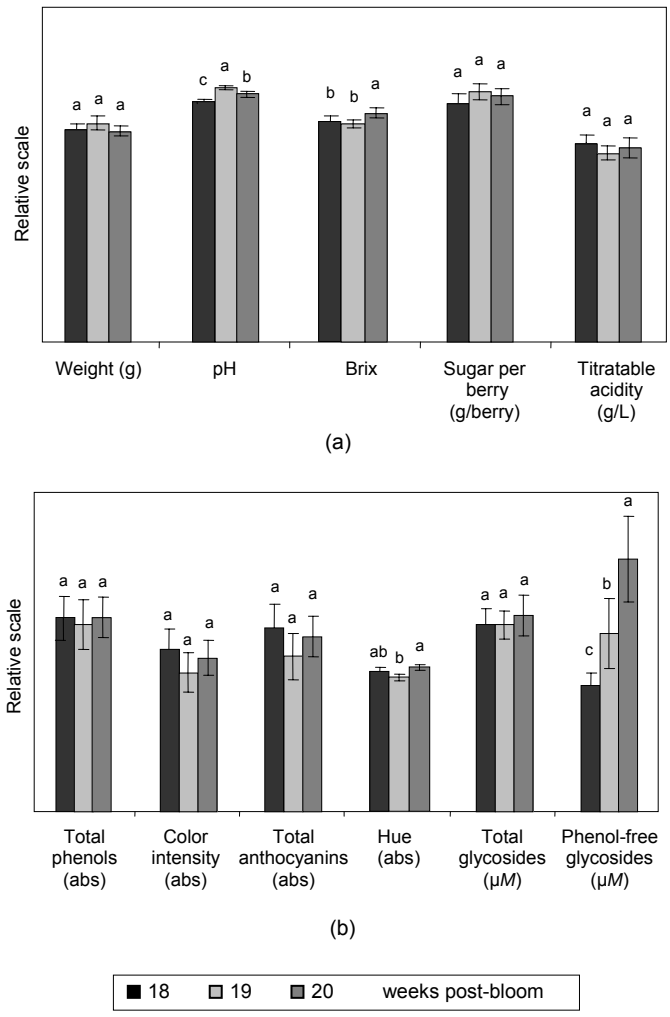
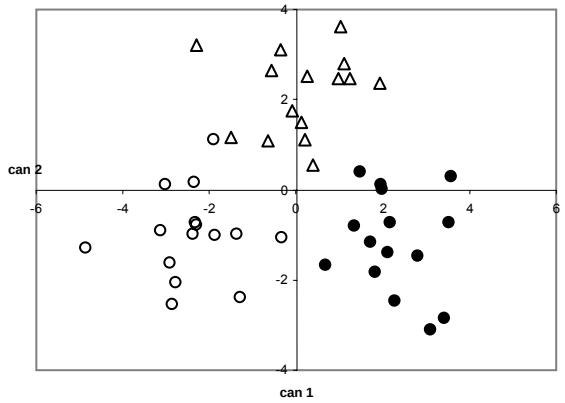
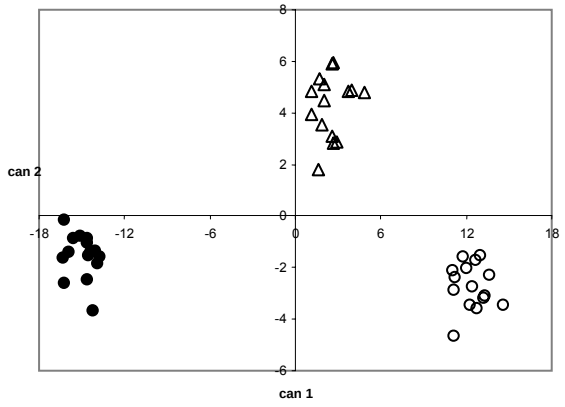


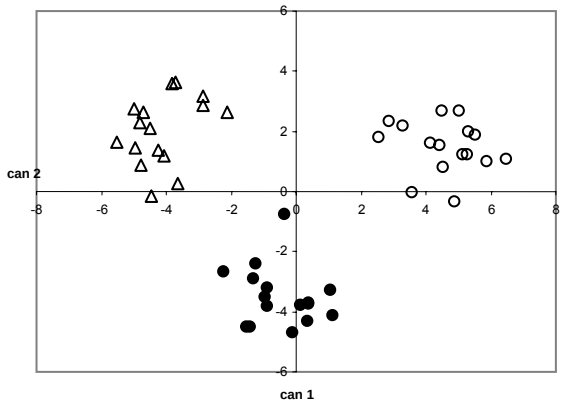
Figure 2. Physicochemical analyses for Cabernet Sauvignon grapes sampled 18, 19, and 20 weeks post-bloom. Means associated with different letters are significantly different, α = 0.05, by least significant difference. Error bars represent 95% confidence intervals.



(a)



(b)



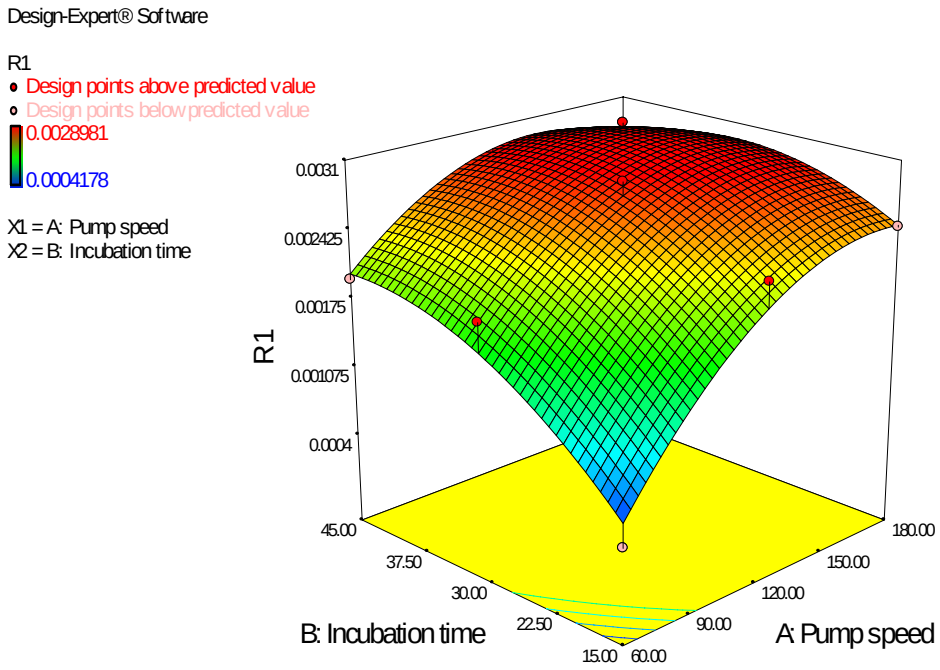
(c)

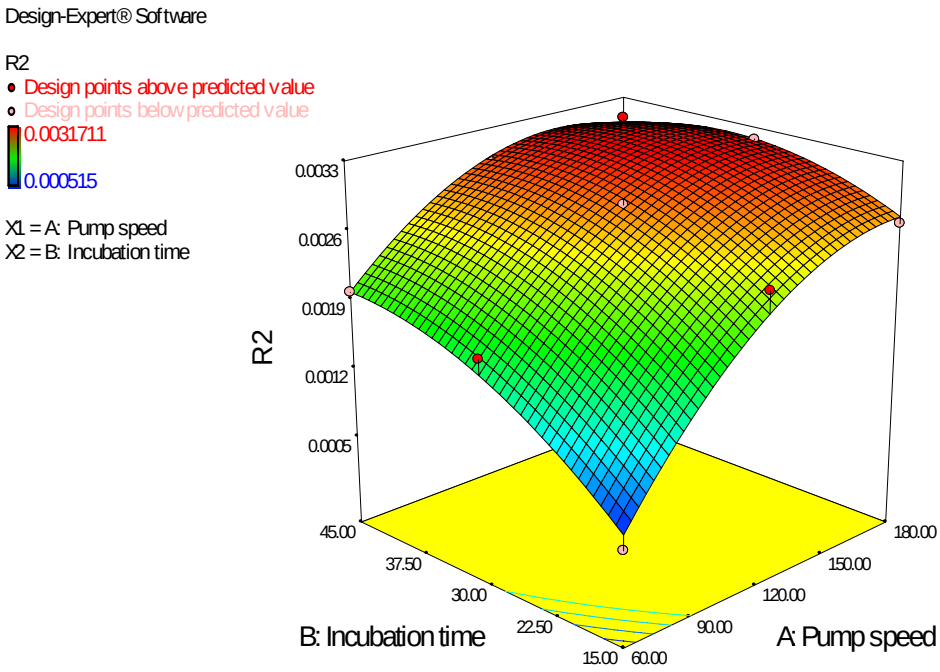
Figure 3. Canonical plot of (a) physicochemical analyses data, (b) electronic nose data measured in the laboratory, and (c) electronic nose data measured in the vineyard, for Cabernet Sauvignon grapes sampled 18, 19, and 20 weeks post-bloom.

APPENDIX

A.1 RESPONSE SURFACE CHARACTERIZING THE RESPONSE OF ALL SENSORS AS A FUNCTION OF SAMPLE INCUBATION TIME AND PUMP SPEED.

Rx: Response from Sensor x.





Design-Expert® Software

R3

Design points above predicted value

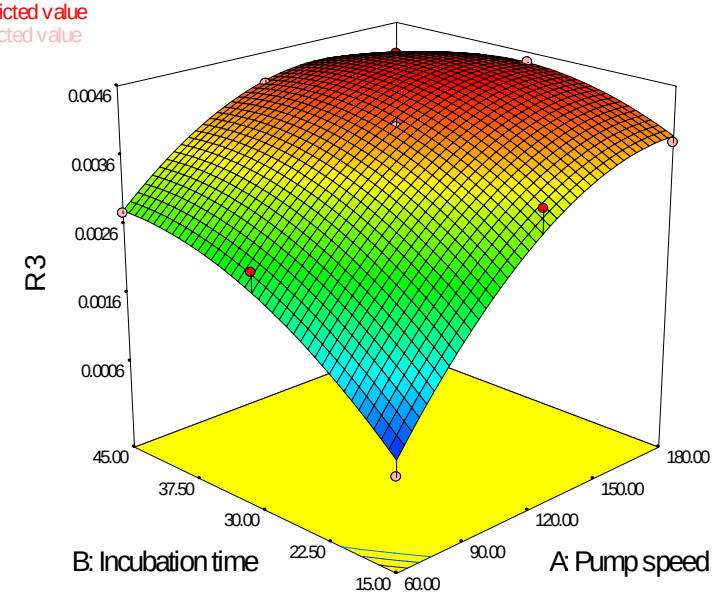
Design points below predicted value

0.00445

0.0006012

X1 = A: Pump speed

X2 = B: Incubation time



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R4

Design points above predicted value

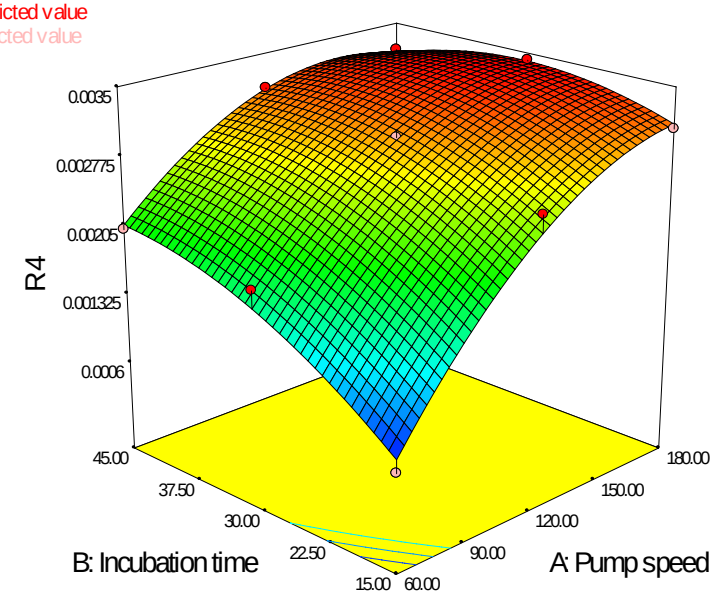
Design points below predicted value

0.0034255

0.0006475

X1 = A: Pump speed

X2 = B: Incubation time



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R5

Design points above predicted value

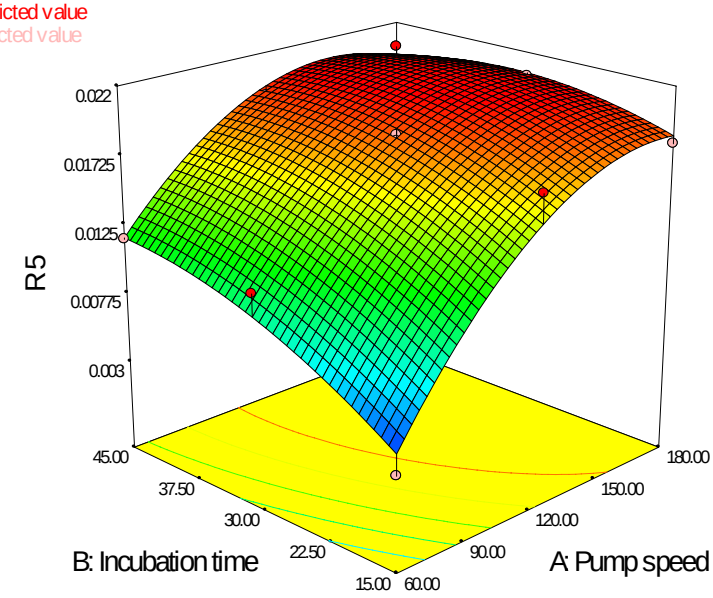
Design points below predicted value

0.020331

0.0030945

X1 = A: Pump speed

X2 = B: Incubation time



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R6

Design points above predicted value

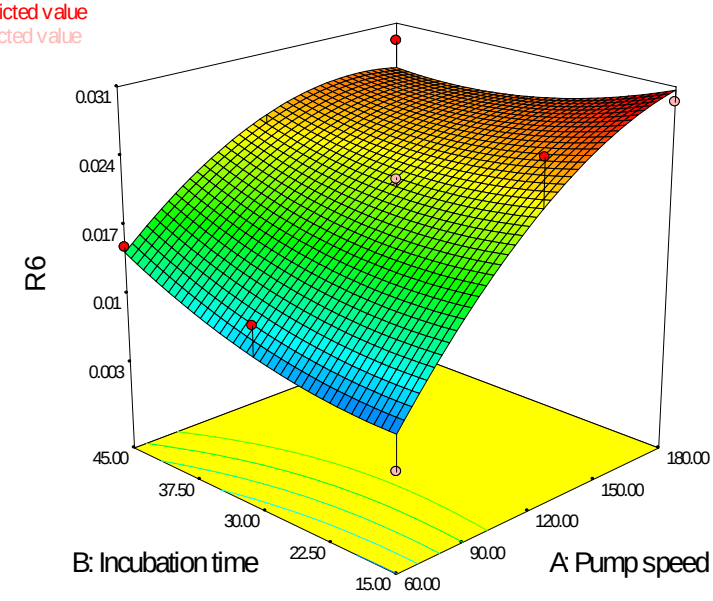
Design points below predicted value

0.0294993

0.0036176

X1 = A: Pump speed

X2 = B: Incubation time



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R7

Design points above predicted value

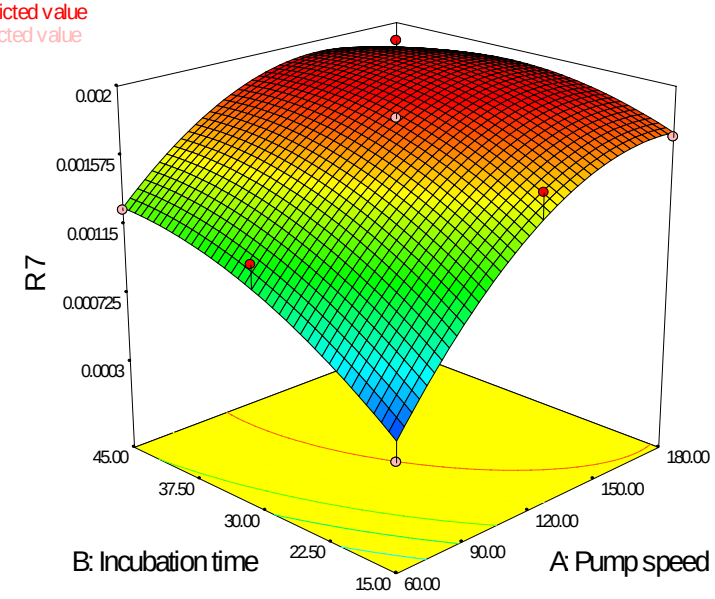
Design points below predicted value

0.0018788

0.0003866

X1 = A: Pump speed

X2 = B: Incubation time



Design-Expert® Software

R8

Design points above predicted value

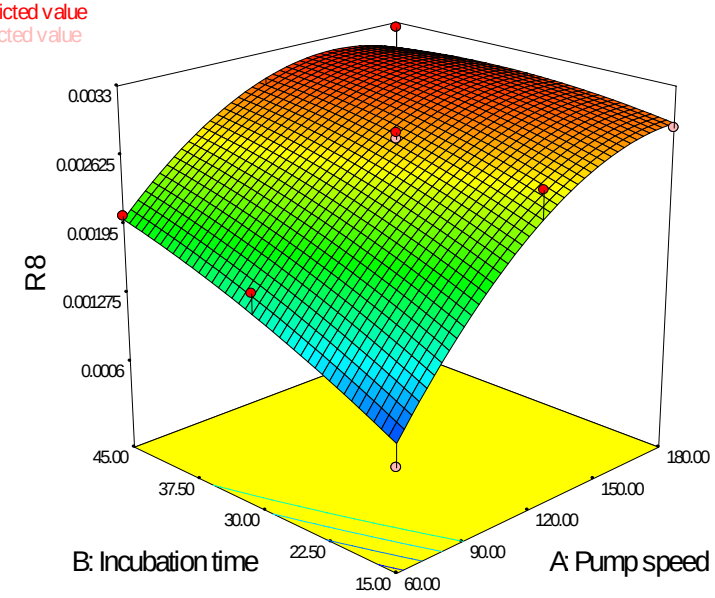
Design points below predicted value

0.0032451

0.0006886

X1 = A: Pump speed

X2 = B: Incubation time



Design-Expert® Software

R9

Design points above predicted value

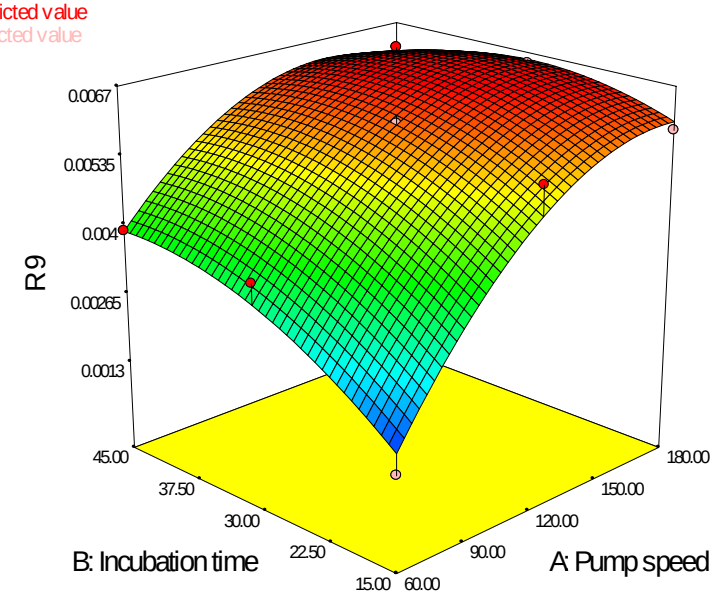
Design points below predicted value

0.0064866

0.0013377

X1 = A: Pump speed

X2 = B: Incubation time



Design-Expert® Software

R10

Design points above predicted value

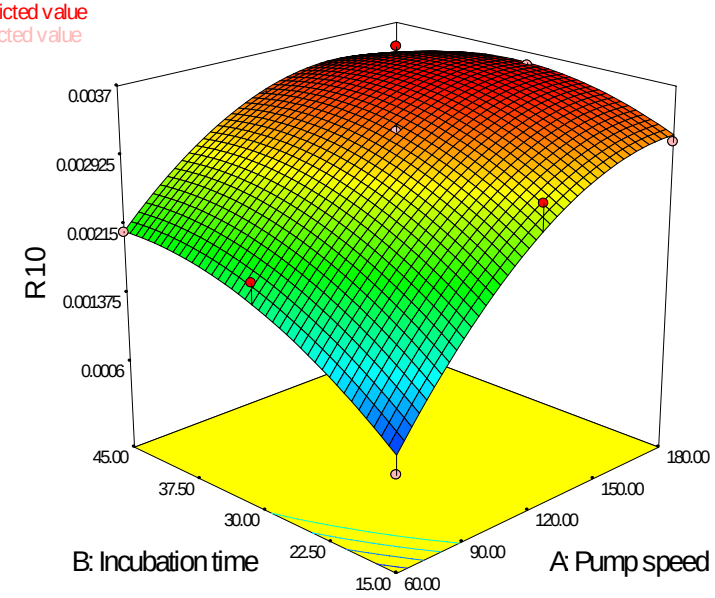
Design points below predicted value

0.003548

0.0006242

X1 = A: Pump speed

X2 = B: Incubation time



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R11

Design points above predicted value

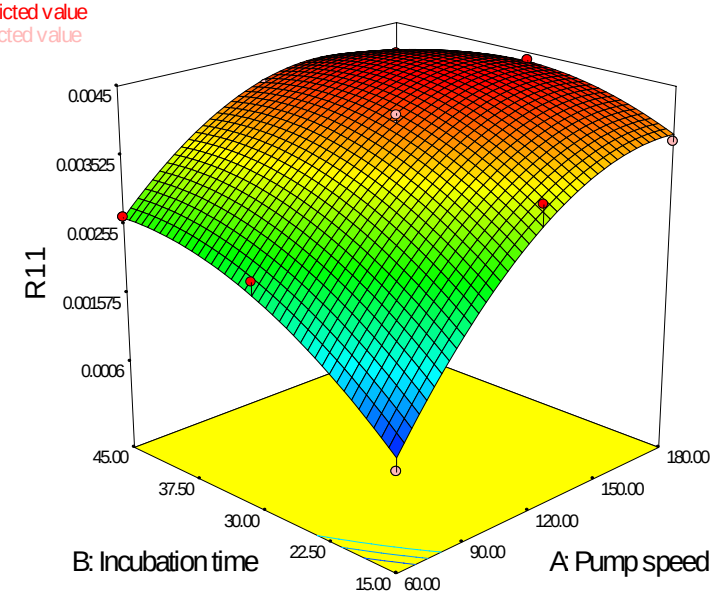
Design points below predicted value

0.0043892

0.0006764

X1 = A: Pump speed

X2 = B: Incubation time



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R12

Design points above predicted value

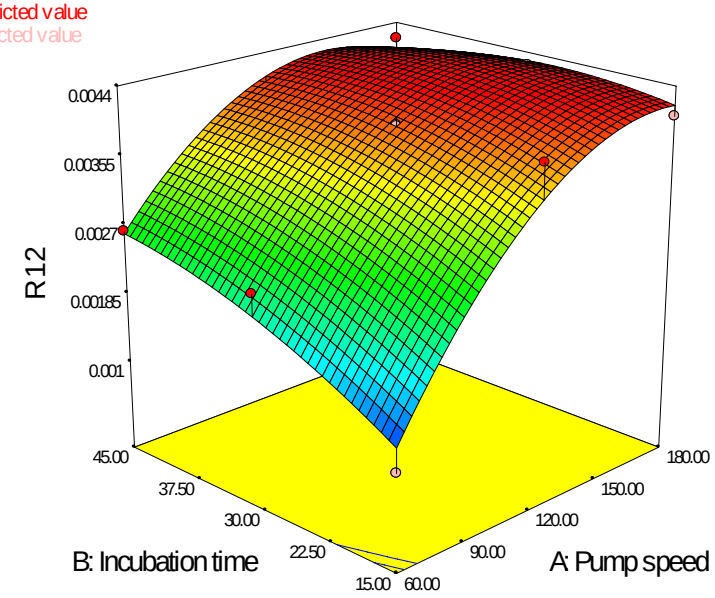
Design points below predicted value

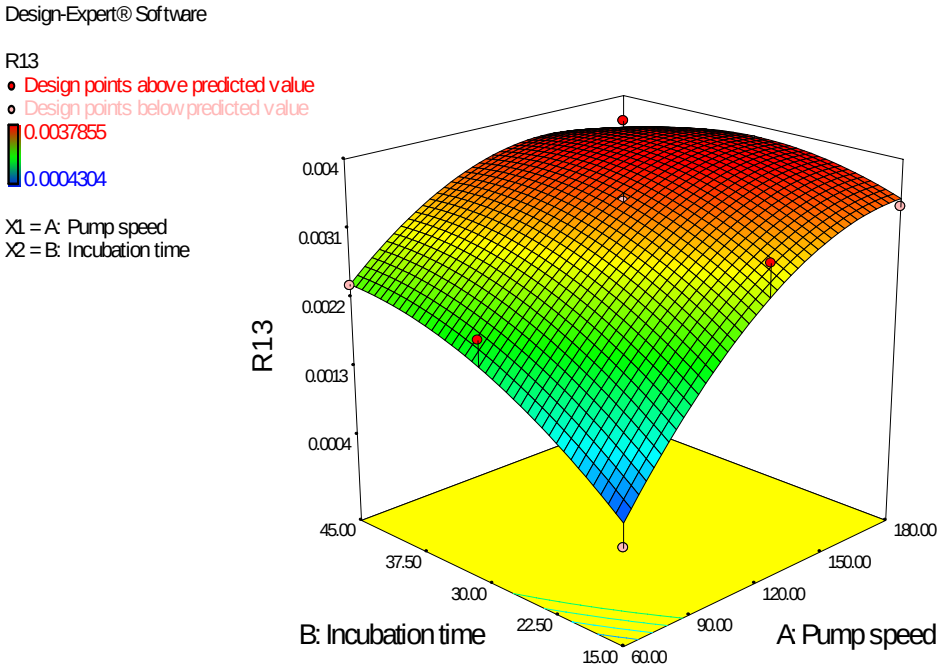
0.0042389

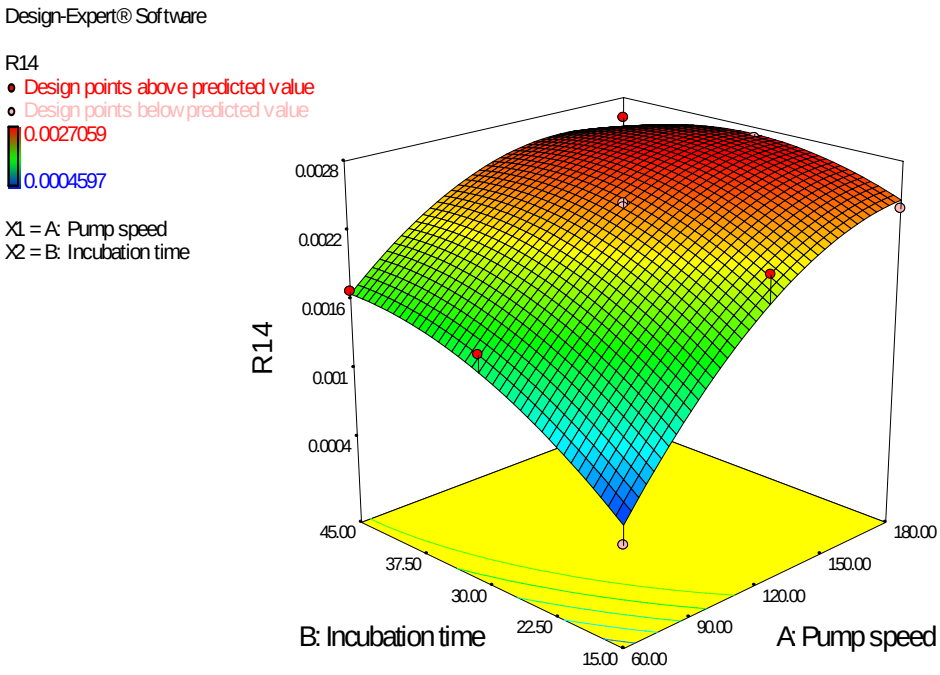
0.001047

X1 = A: Pump speed

X2 = B: Incubation time




Design-Expert® Software
R13
Design points above predicted value
Design points below predicted value
0.0037855
0.0004304
X1 = A: Pump speed
X2 = B: Incubation time
R13
0.004
0.0031
0.0022
0.0013
0.0004
45.00
37.50
30.00
22.50
15.00
B: Incubation time
60.00
90.00
120.00
150.00
180.00
A: Pump speed


Design-Expert® Software
R14
Design points above predicted value
Design points below predicted value
0.0027059
0.0004597
X1 = A: Pump speed
X2 = B: Incubation time
R14
0.0028
0.0022
0.0016
0.001
0.0004
45.00
37.50
30.00
22.50
15.00
B: Incubation time
60.00
90.00
120.00
150.00
180.00
A: Pump speed

Design-Expert® Software

R15

Design points above predicted value

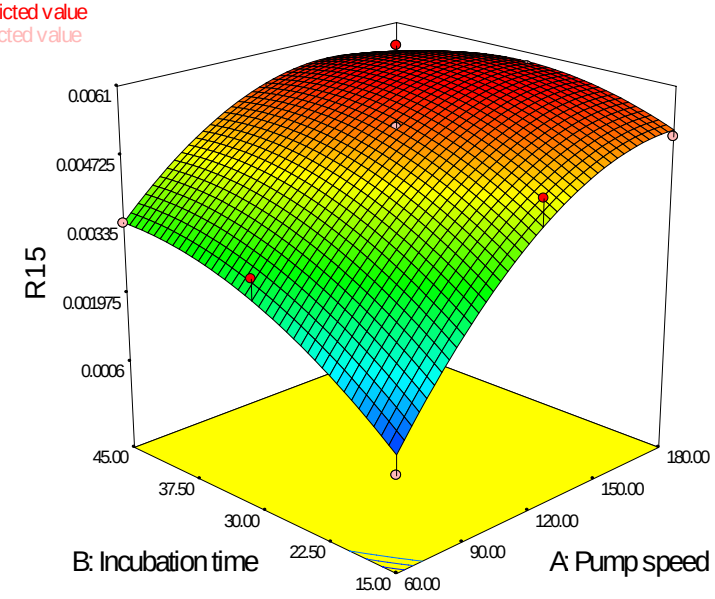
Design points below predicted value

0.0058156

0.000639

X1 = A: Pump speed

X2 = B: Incubation time



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R16

Design points above predicted value

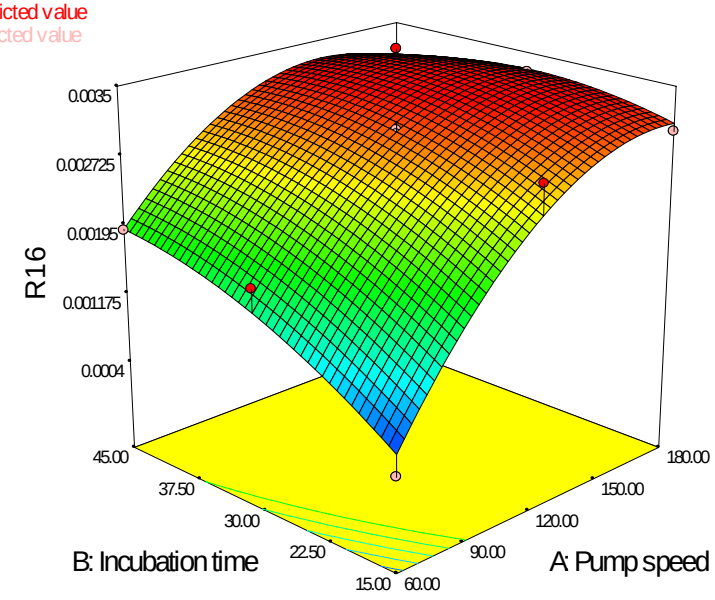
Design points below predicted value

0.0032752

0.0004056

X1 = A: Pump speed

X2 = B: Incubation time



Design-Expert® Software

R17

Design points above predicted value

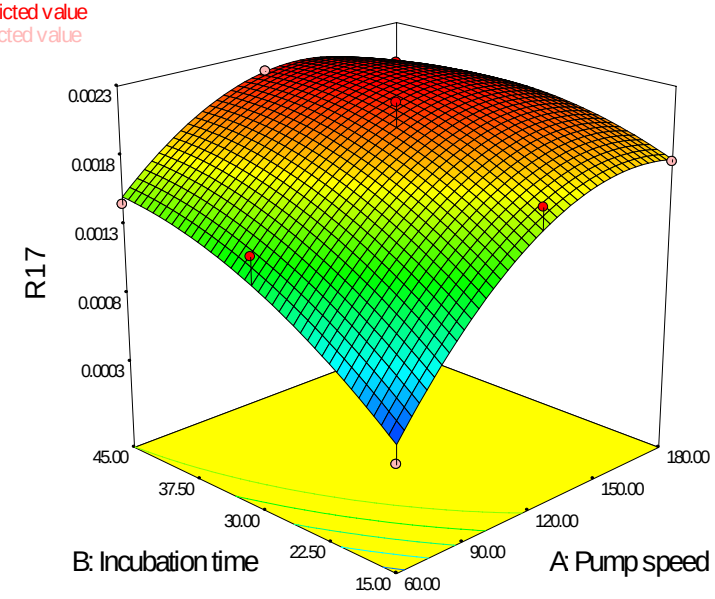
Design points below predicted value

0.0021851

0.0003882

X1 = A: Pump speed

X2 = B: Incubation time



Design-Expert® Software

R18

Design points above predicted value

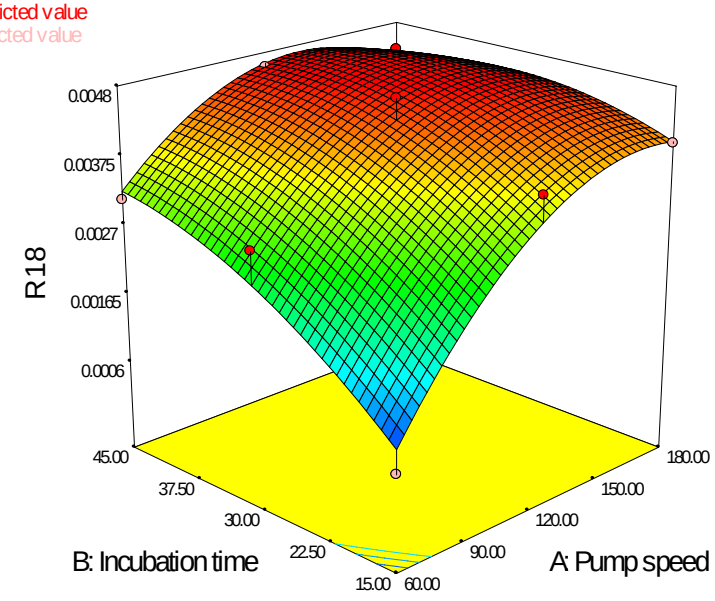
Design points below predicted value

0.0046208

0.0006384

X1 = A: Pump speed

X2 = B: Incubation time



Design-Expert® Software

R19

Design points above predicted value

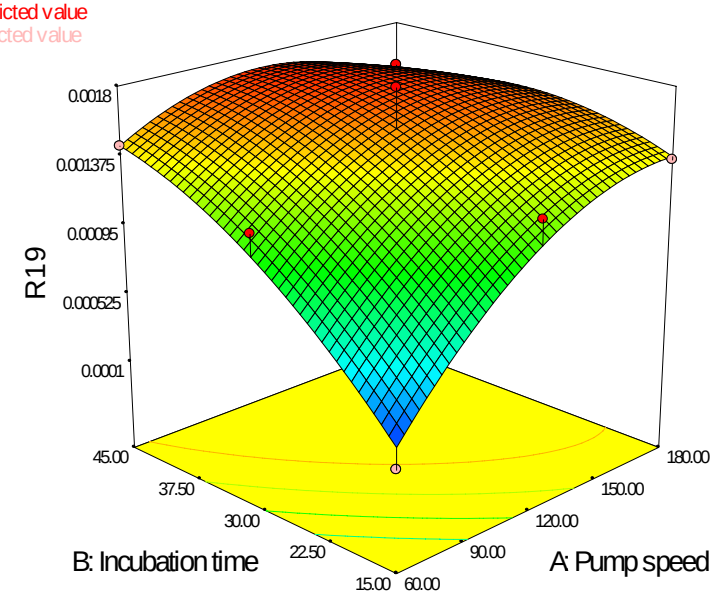
Design points below predicted value

0.0017908

0.0001443

X1 = A: Pump speed

X2 = B: Incubation time



Design-Expert® Software

R20

Design points above predicted value

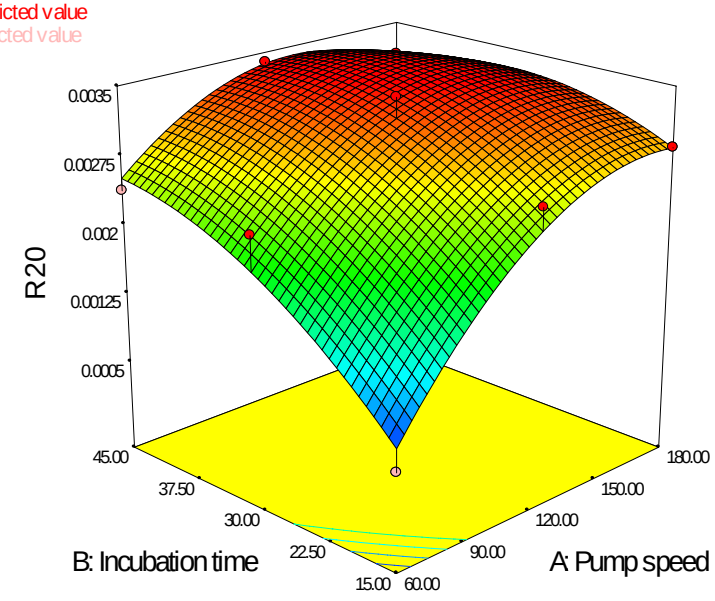
Design points below predicted value

0.0033912

0.0005488

X1 = A: Pump speed

X2 = B: Incubation time



Design-Expert® Software

R21

Design points above predicted value

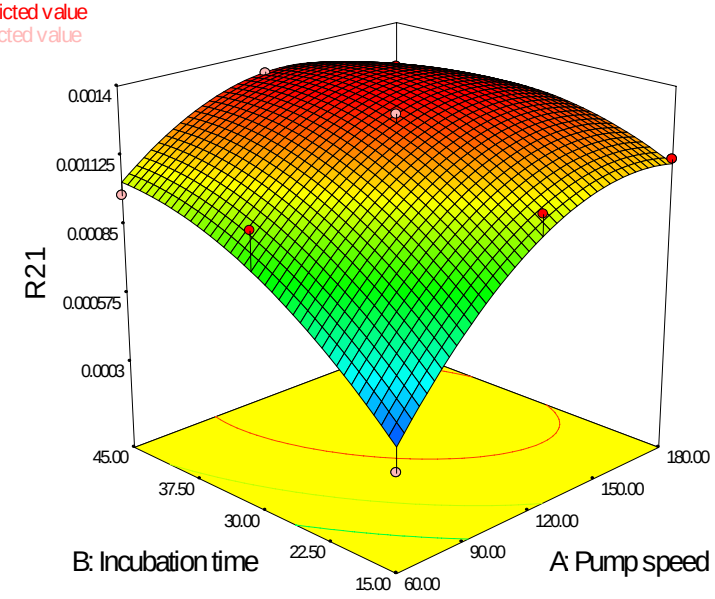
Design points below predicted value

0.001315

0.0003171

X1 = A: Pump speed

X2 = B: Incubation time



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R22

Design points above predicted value

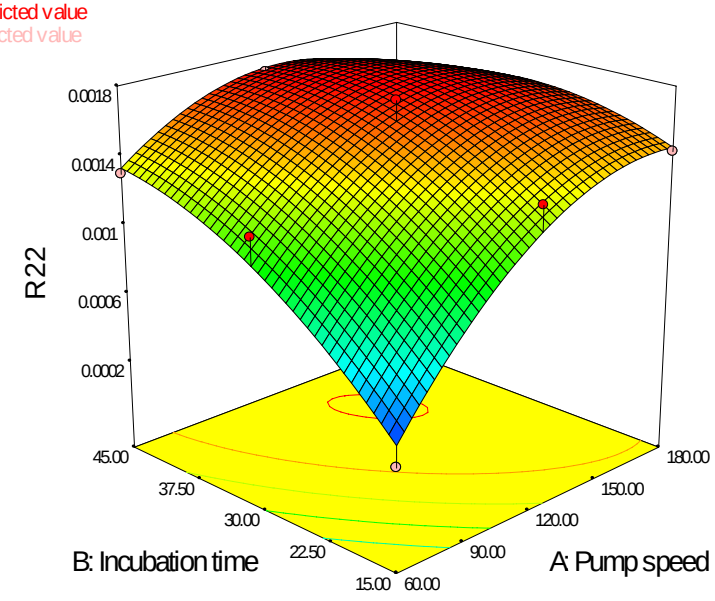
Design points below predicted value

0.0017202

0.0002524

X1 = A: Pump speed

X2 = B: Incubation time



Design-Expert® Software

R23

Design points above predicted value

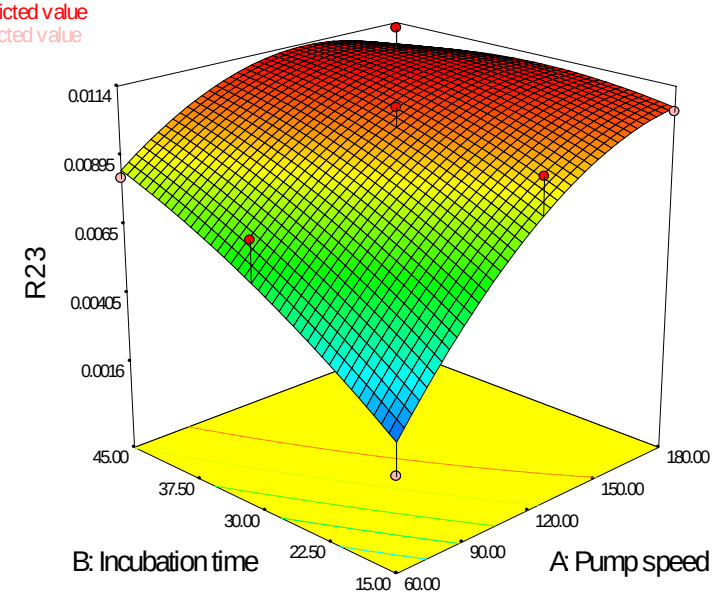
Design points below predicted value

0.0111729

0.0016378

X1 = A: Pump speed

X2 = B: Incubation time



Design-Expert® Software

R24

Design points above predicted value

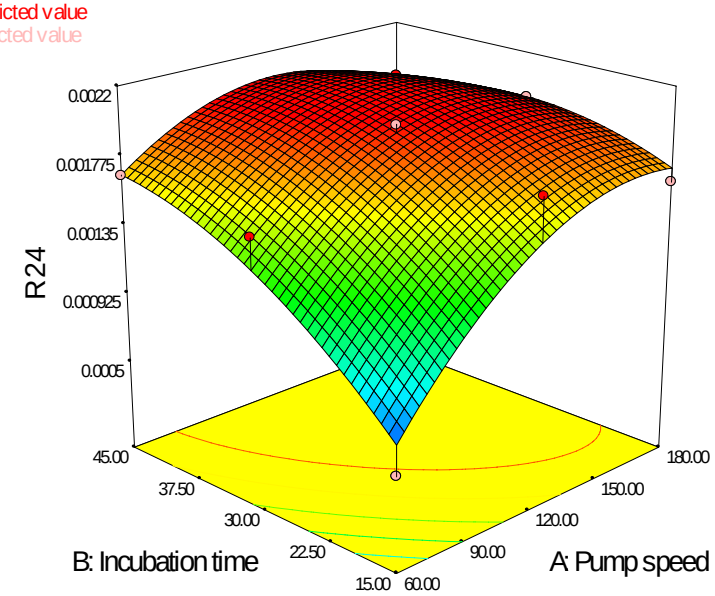
Design points below predicted value

0.0019671

0.0005045

X1 = A: Pump speed

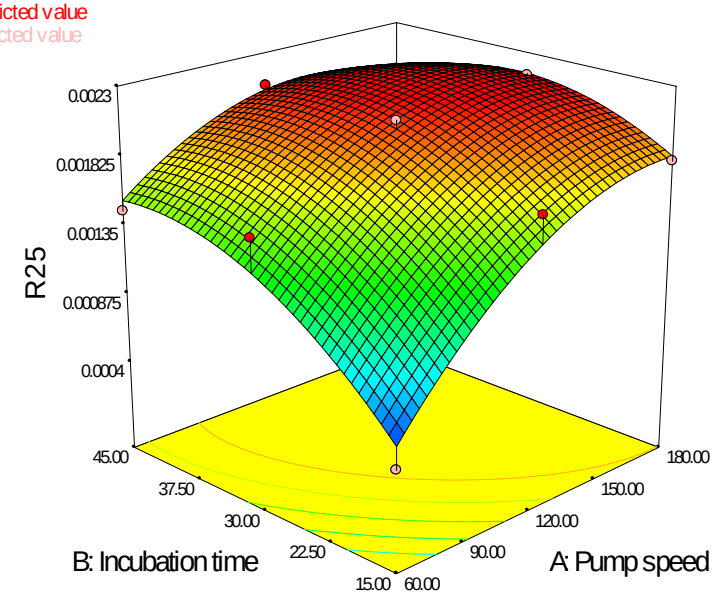
X2 = B: Incubation time



Design-Expert® Software

R25
Design points above predicted value
Design points below predicted value
0.0021415
0.0004473

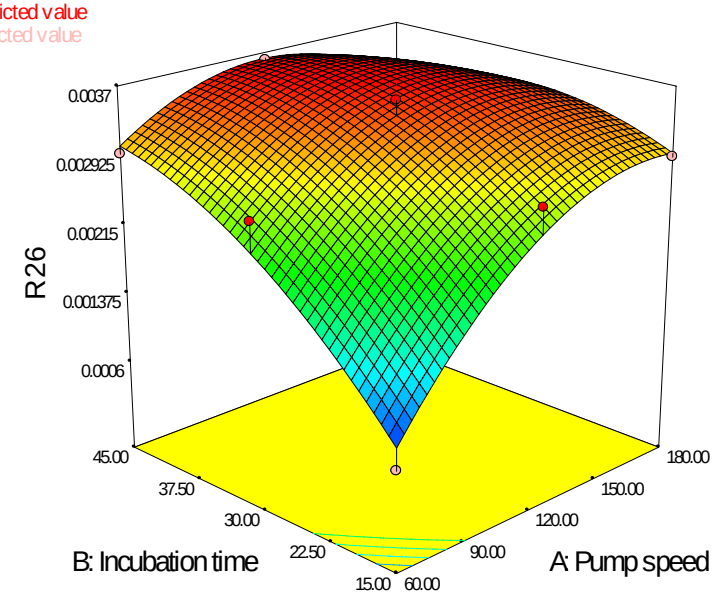
X1 = A: Pump speed
X2 = B: Incubation time



Design-Expert® Software

R26
Design points above predicted value
Design points below predicted value
0.0036111
0.0006668

X1 = A: Pump speed
X2 = B: Incubation time



Design-Expert® Software

R27

Design points above predicted value

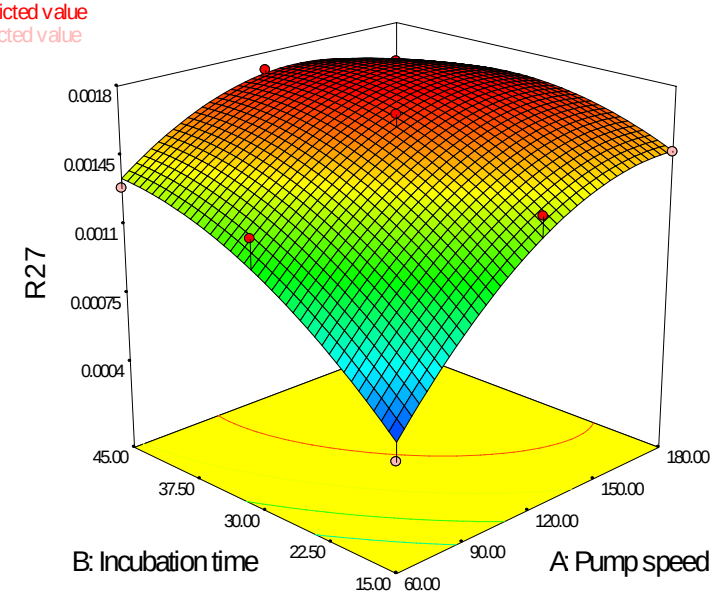
Design points below predicted value

0.001708

0.0004743

X1 = A: Pump speed

X2 = B: Incubation time



Design-Expert® Software

R28

Design points above predicted value

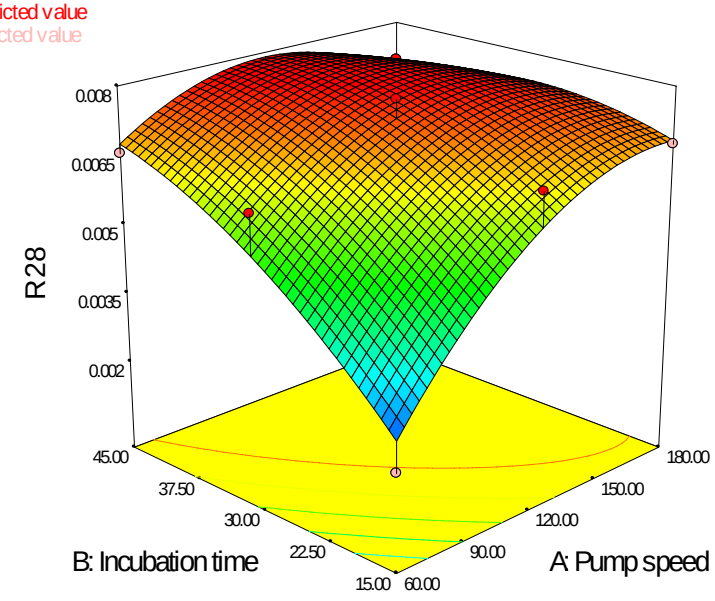
Design points below predicted value

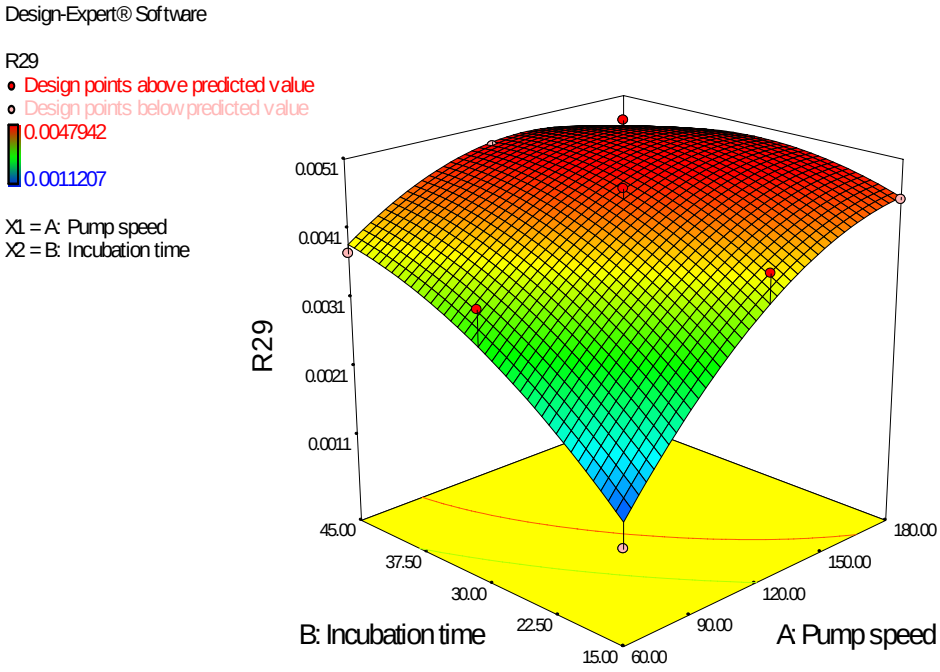
0.0077294

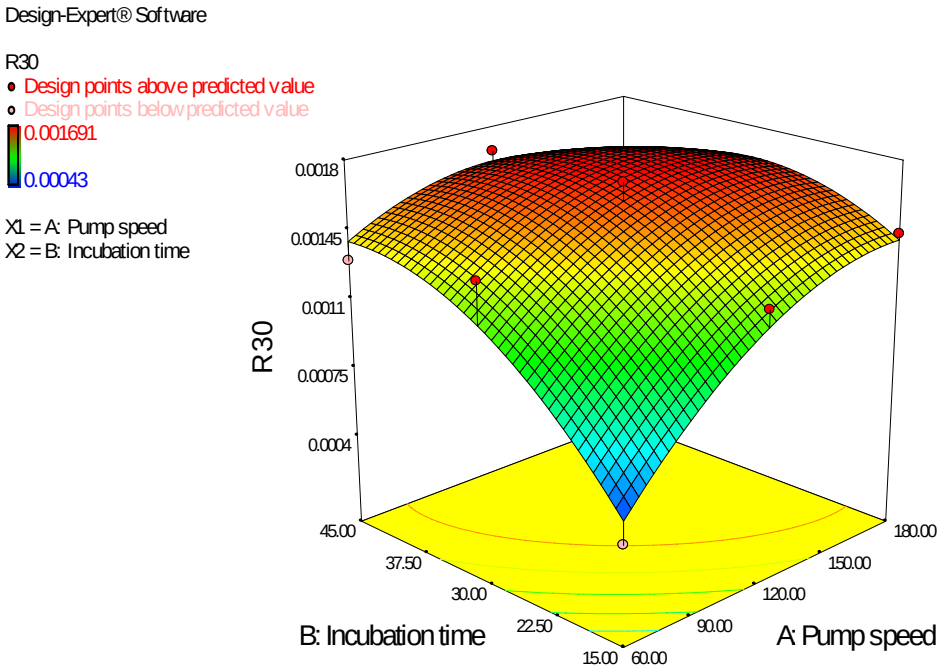
0.0020818

X1 = A: Pump speed

X2 = B: Incubation time



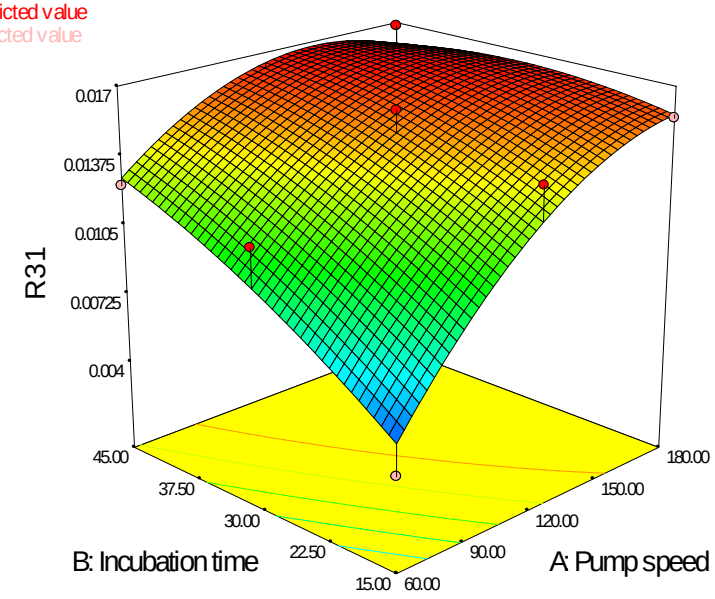

Design-Expert® Software
R29
Design points above predicted value
Design points below predicted value
0.0047942
0.0011207
X1 = A: Pump speed
X2 = B: Incubation time
R29
0.0051
0.0041
0.0031
0.0021
0.0011
45.00
37.50
30.00
22.50
15.00
B: Incubation time
60.00
90.00
120.00
150.00
180.00
A: Pump speed


Design-Expert® Software
R30
Design points above predicted value
Design points below predicted value
0.001691
0.00043
X1 = A: Pump speed
X2 = B: Incubation time
R30
0.0018
0.00145
0.0011
0.00075
0.0004
45.00
37.50
30.00
22.50
15.00
B: Incubation time
60.00
90.00
120.00
150.00
180.00
A: Pump speed

Design-Expert® Software

R31
Design points above predicted value
Design points below predicted value
0.0168325
0.004044

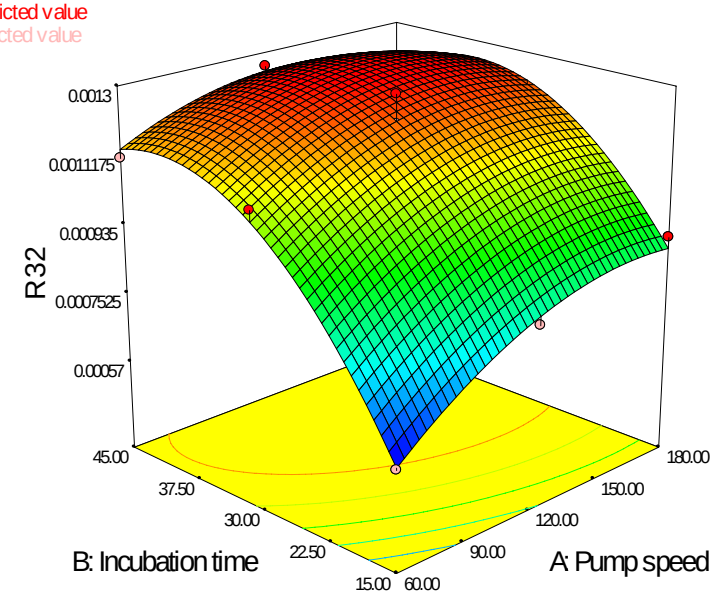
X1 = A: Pump speed
X2 = B: Incubation time

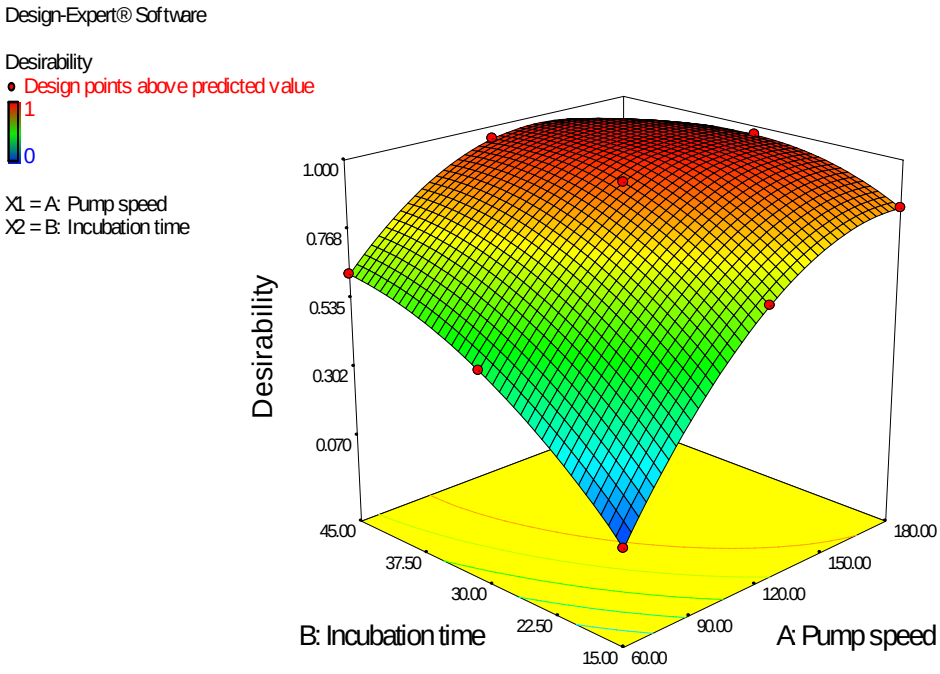


Design-Expert® Software

R32
Design points above predicted value
Design points below predicted value
0.0012794
0.0005879

X1 = A: Pump speed
X2 = B: Incubation time




Design-Expert® Software
Desirability
Design points above predicted value
1
0
X1 = A: Pump speed
X2 = B: Incubation time
Desirability
1.000
0.768
0.535
0.302
0.070
45.00
37.50
30.00
22.50
15.00
B: Incubation time
60.00
90.00
120.00
150.00
180.00
A: Pump speed

A.2 NUMERICAL OPTIMIZATION

Constraints

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Pump speed	is in range	60	180	1	1	3
Incubation time	is in range	15	45	1	1	3
Sensor1	maximize	0.0004178	0.0028981	1	1	3
Sensor2	maximize	0.000515	0.0031711	1	1	3
Sensor3	maximize	0.0006012	0.00445	1	1	3
Sensor4	maximize	0.0006475	0.0034255	1	1	3
Sensor5	maximize	0.0030945	0.020331	1	1	3
Sensor6	maximize	0.0036176	0.0294993	1	1	3
Sensor7	maximize	0.0003866	0.0018788	1	1	3
Sensor8	maximize	0.0006886	0.0032451	1	1	3
Sensor9	maximize	0.0013377	0.0064866	1	1	3
Sensor10	maximize	0.0006242	0.003548	1	1	3
Sensor11	maximize	0.0006764	0.0043892	1	1	3
Sensor12	maximize	0.001047	0.0042389	1	1	3
Sensor13	maximize	0.0004304	0.0037855	1	1	3
Sensor14	maximize	0.0004597	0.0027059	1	1	3
Sensor15	maximize	0.000639	0.0058156	1	1	3
Sensor16	maximize	0.0004056	0.0032752	1	1	3
Sensor17	maximize	0.0003882	0.0021851	1	1	3
Sensor18	maximize	0.0006384	0.0046208	1	1	3
Sensor19	maximize	0.0001443	0.0017908	1	1	3
Sensor20	maximize	0.0005488	0.0033912	1	1	3
Sensor21	maximize	0.0003171	0.001315	1	1	3
Sensor22	maximize	0.0002524	0.0017202	1	1	3
Sensor23	maximize	0.0016378	0.0111729	1	1	3
Sensor24	maximize	0.0005045	0.0019671	1	1	3
Sensor25	maximize	0.0004473	0.0021415	1	1	3
Sensor26	maximize	0.0006668	0.0036111	1	1	3
Sensor27	maximize	0.0004743	0.001708	1	1	3
Sensor28	maximize	0.0020818	0.0077294	1	1	3
Sensor29	maximize	0.0011207	0.0047942	1	1	3
Sensor30	maximize	0.00043	0.001691	1	1	3
Sensor31	maximize	0.004044	0.0168325	1	1	3
Sensor32	maximize	0.0005879	0.0012794	1	1	3

Solutions

1 solution found:

Pump speed	151.68
Incubation time	37.13
Sensor1	0.003061
Sensor2	0.003242
Sensor3	0.004526
Sensor4	0.003391
Sensor5	0.021134
Sensor6	0.025507
Sensor7	0.00199
Sensor8	0.003159
Sensor9	0.006634
Sensor10	0.003605
Sensor11	0.004473
Sensor12	0.004364
Sensor13	0.003895
Sensor14	0.00273
Sensor15	0.005987
Sensor16	0.003399
Sensor17	0.002209
Sensor18	0.004759
Sensor19	0.001692
Sensor20	0.003472
Sensor21	0.001347
Sensor22	0.001739
Sensor23	0.011327
Sensor24	0.002049
Sensor25	0.0022
Sensor26	0.003608
Sensor27	0.001744
Sensor28	0.007806
Sensor29	0.005013
Sensor30	0.001691
Sensor31	0.016774
Sensor32	0.001294
Desirability	0.991229

Number of Starting Points: 39

Pump speed	Incubation time
180	45
120	30
60	30
180	30
60	45
120	45
180	15
60	15
120	15
133.956	38.502
118.164	19.53
84.684	23.595
131.028	35.538
171.048	30.384
146.568	44.496
136.116	32.229
175.98	44.316
109.536	18.45
88.704	41.889
80.388	18.429
178.296	37.629
97.704	29.919
117.3	17.79
137.556	17.496
71.172	38.04
75.324	42.099
118.824	16.656
142.116	27.534
69.12	40.908
143.58	35.004
142.236	24.861
100.56	41.448
163.98	32.313
109.824	32.745
142.536	43.005
163.692	37.689
115.968	25.767
127.548	43.623
99.288	26.961

VITA

Ahmad Ibrahim Athamneh was born August 4th, 1978 in As Sarih, Jordan. He received his B.S. in Biosystems Engineering at Jordan University of Science and Technology (JUST), Irbid, Jordan, in January, 2001. Ahmad took a break from engineering to work, with a team of faculty, on establishing the Civil Society Development Center at JUST, and then pursuing M.A. degree in Politics and Social Policy at the University of Nottingham, Nottingham, UK, 2004. He began graduate work at Virginia Tech in Biological Systems Engineering in January, 2005.