Processing Characteristics and Utilization of Germinated Lentil for Product Development

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By
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Abstract

Lentils (*Lens culinaris* Medikus) is among the oldest crops, consumed by humans since ancient civilization and are known for its affordability in terms of protein, carbohydrates and other vital micro-nutrients. The presence of bioactive compounds make them highly nutritive; however, they also contain antinutrients some of which can be easily removed or reduced by simple processes. Germination improves the bioavailability of nutritional compounds, along with removal of antinutritional compounds. The optimum number of days for germination of green and red lentils (CDC Greenland and CDC Maxim) was investigated, based on the highest value total phenolic compounds (TPC), total antioxidant activity (TAA) and *in vitro* starch digestibility (SD). The TPC and TAA increase until 5 days of germination and tended to decline on day 6 of germination. The SD increased steadily, even after 5 days of germination. However, the increase was much less after germinating for 4 days. Therefore, lentil after 5 days of germination was selected for further studies and utilization.

Germinated lentil was dried using a pulsed-mode, microwave-vacuum drying (MVD) technique, for 2 s, 5 s and 8 s of microwave pulse level (rated capacity, 2000 W) at 0, 15 and 45 kPa vacuum pressure to understand the effect of microwave and vacuum on drying kinetics and energy consumption. Overall, the Modified Page model II showed the highest R^2 and lowest RMSE values. The drying rate constant k and effective moisture diffusivity (D_{eff}) increased with increased microwave pulse and vacuum pressure level, resulting in reduction of drying time. The optimization of processing parameters for pulse-mode, microwave-vacuum drying of germinated lentil was based on the total phenolic content (TPC), total antioxidant activity (TAA) and *in vitro* starch digestibility (SD). *In vitro* starch digestibility increased significantly with increased microwave pulse level. The TPC and TAA may vary distinctively with changing varieties of selected lentils. Vacuum pressure levels did not significantly ($p>0.05$) affect any responses. Green lentil can be dried at 8 s microwave pulse and 45 kPa vacuum pressure and red lentils can be dried at 5.5 s microwave pulse and 42.19 kPa vacuum pressure.

Dried germinated green and red lentil flours were extruded with corn flour using a single-screw extruder. Response surface methodology was employed to study the effect of moisture, percent blend of lentils and screw speed at 150℃ barrel temperature on expansion ratio (ER), extrudate density (ED), hardness (HD), water absorption index (WAI), water solublility index (WSI), TPC, TAA and SD. The percentage of germinated lentil flour in the blend had more significant effects on the response variables as compared to moisture and screw speed.

Optimum process parameters were observed at 20% blend, 19.5% moisture and 147 rpm screw speed for germinated green lentil extrudates, whereas 20.7% blend of germinated red lentil flour, 19.7% moisture and 141 rpm screw speed for germinated red lentil extrudates.

The technoeconomic analysis (TEA) of germinated, lentil-based, extruded products was carried out. Extrusion of germinated red lentil was more profitable (3.8%) and had shorter break-even point (BEP of 949.3 t/h) as compared to germinated green lentils. Net present value (NPV) and internal rate of return (IRR) for red lentil type was higher than green lentil type, CAD $ 392612.9 and 21% respectively. Germination of lentil followed by microwave vacuum drying (MVD) and extrusion can enhance the nutritive value and can be utilized in new product development.

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LIST OF AABBREVIATION

ΔT	Temperature difference (°C)
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
BEP	Break-even point
C_a	Specific heat of air (kJ/kg °C)
CCRD	Central composite rotate design
CDC	Crop Development Centre
d.b.	Dry basis
D_{eff}	Effective moisture diffusivity (m^2/s)
DM	Dry matter
DPPH	2, 2-diphenyl-1-picrylhydrazyl
E	Energy consumed (kWh)
ED	Extrudate density (g/cm^3)
E_{MVD}	Energy consumed during MVD (kWh)
E_{MWD}	Energy consumed during MWD (kWh)
ER	Expansion ratio
FAO	The food and agriculture organization of the united nations
GAE	Gallic Acid equivalent
GOPOD	Glucose oxidase/peroxidase
h	hour
HA	Hot air drying
IRR	Internal rate of return
k	Drying constant (s^{-1})
KOH	Potassium hydroxide
l	Length of extrudate, (cm)
m	Mass of extrudate, (g)
M_e	Equivalent moisture content (%)
M_i	Initial moisture content (%)
MR	Moisture ratio
M_t	Moisture content at time t (%)
MV	Microwave vacuum

MVD	Microwave vacuum drying
MW	Microwave
MWD	Microwave drying
NPV	Net present value
NRS	Non-resistant starch
P_{MW}	Microwave rated power output (W)
P_V	Vacuum pump rated power output (W)
RMSE	Root mean square error
RS	Resistant starch
R^2	Coefficient of determination
SD	Starch digestibility (%)
t	Tonne
TAA	Total Antioxidant activity (Teq mg/g DM)
TEA	Technoeconomic Analysis
Teq	Trolox equivalent
TPC	Total phenolic content (GAE mg/g DM)
TS	Total Starch
USDA	United States department of agriculture
v	air velocity (m/s)
WAI	Water absorption index
WSI	Water solubility index
ρ_a	air density (kg/m^3)

GENERAL INTRODUCTION

Pulses are not only excellent and inexpensive sources of protein, carbohydrates, some vitamins, minerals and dietary fibre, but also contain a wide range of bioactive compounds, such as phenolics, having good antioxidative and nutraceutical properties (Gharachorloo et al. 2013; Shivashankara and Acharya 2010; Gawlik-Dziki and Swieca 2011). Lentil is a pulse (legume crop) which is gaining interest as an excellent source of bioactive compounds and phenolics with the lowest glycaemic index among major staple foods. These bioactive and phenolic compounds with antioxidative abilities help in preventing oxidative damage to human cells by free radicals, which can cause chronic diseases, viz. cardiovascular disease, obesity, diabetes, inflammation, and cancer (Swieca et al. 2012). In addition, a low glycaemic index, leading to a significant reduction in fluctuation in the blood glucose level and a more stable insulin response has been shown to benefit in managing type II diabetes (Rizkalla et al. 2002). However, pulses, including lentils, contain anti-nutritional components, which include trypsin inhibitors, phytic acid, tannins and oligosaccharides. Therefore, they should be processed to remove these antinutritional compounds so as to increase their utilization as health and wellness food products.

Germination of seeds is a post-harvest processing method which is rather inexpensive, can improve nutritive value, and reduce or remove anti-nutritional factors (Vidal-Valverde et al. 2002). It has been reported that trypsin inhibitor activity is reduced by soaking of lentil in distilled water (Sampathkumar 2011), and also that phytic acid is utilized to generate phosphates by the action of phytase, for the developing seedling (Esonu et al. 1998). Apart from reduced level of antinutritional compounds, germinated seed shows significant increase in the phenolic antioxidant content of lentil when compared to non-germinated seeds. The germination process, it creates an ideal and optimal condition for bacterial proliferation. Therefore, consumers are recommended to cook (thermal) the germinated seed thoroughly before consumption (US FDA-1999 2012). Heat treatment is an important method to improve nutritional quality and reduce microbial contamination.

Drying is among the oldest and most cost effective heat treatment technology to increase the shelf life of a commodity by lowering the water activity and enhance or maintain quality. In addition, drying can drastically reduce or completely inactivate physiological, microbial and enzymatic degradation, as well as antinutritional compounds. Drying is also considered as the most common preliminary unit operation required before further processing and value addition of food commodities such as extrusion cooking. Among the electro-technology-based heating technologies, viz. radio frequency (RF), microwave (MW), ohmic and infrared (IR), microwave shows the most promise (Sham et al. 2001). With the addition of vacuum to the microwave drying system, materials can be dried in a shorter duration and at lower temperatures allowing better retention of colour, texture and nutrients (Mitra and Meda 2009). Vacuum-assisted microwave drying has been applied to reduce the moisture content of various plant materials, such as cranberries (Sunjka et al. 2004), tomatoes (Durance and Wang 2002), garlic (Figiel 2009), rosemary (Szumny et al. 2010; Calin-Sanchez et al. 2011), Saskatoon Berries (Mitra and Meda 2009) and sour cherries (Wojdylo et al. 2014). Hence, microwave-assisted drying shows potential for drying of germinated seeds as the operation is rapid, more uniform and more energy efficient as compared to conventional hot air drying.

For drying of food commodities, process conditions such as drying temperature, pressure, air velocity, relative humidity and product retention time, in addition to energy requirement, are to be determined according to the nature of the input material (feed), product, purpose and method (Erbay and Icier 2009). Effective models are necessary for process design, optimization, energy integration and control in drying of food or biological (plant-based) substances. Considering the fact that drying operations accounts for the highest energy consumption in agro-processing, for drying a particular product, effective models are necessary for process design and optimization of several drying methods including combination methods (hybrid technologies) based on nutritional characteristics (especially phenolics and glycaemic index) and energy requirements.

Although there has been a steady increase in overall global consumption of germinated lentil, it is very limited in Western countries. Incorporation of lentil in the western diets will enhance in the diversification effort of novel, health-beneficial, food products. The utilization of dried germinated lentil in extrusion is another way of supplementing bioactive compounds into our food system. Since the 1930s, extrusion cooking has been one of the most important processing technologies that has been used with different food/feed formulations. However the use of germinated (sprouted) pulses has not been reported often. Because of the healthy nutritive value of pulses, there has been growing interest in using pulse flours or blends during extrusion. Studies have shown that variation in feed moisture, screw speed and temperature during extrusion cooking may change the nutritive value of extruded products (Berrios et al. 2010), such as improvement in bioavailability of bioactive compounds by formation of complexes with protein, which can be further broken down in human body thus yielding antioxidant activity (Brennan et al. 2011). An attempt to produce an extruded product with germinated lentils may be a feasible way to increase phenolic content and antioxidant activity. This might add value to Saskatchewan grown crops as well as lentil consumers and processors across the globe. Also, with the fast changing demography in Canada and influx of the ethnic communities, the consumption of lentil and lentil based diet has been on rise.

According to our knowledge, no scientific work has been reported yet on drying of geminated lentil for extending shelf life and potential product development opportunity via extrusion technology. Therefore, this research work was focused on the effects of germination on drying of germinated lentils in terms of phenolics, antioxidant activity and starch digestibility. The dried germinated lentil will be utilized for formulation of new products using extrusion technique. The overall objective was to maximize the availability of bioactive compounds and increase the nutritional value of our diet by incorporating and utilization of germinated lentils. The specific objectives of this study include:

i. to study the chemical changes during germination of CDC green/red lentils;

ii. to investigate the drying kinetics of microwave and microwave-vacuum drying of germinated lentil and its energy consumption;

iii. to optimize drying parameters of microwave-vacuum drying of germinated lentils based on total phenolic content, antioxidant activity and starch digestibility;

iv. to determine the physico-chemical properties and starch digestibility of germinal lentil extrudates; and

v. to conduct technoeconomic and break-even analysis of processing germinated lentil extrudates.

Outline of the thesis

This thesis is manuscript based and is comprised of six chapters.

The first chapter is a literature review on utilization of lentil in various food processes. This chapter helps the readers to understand the diversity of lentil's role in our food system. It describes the overall physico-chemical properties of lentil, antinutritional compounds, value addition by the germination process and other miscellaneous uses. This chapter was published in *Research and Reviews: Journal of Food Science and Technology*. The second chapter determines the optimum number of days for lentils to be germinated for further studies based on chemical changes (total phenolic content, total antioxidant activity and *in vitro* starch digestibility). This chapter is based on specific objective 1. The third chapter determines the drying kinetics, effective moisture diffusivity and energy consumption of microwave and microwave-vacuum drying of germinated lentil (specific objective 2). This chapter was published in *Ecology, Environment and Conservation*. The fourth chapter is focused on optimizing the microwave-vacuum drying parameters based on total phenolic content, total antioxidant activity and *in vitro* starch digestibility for germinated lentil (specific objective 3). This chapter was published in *International Journal of Food Studies*. In the fifth chapter , the optimally germinated and dried lentil was converted to flour and was used for new product development employing extrusion technology. Physical and chemical aspects were investigated to determine the optimum processing parameters to develop a new extruded product based on maximum values of expansion ratio, water absorption index and *in vitro* starch digestibility (specific objective 4). This chapter is published in *Measurement: Food*. The last chapter (sixth) explains the technoeconomic feasibility of the extruded product developed from optimally germinated and dried lentil as well as the analysis of break-even point (specific objective 5). This chapter was presented at the *2018 Annual technical conference of the Canadian Society for Bioengineering/La Société Canadienne de Génie Agroalimentaire et de Bioingénierie (CSBE-SCGAB).*

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CHAPTER 1

LENTIL AND ITS UTILIZATION: A REVIEW

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Contribution of this paper to the overall study

Understanding the depth of study that has been conducted by various researchers was essential before proceeding to experiments. This study gave general idea with respect to conducting research on lentil using various unit processing techniques and also to know the initial values for different processing parameters to be considered in the following studies (Chapter 2-5). This chapter helped in determining the parameters to be used during germination and extrusion (specifically, objective 1 and 4). The manuscript was drafted by myself.

1.1 Abstract

Lentils (*Lens culinaris* Medikus) is among the oldest crops, and has been consumed by humans since ancient civilization. Lentil is consumed mostly in Asia, Africa and Latin America and is known for its affordability in the terms of protein, carbohydrates and vital micro-nutrients. It is considered highly nutritive due to the presence of bioactive compounds; however, it also contains antinutrients, some of which can be easily removed or reduced by simple processes. Hence, many researchers have been attempting to improve the nutritional quality and the utilization of lentil in different aspects of our food system. This concise review will help further understanding of simple techniques employed to reduce, the level of antinutritional compounds in the lentil, highlight the potential to use lentil in innovative food products.

1.2 Nomenclature

FAO	The food and agriculture organization of the united nations	ATCC	American type culture collection
d.b.	Dry basis	USDA	United States department of agriculture

1.3 Introduction

Pulses are among the most basic of staple foods for humans providing not only an excellent and inexpensive source of protein, carbohydrates, some vitamins, minerals and dietary fibre, but also a wide range of bioactive compounds, such as phenolics, having antioxidative and nutraceutical properties (Gharachorloo et al. 3013; Shivashankara and Acharya 2010; Gawlik-Dziki and Swieca 2011). Lentils (*Lens culnaris* Medikus) is the oldest cultivated pulse crop in many civilizations. Many inhabitants in Latin America, Africa and the Asian subcontinent consume lentil daily and it is considered a vital ingredient in their diet (Yadav and McNeil 2007). Lentil is an important commercial crop grown in Canada which provide over 40% of total international production followed by India with 22%. Saskatchewan is the largest producer and exporter of lentil in Canada (FAO 2014).

As in the case of most pulses, lentil also contains some antinutritional compounds such as tannin, phytic acid, trypsin inhibitors and oligosaccharides (Bhatty 1988; Wang and Daun, 2006; Ghavidel and Prakash 2007; Hefnawy 2011). However, these can be greatly reduced by heat treatment and germination (Reddy et al. 1982; Habiba 2002). Several studies have been conducted on lentil based on their nutritional composition, changes in nutrients due to various processing methods and also the utilization of lentil in different food systems. This review article focuses on the diverse utilization and different processing steps involving lentil. The main objective was to attain relevant and appropriate information on lentil utilization and processing from the available literature.

1.4 The Lentils

1.4.1 Physical and Chemical Composition of Lentils

Lentils ia a small seed having a round and lens shape which range in weight from 2–8 g per 100 seeds (Duke 1981; Muehlbauer et al. 1985) as shown Figure 1.1. Lentil can be divided into large and small according to their size and also by their colour. There are two main types of lentil grown in Canada i.e., red and green. Red lentil has either a brown or grey seat coat with orange to red cotyledons, whereas green lentil has a light green seed coat with yellow cotyledons. The demand for large green lentil is increasing in today's ever growing market. The large green lentils variety Laird is about 7.25 mm in its major axis, having a bulk density of about 750-780 kg/m^3 with 46-48% porosity at 10-12% (w.b.) moisture content (Tabil et al. 1999).

The approximate chemical composition of lentil is shown in Table 1.1. Carbohydrates was the main component of lentil which accounted for approximately 60.1 g/100 g dry basis (d.b) for green lentil and 59.1 g/100 g (d.b) for red lentil. Starch constituted 34.7-65% (d.b) of the total carbohydrate (Vidal-Valverde et al. 2015; Iqbal 2006). The total fibre content of lentil was 14 and 14.2 g/100 g (d.b) for green and red lentil respectively. Another important constituent of the lentil seed is protein which accounted for 25.8 g and 28.4 g/100 g (d.b) in green and red lentil respectively. Level of essential amino acids such methionine and tryptophan are low in lentil.

Table 1.1: Nutrient composition of lentil per 100g dry matter.

Component	Whole green lentil	Whole red lentil
Fat	1.1 g	1.0 g
Carbohydrate	60.1 g	59.1 g
Total fibre	14.0 g	14.2 g
Insoluble fibre	12.3 g	12.4 g
Soluble fibre	1.7 g	1.81 g
Sucrose	1.95 g	1.79 g
Protein	25.8 g	28.4 g
Calcium	73.9 mg	97.3 mg
Iron	8.1 mg	7.3 mg
Potassium	695 mg	1135 mg
Thiamin	0.29 mg	0.34 mg
Vitamin C	0.71 mg	0.73 mg
Riboflavin	0.33 mg	0.1 mg
Niacin	2.57 mg	1.73 mg
Vitamin B6	0.23 mg	0.28 mg
Folate	180 µg	186 µg

Source: http://www.pulsecanada.com/uploads/c0/59/c059dac65dad764f4b94986a704af828/Canadian-Lentil-Composition.pdf (accessed on 29 September 2015)

However, it contains a high amount of lysine, which is deficient in cereal protein (Adsul et al. 1989). In many developing countries, pulses, including lentil are important sources of low-cost protein in the daily diet, where high cost animal proteins have limited availability (Sampathkumar 2011). Potassium is among the minerals present at the highest level in lentil (695 mg and 1135 mg per 100 g (d.b) for green and red lentil, respectively). Shelhub and Resenburg (1996) mentioned that the deficiency of folate in infants resulted in neural tube defects and may cause certain types of cancer and heart disease in adults. Lentil contains a relatively high amount of folate, upto 186 µg in red lentils, therefore inclusion of lentil in our daily diet may help preventing these diseases.

Fig.1.1: Large green and red lentil.

1.4.2 Antinutritional Components

Although lentil plays a vital role in supplementing various nutrients in our daily diets, it also contains some antinutritional compounds such as tannins, phytic acid, trypsin inhibitors and oligosaccharides. The level of the above mentioned antinutritional elements are relatively low when compared to other components of legume crops. Wang and Daun (2006) reported the contents (in dry weight basis) of antinutritional compounds as follows: tannins: 0.4% to 1%, phytic acid: 0.3-1.2%, oligosaccharides: 2.5-3.3% and trypsin inhibitor activity: 1.9-3.1 mg/g sample. Tannins are water-soluble polyphenols. They reduce the availability of protein by binding proteins through hydrogen bonding and hydrophobic interaction (Hahn et al. 1984).

Tannin content can be reduced by soaking, hulling and heat treatment processes (Ghavidel and Prakash 2007; Hefnawy 2011; Singh 1988). Phytic acids (phytates) may also reduce protein utilization by forming phytate-protein and phytate-mineral protein complexes. Phytates are also responsible for lowering mineral availability, in addition to inhibiting digestive enzymes (Reddy 1982). Trypsin is a protein digestive enzyme.

Trypsin inhibitors are also protein with lower molecular weight. They are responsible for inactivating the trypsin enzyme, thereby reducing protein metabolism in our digestive system (Oomah et al. 2011). The oligosaccharides in lentil are mainly raffinose, stachyose and verbascose. Their metabolism takes place in the large intestine, as they are not digested in the small intestine, resulting in flatus. However, the release of less flatulence was observed for lentil as compared to red kidney bean, chickpea and pea (Reddy et al. 1982).

These antinutrients, such as indigestible oligosaccharides (α-galactosides), protease inhibitors and trypsin inhibitors may be greatly reduced during processing by the application of heat. Further, levels of phytic acid, which is heat stable, can be greatly reduced by germination, fermentation or soaking in water (Habiba 2002). According to Hefnawy (2011), trypsin inhibitor can be reduced by 95% by boiling in water, up to a 36% reduction in tannins by autoclaving and microwave cooking and up to 42% reduction of phytic acid by autoclaving of raw lentil. Therefore, lentil should be processed to increase its nutritional value and for better utilization in human health.

1.5 Value addition and Utilization of Lentil

Various attempts have been made to add nutritional value to lentil. The most widely studied process for value addition is germination, which is quite simple and economic. In this section, we focus on value addition and various attempts made to develop products which include lentil as an ingredient.

1.5.1 Value addition by Germination

The process by which a seed imbibe water, sprouts and develops into a new seedling is called germination. The reserve materials (carbohydrates, protein and fats) of the seed are degraded for synthesis of new cell constituents for the developing embryo, and some of the degraded materials are used up in respiration, causing a change in nutritional composition as well as in the constituents (Muelhlbauer et al. 1985). The changes in nutrients vary with time of germination, oxygen, light, moisture, temperature, seed variety and the type of drying process employed (Danisova et al. 1994, Bau et al. 1997).

Initially, seeds are soaked for 5-7 h at 20-30 °C, which leads to water imbibition. This further leads to swelling and breakage of seat coats allowing hydrolytic enzymes to activate and break down the stored food (such as starch in cotyledons) into useful biochemical metabolites. The imbibed seed may be allowed to germinate under light or dark conditions depending upon the purpose of utilization (Vidal-Valverde et al. 2002; Ayet et al. 1997; Troszynska et al. 2002; Lin and Lai 2006). Germination of seed produces bioactive compounds, usually phenolics, through the pentose phosphate pathway, shikimate and phenylpropanoid pathway. These phenolics help in acting as signaling molecules in regulation of plant metabolism. Plant-based bioactive compounds (natural health products) are gaining in popularity among researchers and due to their wide uses in pharmaceutical and food preservatives.

Gharachorloo et al. (2013) reported that lentil germinated for 5 days in dark conditions at 20°C and 99% relative humidity increases its phenolics content significantly ($P < 0.05$) as compared to dormant seeds. Antioxidant activity was maximum on the 5th day of germination, which was analyzed in terms of peroxide value. Similarly, in other pulses such as mung beans, Pajak et al. (2014), found that soaking for 12 h in deionized water at a ration of 1:10 (m/v) and germinating at room temperature (22±1° C) for 5 days (12/12, light/dark), increased the total phenolics content by 841%, and total flavonoids to 13.7 mg/g (d.b), which was 50% higher than in seeds. Also, antioxidant activity increased from 0.11±0.00 to10.52±0.1 mg trolox/g dm for sprouts as determined by DPPH method.

Swieca et al. (2012) reported that a steady decline in phenolics and free radical scavenging, metal chelating and reducing properties in lentil sprouts with germination. Lopez-Amoros et al. (2006) soaked lentil for 5 h and 30 min in distilled water followed by germination at 20° C and 99% relative humidity for 6 days in both light and dark conditions, resulting in a negative antioxidative activity. This may have been due to the presence of other compounds during extraction of phenolic process. However, Swieca and Baraniak (2013) observed, elicitation with H_2O_2, increase in caffeic acid, salicylic acid and genistein contents in 4 day old sprouts, and *p*-hydroxybenzoic, chlorogenic, *o*-coumeric, *p*-coumeric acids and naringenin, and (+)- catechin contents in 6 day old sprouts when compared to control samples. In addition, the antioxidant potential was elevated.

According to Esonu et al. (1998), germination initiates chemical changes by breaking down certain materials and transporting these materials from the endosperm to the embryo and cotyledons to growing parts. Stored proteins are hydrolyzed and the amino acids are transported into the growing seedling axis during germination. Phytic acids also are hydrolyzed during germination, enhancing the bioavailability of protein (Reddy, 1982). As the germination process utilizes carbohydrates (starch) and protein for plant metabolic activities, the glycaemic index and protein digestibility levels change. Numerous studies have shown that the digestibility of starch is related to chronic diseases such as diabetes, cardiovascular diseases and obesity (Brand-Miller 2007; Rizkalla et al. 2002). A low glycaemic index food (GI <55), such as lentil, delivers glucose to the blood stream at a lower rate by triggering a more stable insulin response (Rizkalla et al. 2002; Sparti et al. 2000). A large portion of the carbohydrate in lentil are starch, hence their digestibility and availability to our body has been widely reported. El-Adawy (2003) reported that lentil, after germination, showed a significant ($p < 0.05$) reduction in starch and reducing sugars with an increase in germination time. This might be due to their usage as an energy source during germination.

Sweica et al. (2013) observed that elicitation by solutions with high osmotic potential (Mannitol and NaCl) in germination of lentil sprouts significantly decreased total starch, resistant starch and potentially bioavailable starch. However, the calculated hydrolysis index and predicted glycemic index of sprouts were significantly lower than the dry seeds. Also, Swieca and Baraniak (2013) reported that elicitation with 20 mmol/L H_2O_2 showed the highest content of resistant starch in lentil sprouts, i.e. 53.3% of total starch. Elicitation increased α-amylase inhibitor activity, resulting in a reduction in starch digestibility and the expected glycemic index. In another study, germinating lentil up to 75 h resulted in improved protein digestibility and reduction in tannins, phytic acid and lead. Similarly, protein digestibility increased by 1.1% and 3.8% during germination of lentil for 72 and 120 h. Respectively, the reason being reduction of tannin and trypsin inhibitor during germination, which increased proteolytic enzyme efficiency. The impact of germination on digestibility of starch and protein is shown in Figure 1.2.

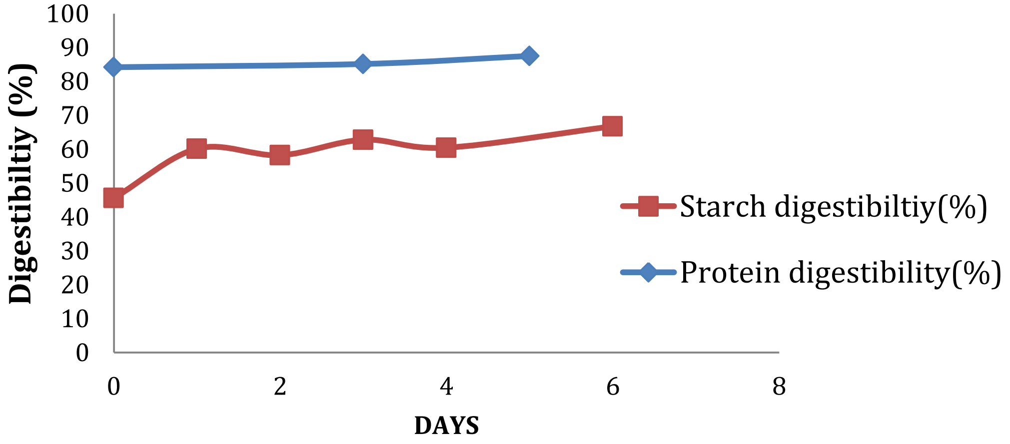

Fig. 1. 2: Effect of germination on digestibility of starch and protein.

Source: Swieca and Gawlik-Dziki (2015) and El-Adawy et al. (2003).

This shows that germination of lentil improved the nutritional value in terms of increasing antioxidant activities and protein digestibility, in addition to lowering the expected glycemic index and level of antinutritional compounds. Other antinutritional compounds present in germinated lentil can be removed by heat treatment such as drying (thermal treatment) before further utilization. Moreover, heat treatment can have a significant impact on the shelf-life of germinated lentil, as germinated seed is more perishable and prone to microbial contamination and losses.

1.5.2 Probiotics and Prebiotics

Lentil is considered as inexpensive source of protein, carbohydrates, minerals and vitamins in addition to their housing of several bioactive compounds. These constituents also serve as nutrients for the growth of the starter culture in yogurt and other probiotics. Probiotics are microorganisms which provide health benefits when consumed in adequate amounts (Kailasapathy et al. 2008; Zare et al. 2012). Enhancement of level of probiotics in food items is mainly associated with milk, as shown by numerous researchers. *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* are the main starters used for fermentation of milk e.g., yogurt. These microorganisms grow at a slow rate in milk (Klaver et al. 1993; Roy 2005), and therefore numerous studies have been conducted to enhance the growth rate by fortifying with different commodities with an aim to enrich the nutrient content of milk and milk products. From the yoghurt production point of view, acid production rate is an important processing parameter (El-Adawy et al. 2003) and can be related to the processing time, which is of economic concern. Since pulses (including lentil) fall under the category of nutritive ingredient, Zare et al. (2012) examined properties of yogurt and probiotic type beverages by supplementing with pulse ingredients (viz. pea protein, pea fiber, chickpea flour, soy protein, soy flour and lentil flour). It was found that all the supplements improved the acidification rate by probiotics (*Lactobacillus rhamnosus* AD200 and *Lactobacillus acidophilus* AD200) and among them, lentil flour showed the highest effect on acidification rate.While in yogurt production, the levels of lactobacilli (ratio of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*) were higher for all supplements and growth rate was particularly noticeable with supplementation of lentil flour. Short chain carbohydrates which are not digested by digestive enzymes but can be fermented by gut bacteria, are considered as prebiotics and promote the activity of beneficial microorganisms (Rastall and Gibson, 2006). Prebiotics normally used in the human diet are galactooligosaccharides, fructooligosaccharides, lactulose, inulin, maltooligosaccharides and resistant starches. When consumed, they pass through the small intestine and become accessible to probiotics in the large intestine (Al-Sheraji et al. 2013). Therefore, a mixture of prebiotics and probiotics are frequently used to benefit their synergic effect. Although the antioxidant activity of bioactive compounds (mostly phenolic) has been widely reported, the antioxidant activity of prebiotics had been reported by Agil et al. (2013). In this study, yoghurts were prepared using both starter cultures (*Lactobacillus delbrueckii ssp. bulgaricus* (B-548; USDA) and *Streptococcus salivarius ssp. thermophilus* (14485; ATCC) and probiotic bacteria (*L. acidophilus* (B-4495; USDA) and *B. lactis* (41405; USDA) with fortified milk containing 0-6% (w/v) lentil flour. Results showed that, bacterial growth in yogurt containing lentil was higher as compared to the control (without lentil flour) and the antioxidant activity of water-extracted polysaccharides from whole lentil flour was higher that that of dehulled lentil flour, which may be due to the presence of phenolic compounds in the seed coats of lentil (Wang 2008).

1.5.3 Extrusion Cooking of Lentil Flour

Although lentil is a good source of vegetable protein and is highly nutritious, its antinutritional factors limits its consumption (Adsule et al. 1989; Liener and Kakade 1969). This can be improved by employing efficient thermal processing techniques such as extrusion, thereby changing their composition and increasing or decreasing their nutritional value (Sathe et al. 1984). Extrusion cooking involves high heat, pressure and shear forces, causing various changes in the physio-chemical and functional properties of the flour, including the compounds of polyphenolics and their antioxidant activity. Depending on the type of raw material and extrusion cooking variables such as feeding rate, feed moisture, screw speed and configuration, die geometry, temperature and time (Brennan et al. 2011; Sarawong et al. 2014), nutritional quality can be improved.

Extrusion cooking has been studied extensively for production of a variety of ready-to-eat or ready-to-cook food items such as pasta products, breakfast cereals, baby foods, snacks, pet foods, dried soups and dry beverage mixes, as it improves nutrient bioavailability compared to conventional cooking (Singh et al 2010; Gu et al. 2008). Moreover, extrusion enhances the processing ability to develop a range of products with distinct textural advantages, high productivity, low operating cost, and energy efficient and shorter cooking times. A typical schematic of an extrusion system is shown in Figure 1.3.

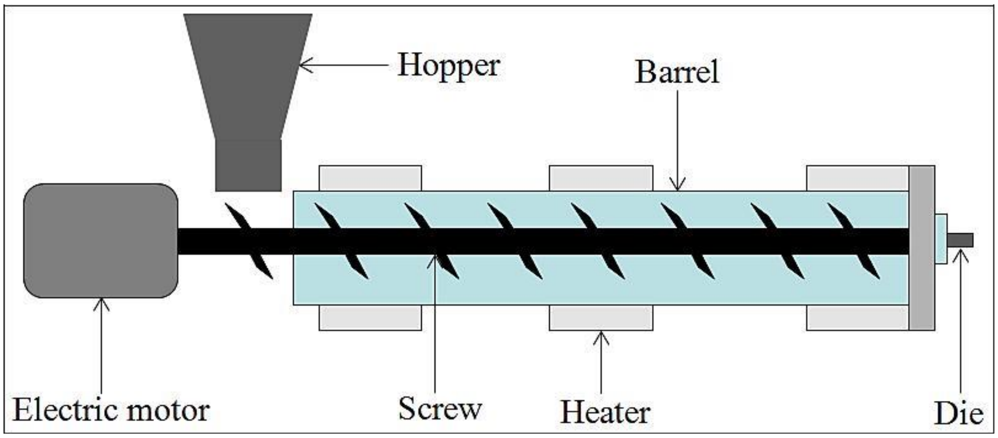

Fig 1.3: Typical schematic of extrusion system.

Use of extrusion technology has been reported in development of expanded, novel, value-added pulse based foods (Patil et al. 2007; Berrios et al. 2008; Berrios et al. 2010). It is a high-temperature, short-time process which can inactivate harmful microorganisms and enzymes without affecting nutritional and sensory characteristics. During extrusion, simultaneous mixing, kneading and heating of ingredients takes place. These processes include hydration, gelation and shearing of starch and protein, fats get melted, proteins are denatured or re-oriented, materials are plasticised forming a fluid melt followed by formation of a glassy state which expands and solidifies the ingredients when they are extruded through a die. Temperature, screw speed and moisture content are among several extrusion process variables which can influence the composition of the finished product and the desirability of extruded products (Brennan et al. 2010).

Gonzalez and Perez (2002) reported that microwave irradiation followed by extrusion reduced the retrogradation tendency of lentil starch, which is beneficial for the processing industry as this is a limiting factor for its commercial use. It also was observed that microwave irradiation and extrusion processing caused significant reductions in its functional properties viz. water absorption, water solubility and swelling power; microwave irradiation had a greater effect. Lazou and Krokida (2010a) claimed the possibility to produce acceptable whole lentil extruded snacks by varying feed composition and extrusion process parameters precisely, which had a crunchy, crispy and soft texture. However, their functional properties such as water absorption, water solubility and oil absorption index were generally low (Lazou and Krokida 2010b). Dongan et al. (2013) also claimed the potential of producing novel, extruded, snack foods from lentil flour. The optimum composition for extruding lentil flour mixed with corn flour and corn oil was 67%, 30% and 3% respectively based on their functional properties, which were processed at a temperature of 178° C and 15% moisture content. Wang et al. (2014), reported that lentil starch can be used for producing starch noodles and can be an alternative to commercially available mung bean starch noodles. Moreover, it exhibited superior textural characteristics, when prepared by high-temperature extrusion (up to 95° C at certain zone of extruder barrel).

1.5.4 Other (Miscellaneous) Application

Various attempts have been made to incorporate lentil into our diet. Among them, de la Hera et al. (2012) added lentil flour to layer and sponge cakes. Results showed that the use of fine particle size lentil flour increased cake volume and reduced hardness in sponge cake. Volume, cohesiveness, springiness and symmetry index were reduced with the addition of lentil flour in layer cake, whereas no relation was observed for sponge cake. Complete substitution of wheat flour with lentil flour resulted in a harder and less cohesive sponge cake. Nutritive value of cakes was improved with the addition of lentil flour, preferably one with fine particle size. Moreover, it also was suggested that the use of fillings and toppings could mask the flavor and increase the acceptability of cakes.

In another study, Bugera et al. (2013) incorporated lentil into a tomato pasta sauce to increase its nutritive value, as lentil is considered highly nutritive food with diverse health benefits. The tomato lentil pasta sauce was tested for acceptability to baby boomer generations (born 1946 to 1965) in Manitoba, Canada. It was observed that the colour, texture, aroma and flavour were appreciated by participants, who were willing to purchase it if it were available in today's market. As consumers demand for a healthy life style, food industry companies have started to focus on natural and ready to eat pulse ingredients. Among the conventional foods items, lentils are gaining interest to consumer as more researchers have revealed their potential to prevent diet related diseases by different mechanisms. The limiting factor for excessive utilization and consumption being the presence of antinutritional factors, through processing viz. germination, drying and extrusion (thermal treatments) can remarkably reduce antinutrients. These methods upgrade the nutritional value of lentils by improving protein digestibility and lowering glycaemic index. Table 1.2 summarize selected processes and utilization of lentils.

Table 1.2: Processes and mode of utilization of lentil.

Process	Mode of utilization and its investigation	Reference
Baking	Lentil flour was used for making sponge cake and layer cake. Wheat flour was substituted upto 100% and its quality was analyzed	Hera et al. (2012)
Germination and baking	Germinated lentil flour was blended with wheat flour to see changes during baking	Hsu et al. (1980)
Germination and fermentation	Lentil sprouts were incorporated during the production of vegetable juice and fermented vegetable juice and evaluated for various health benefits	Simsek et al. (2014)
Germination	Ready-to-eat lentil sprout production was optimized by elicitation with hydrogen peroxide	Swieca (2015)
Fermentation	Lentil flour was added to skim milk for production of yogurt. Probiotic characteristics exhibited due to blending were investigated	Zare et al. (2012)
Fermentation	Yogurt was blended with lentil flour. Probiotic growth enhancement and the antioxidant activity of lentil's polysaccharide were examined	Agil et al. 2012
Extrusion cooking after microwave irradiation	After microwave irradiation of lentil starch, it was extruded and evaluated for changes resulting microwave irradiation ad extrusion	Gozalez and Perez (2002)
Extrusion	Blend of lentil flour and corn flour was extruded to produce snacks. Structural and textural characteristics of extruded snacks was studied	Lazou and Krokida (2010a)
Extrusion	A lentil and corn flour mixture were extruded and investigated for water absorption, water solubility and oil absorption indexes based on different extrusion conditions	Lazou and Krokida (2010b)
Extrusion	A mixture of lentil flour and corn flour was extruded to produce extruded snacks. Phase transition during the different extrusion processes were evaluated	Lazou and Krokida (2011)
Extrusion	Lentil flour, corn starch and corn oil were extruded and levels optimized based on their functional and physical properties	Dogan et al. (2013)
Extrusion	Lentil starch was isolated and extruded to produce starch noodles and their quality was analysed	Wang et al. (2014)
Thermal cooking	Lentil was added to pasta sauce and its acceptability was evaluated among baby boomers in Manitoba, Canada (born between 1946 to 1965)	Bugera et al. (2013)

1.6 Conclusion

Although there has been a substantial increase in lentil production worldwide, its consumption is limited in certain regions. Its utilization can be enhanced by blending of lentil flours in confectionery, breakfast snacks, pasta, etc. in the correct proportions, with improved bioavailability of nutrients. An attempt also can be made to produce an extruded product with germinated lentils to increase starch digestibility and antioxidant activity. Therefore, still there exists room for research on the utilization of lentil in our diverse food system.

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CHAPTER 2

CHEMICAL CHARACTERIZATION OF LENTILS DURING GERMINATION

Contribution of this chapter to the overall study

This study was conducted to select the optimum number of days (period) for germination of selected varieties of green and red lentils. The selection was based on total phenolic content, total antioxidant activity and starch digestibility. The study is related to specific objective 1. The remaining chapters (3-5) are based on the findings of this chapter. All the experiments were conducted and the chapter manuscript was drafted by myself.

2.1 Abstract

This work studied the effect of germination on total phenolic compound (TPC), total antioxidant activity (TAA) and *in vitro* starch digestibility (SD) of lentils (green lentil and red lentil types).The TPC, TAA and *in vitro* SD increases significantly ($p<0.05$) with days of germination. The highest level was observed for 5 day germinated lentil with 8.77 GAE mg/g DM and 9.43 GAE/mg/g DM for green and red lentil, respectively. The increase in TAA during 5 days of germination was higher for red lentil compared green lentils, 16.29% and 11.59%, respectively. The increment in SD was 8.41% for germinated green lentils and 12.01% for red lentils respectively. Based on the highest TPC and TAA, the number of days of germination seems to be optimum on 5 day of germination as TPC and TAA tended to decline on day 6. The SD increased steadily even after 5 days of germination; however the increment was much less after germinating for 5 days. Therefore, lentil after 5 days of germination was selected for further studies and utilization.

2.2 Nomenclature

CDC	Crop Development Centre	d	day
DM	Dry matter	RS	Resistant starch
DPPH	2, 2-diphenyl-1-picrylhydrazyl	SD	Starch digestibility (%)
GAE	Gallic Acid equivalent	TAA	Total Antioxidant activity (Teq mg/g DM)
GOPOD	Glucose oxidase/peroxidase	Teq	Trolox equivalent
KOH	Potassium hydroxide	TPC	Total phenolic content (GAE mg/g DM)
NRS	Non-resistant starch	TS	Total Starch

2.3 Introduction

Lentil (*Lens culinaris* Medikus) is an inexpensive source of protein, yet it contains some amount of anti-nutritional compounds. Germination of seeds is a post-harvest processing method which is rather inexpensive, can improve nutritive value, and reduce or remove anti-nutritional factors (Vidal-Valverde et al. 2002). It has been reported that trypsin inhibitor activity is reduced by soaking of lentil in distilled water (Mulimani and Prarjyoti 1994; Sampathkumar 2011), and also that phytic acid is utilized to generate phosphates by the action of phytase, for the developing seedling (Esonu et al. 1998). Apart from reduced level of antinutritional compounds, germinated seed shows significant increase in the phenolic antioxidant content of lentil when compared to non-germinated seeds. According to Esonu et al. (1998), germination initiates chemical changes by breaking down certain materials and transporting these materials from the endosperm to the embryo and cotyledons to growing parts. Stored proteins are hydrolyzed and the amino acids are transported into the growing seedling axis during germination. Phytic acids also are hydrolyzed during germination, enhancing the bioavailability of protein (Reddy et al. 1982). As the germination process utilizes carbohydrates (starch) and protein for plant metabolic activities, the glycaemic index and protein digestibility levels change.

Ellis and Barrett (1994) germinated lentil at different temperature conditions and studied its effect on seed germination rate. The result showed that alternation in temperature had instantaneous respond on rate of germination, but longer exposure to sub-zero temperature damage the seed resulting in delay of seed germination when kept at warmer temperature for germination. Śweica et al. (2013) germinated the lentil seeds with elicitation of manitol and NaCl for two days. The study concluded that starch digestibility was connected with amylase inhibitor activity and resistant starch content and germination can modify the bioavailability of starch and expected glycemic index. EL-Adawy et al. (2003) studied the changes in chemical composition, antinutritional factor, *in vitro* digestibility and functional properties during germination of mung bean, pea and lentil for 72 h and 120 h at room temperature (25 °C). The result showed that there was significant reduction in total protein, fat and carbohydrate with significant reduction in antinutritional factor of all germinated legumes seeds.

While lentil output has increased significantly globally, some countries still consume relatively little of it. By blending lentil flours in the optimum amounts for pasta, breakfast snacks, confections, etc., it can be used more effectively and have better nutritional bioavailability. To improve starch digestibility and antioxidant activity, an extruded product produced with germinated lentils can also be attempted (Nongmaithem and Meda (2016)). Although there have been various reports on changes of TPC, TAA and *in vitro* SD of lentil during germination, little is known about the changes of CDC Greenland and CDC Maxim lentil types. The objective of the study was to investigate the changes in TPC, TAA and *in vitro* SD of CDC Greenland and CDC Maxim lentil types during germination.

2.4 Materials and Methods

The lentil sample (CDC Greenland and CDC Maxim) were supplied by the University of Saskatchewan's Crop Development Centre (CDC). The CDC Greenland (green coat with yellow cotyledon) type had 6.84±0.64 mm major axis diameter and 3.02±0.22 mm thickness whereas CDC Maxim (grey seed coat with orangey-red cotyledon) type had major axis diameter of 4.63±0.71 mm and 2.42±0.12 mm thickness. The seeds were soaked in distilled water for 8 h and then transferred to aluminum trays for germination for up to 6 d. During the germination period, they were watered (rinsed with distilled water) every 12 h. The length of the sprout was about 4-6 cm after 6 days of germination. The germinated seeds were frozen at -20 °C after each harvest (1, 2, 4, 5 and 6 days) and freeze dried. The freeze-dried samples were grounded using multipurpose hammer mill (equipped with a sieve having aperture of 1 mm diameter) and kept in storage at -20 °C for further analysis.

2.5. Chemical analysis

2.5.1 Extraction

Ground samples were extracted using 70% aqueous ethanol (1/10 w/v) at 25 °C for 1 h. The supernatants were decanted to a glass vial following centrifuging at 3500 rpm for 10 minutes. This process was repeated three times to maximize the extraction. On the third repetition, the supernatant was a clear solution. The extracts were stored at -20 °C for further analysis.

2.5.2 Total phenolics content

Total phenolics content was determined based on the colour reaction of Folin-Ciocolteu reagent with hydroxyl groups (Swieca et al. 2014). To 0.5 mL of extract, 0.5 mL of water and 2 mL of Folin-Ciocalteau regent (the reagent is diluted to 1:5 with distilled water) were added. After 3 min, 10 mL of 10% sodium carbonate solution was added and allowed to stand for 30 min. Absorbance was measured using a spectrophotometer (Model: 6305, Jenway, Bibby Scientific Limited, Staffordshire, UK) at 725 nm. A standard (calibration) curve of gallic acid (50, 100, 150, 250 and 500 mg in 1 L) was plotted. The amount of total phenolic compounds was calculated as gallic acid equivalents (GAE) in mg per g of dry matter.

2.5.3 Total antioxidant activity

A DPPH (2, 2-diphenyl-1-picrylhydrazyl) stock solution of 500 µM was prepared by dissolving 19.6 mg of DPPH in 100 mL of 70% aqueous methanol and allowed to stand for 20 minutes at 25°C (Mitra et al. 2013). Three sample solutions of crude extract, 5-fold and 10-fold were prepared using 70% aqueous methanol. An aliquot of 0.25 mL was added to 2 mL DPPH solution and vortexed for 15 s and then held at room temperature for 15 min. A blank solution was prepared by adding an equal amount of 70% aqueous methanol to DPPH. The absorbance was measured at 517 nm for the sample and blank, which was calibrated with 70% methanol. Percentage DPPH inhibition was determined using the following equation:

$$\%\,DPPH\ inhibition = \left(1 - \frac{Abs_{sample}}{Abs_{blank}}\right) x100 \tag{2.1}$$

where,

Abs = absorbance for both sample and blank.

The level of DPPH radical scavenging capacity was expressed in terms of mg-Trolox equivalents per g dry matter by plotting a standard curve of Trolox.

2.5.4 *In vitro* starch digestibility

In vitro starch digestibility (% SD) was determined using a Megazyme^{TM} resistant starch assay kit, Magazyme International Ireland, Co. Wicklow, Ireland. One hundred milligram sample was hydrolysed using pancreatic α-amylase and amyloglucosidase for 16 h at 37 °C for determination of resistant starch (RS), non-resistant starch (NRS) and Total Starch (TS) content. With addition of ethanol, the reaction was terminated and the supernatants were collected after centrifugation for 10 min at 3000 rpm for NRS determination. Supernatants were removed by decantation. RS (remaining pellets in glass vials) was dissolved in 2 M KOH by vigorously stirring in an ice water bath over a magnetic stirrer for 20 min, which was then neutralised with acetate buffer and hydrolysed to glucose with amyloglucosidase. Using glucose oxidase/peroxidase reagent (GOPOD) D-glucose was quantified as the RS content of the sample. Non-resistant starch (solubilised starch) was determined with a GOPOD, using supernatant collected previously. Absorbance was measured using a spectrophotometer at 510 nm against a reagent bank. Total starch and *in vitro* starch digestibility was calculated using the following equation (Swieca et al. 2013):

Total starch (TS) = Resistant starch (RS) + Non-resistant starch (NRS) (2.2)

$$\%SD = 100 - \left(\frac{RS}{TS}\right) * 100 \tag{2.3}$$

2.6. Results and Discussion

2.6.1 Total phenolics content (TPC)

The changes in total phenolic content during the germination of lentil are shown in Figure 2.1. The highest level was observed for 5 day germinated lentil (Figure 2.1), with 8.77 GAE mg/g DM and 9.43 GAE/mg/g DM for green and red lentil, respectively.

Table 2.1 Changes in total phenolic content, total antioxidant activity and in vitro starch digestibility during germination.

	TPC (GAE mg/g DM)		**TAA** (Teq mg/g DM)		***in vitro* SD** (%)	
Days	**GL**	**RL**	**GL**	**RL**	**GL**	**RL**
0	7.48	6.42	10.44	11.60	64.95	62.18
1	7.61	7.13	10.92	12.12	66.75	63.78
2	7.96	8.32	11.10	12.27	68.21	65.23
4	8.52	9.23	11.34	12.60	69.71	67.83
5	8.77	9.43	11.65	13.49	70.23	68.38
6	7.67	9.16	11.54	12.66	70.41	69.65

GL-green lentil and RL- red lentil

TPC increased with the number of days of germination for both lentil types (green and red). For both types, TPC content decreased after 5 days of germination. However, in all the cases, TPC of germinated lentils was higher than for the raw lentil seeds. The findings are similar to the results reported by Gharachorloo et al. (2013), where all samples (including lentil) had higher phenolic contents as compared to non-germinated samples.

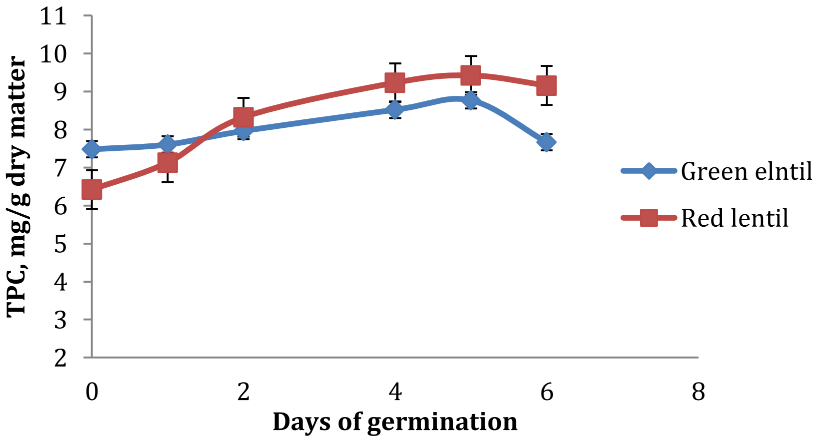

Fig 2.1: Changes in total phenolic content during germination of lentils.

On 5 day of germination, the red lentil had higher TPC contain (7.52%) than green lentil. The increase in TPC was significant ($p<0.05$) in both the lentil types.

Table2.2 Two-factor analysis of variance of total phenolic content.

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Row 1	3.00	13.90	4.63	16.38
Row 2	3.00	15.74	5.25	13.58
Row 3	3.00	18.29	6.10	12.61
Row 4	3.00	21.76	7.25	8.06
Row 5	3.00	23.19	7.73	5.70
Row 6	3.00	22.83	7.61	2.50
Column 1	6.00	18.00	3.00	5.60
Column 2	6.00	48.01	8.00	0.28
Column 3	6.00	49.70	8.28	1.55

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	25.49	5.00	5.10	4.37	0.02	3.33
Columns	106.00	2.00	53.00	45.39	9.62E-06	4.10
Error	11.68	10.00	1.17			
Total	143.17	17.00				

2.6.2 Total antioxidant activity (TAA)

Total antioxidant activity increased with an increase in days of germination, as shown in Fig 2.2.

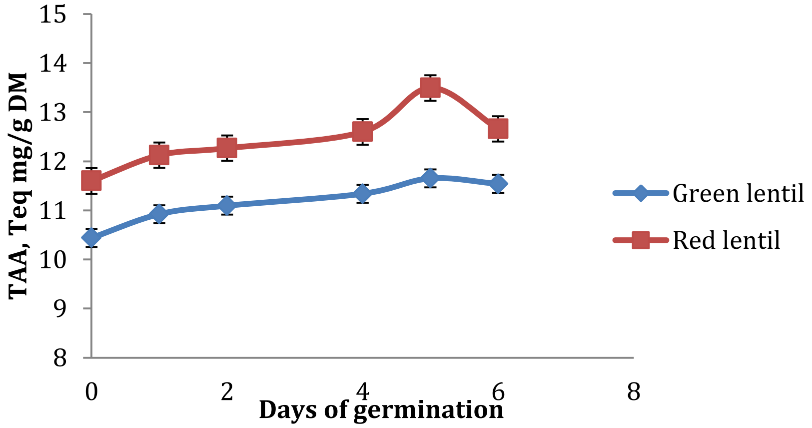

Fig 2.2: Changes in total antioxidant activity during germination of lentils.

The highest TAA was observed after 5 days of germination for both lentil types with 11.65 and 13.42 Teq mg/g DM for green and red lentil, respectively. The increase in TAA during 5 days of germination was higher for red lentil compared green lentils, 16.29% and 11.59%, respectively. This increase in TAA can be related to TPC. There are reports of positive relationships between phenolics and antioxidant activity in lentil (Cevallos-Casals & Cisneros-Zevallos 2010; Świeca 2015). The red lentil was 15.79% higher in TAA compared to green lentil, on 5 day of germination. The increase in TAA was significant ($p<0.05$) for both lentil types.

Table2.3 Two-factor analysis of variance of total antioxidant activity.

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Row 1	3.00	23.31	7.77	46.76
Row 2	3.00	24.05	8.02	37.28
Row 3	3.00	25.36	8.45	31.59
Row 4	3.00	27.94	9.31	21.56
Row 5	3.00	30.15	10.05	19.96
Row 6	3.00	30.20	10.07	12.72
Column 1	6.00	18.00	3.00	5.60
Column 2	6.00	66.99	11.16	0.20
Column 3	6.00	76.01	12.67	0.24

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	15.29	5.00	3.06	2.05	0.16	3.33
Columns	324.83	2.00	162.42	109.06	1.62E-07	4.10
Error	14.89	10.00	1.49			
Total	355.01	17.00				

2.6.3 *In vitro* Starch Digestibility

During germination, the *in vitro* starch digestibility increased with increase an in days of germination (Fig 2.3). However, the level of changes in digestibility corresponding to the number of days of germination was minimal after 4 days of germination, especially for green lentils. The highest level was observed for samples germinated for 6 days. The increment was 8.41% for germinated green lentils and 12.01% for red lentils respectively.

Comparable findings can be observed in Swieca et al. (2013), during the germination of lentils at various conditions. The increment in SD from 5 day to 6 day germination was minimal, 0.26% and 1.8% for green and red lentil types, respectively. The increase in SD for 5 days of germination were significant ($p<0.05$) for both lentil types.

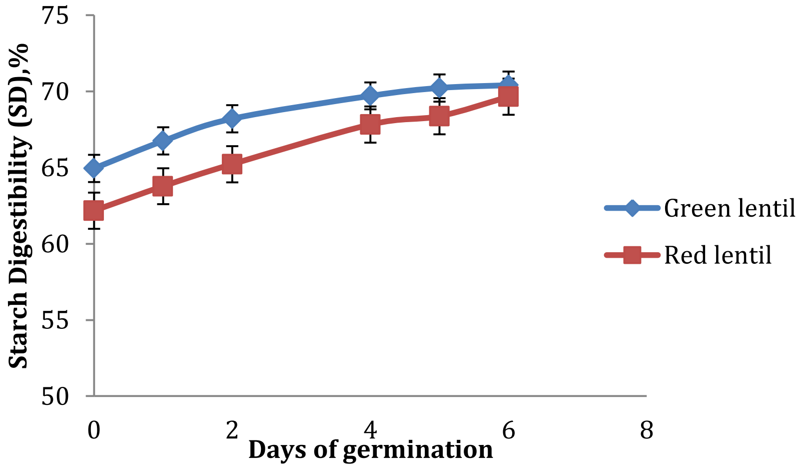

Fig 2.3: Changes in *in vitro* starch digestibility during germination of lentils.

Table2.2 Two-factor analysis of variance of *in vitro* starch digestibility.

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Row 1	3.00	127.13	42.38	1348.75
Row 2	3.00	131.53	43.84	1378.87
Row 3	3.00	135.44	45.15	1398.45
Row 4	3.00	141.54	47.18	1399.27
Row 5	3.00	143.61	47.87	1379.23
Row 6	3.00	146.06	48.69	1366.76
Column 1	6.00	18.00	3.00	5.60
Column 2	6.00	410.26	68.38	4.75
Column 3	6.00	397.05	66.18	8.45

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	91.44	5.00	18.29	70.76	1.78E-07	3.33
Columns	16540.07	2.00	8270.04	31999.14	9.31E-20	4.10
Error	2.58	10.00	0.26			
Total	16634.10	17.00				

2.7. Conclusions

Based on the highest TPC and TAA, the number of days of germination seems to be optimum on 5 day of germination as TPC and TAA tended to decline on day 6. The SD increased steadily even after 5 days of germination; however the increment was much less after germinating for 5 days. Therefore, lentil after 5 days of germination was selected for further studies and utilization.

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CHAPTER 3

EFFECT OF MICROWAVE AND MICROWAVE-VACUUM DRYING ON DRYING KINETICS OF GERMINATED LENTIL AND ITS ENERGY CONSUMPTION

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Contribution of this paper to overall study

No comprehensive research has been reported on drying of germinated lentil. The microwave drying technique has been proven as an effective and efficient drying technique for various food materials. This chapter investigated drying kinetics along with effective moisture diffusivity during drying of germinated lentil to produce dried, germinated lentil flours. The dried germinated lentil flours were used for extrusion, which is described in Chapter 5. The investigation on overall energy consumption during microwave and microwave-vacuum drying of germinated lentil will help in choosing a better drying technique. This paper was specific to objective 2. All the experiments were conducted and the manuscript was drafted by myself.

3.1 Abstract

The present study examined the effect of microwave and vacuum on the drying kinetics of pulsed mode microwave-vacuum drying (MVD) of germinated lentil. The germinated lentil was dried using the pulsed-mode, microwave-vacuum technique for 2 s, 5 s and 8 s of 2000 W microwave power at 0, 15 and 45 kPa vacuum pressure. Overall, the Modified page model II showed the highest R^2 and lowest RMSE values. The drying rate constant *k* and effective moisture diffusivity (D_{eff}) increased with an increase in microwave pulse and vacuum pressure level, resulting in a reduction in drying time. This reduction in

drying time reduced energy consumption from 43.81 kWh in hot air (HA) drying (65 °C at 1.5 ms^{-1}) to 0.7016 kWh in microwave vacuum drying (MVD) at 8s and 15 kPa drying. MVD proved to be a promising technique in drying of germinated lentil.

3.2 Nomenclature

A	Area, (m^2)	MVD	Microwave vacuum drying
C_a	Specific heat of air (kJ/kg °C)	MWD	Microwave drying
D_{eff}	Effective moisture diffusivity (m^2/s)	P_{MW}	Microwave rated power output (W)
E	Energy consumed (kWh)	P_V	Vacuum pump rated power output (W)
E_{MWD}	Energy consumed during MWD (kWh)	RMSE	Root mean square error
		R^2	Coefficient of determination
E_{MVD}	Energy consumed during MVD (kWh)	t	Drying time (h)
HA	Hot air drying	ΔT	Temperature difference (°C)
k	Drying constant (s^{-1})	v	air velocity (m/s);
M_e	Equivalent moisture content (%)		
M_i	Initial moisture content (%)	Greek Symbol	
M_t	Moisture content at time t (%)	ρ_a	air density (kg/m^3)
MR	Moisture ratio		

3.3 Introduction

Lentils (*Lens culnaris* Medikus), plays a vital role in the diet and is consumed daily by many people (Iqbal et al. 2006). It is one of the oldest cultivated crops of many cultures and civilization mostly, in Latin America, Africa, and the Asian subcontinent. At present, Canada is the largest producer of lentil, followed by India, Australia, Turkey and the United States of America. Lentil can be considered an inexpensive source of protein and other bioactive compounds (Nongmaithem and Meda 2016; Gharachorloo 2013). However, it also contains aninutritional compounds such as tannins, phytic acids, oligosaccharides, and trypsin inhibitors (Wang and Daun 2006) which reduce the bioavailability of nutrients.

Germination and heat treatment can greatly reduce the level of antinutrients (Mulimani and Paramjyothi 1994; Sampathkumar 2011). Further (including lentil) are consumed after various types of processing (cooking, sprouting, frying, etc.). Among processing types, germination has been shown to be the most effective in significantly increasing nutraceutical and nutritional quality (Cevallos-Casals and Cisneros-Zevallos 2010; Swieca 2015). Many studies have been reported on changes in antioxidant activity and digestibility of lentil after germination by reducing antinutrients (Gharachorlo et al. 2013; Swieca et al. 2013).

Germinated seed has high moisture content and is among the most perishable of foods. Therefore, drying is an important unit operation for increasing shelf life, as well as increasing the feasibility of utilizing germinated lentil in various food products. The use of microwave in drying of high value crops has been intensively studied in the past few years. Microwave drying is reported to be energy efficient and associated with shorter drying time as compared to conventional hot air drying (Bal et al. 2011; Kantrong et al. 2016). Addition of vacuum in microwave drying allows both the volumetric heating effect of microwave and the rapid transfer of moisture to the surrounding vacuum, which helps in the retaining high quality food products (Calín-Sánchez et al. 2011). Microwave vacuum drying techniques have been applied widely in food products, such as garlic (Figiel 2009), rosemary (Calín-Sánchez et al. 2011), beetroot (Figiel 2010) and apples (Sham et al. 2001).

Considering the fact that drying operations account for the highest energy consumption in agro-processing, effective models are necessary for process design, optimization, energy integration and control in drying of food or biological (plant-based) substances (Erbay and Icier 2009). Drying is a simultaneous heat and mass transfer process. Although extensive research has been done on drying models, there is no model to unify the calculations. Hence, thin-layer drying equations are simple, but they are practical and provide information necessary to describe the mechanism of drying mathematically (McMinn 2004). To describe the mass transfer mechanism, effective moisture diffusivity, which describes all the diffusion mechanisms, is to be determined for designing and modeling mass transfer processer such as dehydration, adsorption and desorption of moisture.

Therefore, the main objective of the current study was to investigate the drying kinetics of microwave and microwave-vacuum drying of two types of germinated lentil based on five existing thin-layer drying models. The effective moisture diffusivity and energy consumption during the drying of germinated lentil also was determined.

3.4 Materials and Methods

Samples of two lentil types namely green lentil (CDC Greenland) and red lentil (CDC Maxim) were supplied by the University of Saskatchewan's Crop Development Centre, Canada. Samples were germinated in aluminum containers for 5 days (120 h) under dark condition at room temperature (25°C). Initially lentil seed was soaked in distilled water for 8 h. Sprouting samples were rinsed every 12 h. The numbers of days of germination was selected based on preliminary trials on the quality of germinated seed (based on Chapter 2).

3.4.1 Drying and drying equipment

Three hundred gram of germinated lentil was dried using a microwave-vacuum dryer (MVD) (Model: VMD 1.8, EnWave^{TM} Corp., Vancouver, Canada). The dryer had a rated output power of 2000 W and maximum vacuum pressure of 101.592 kPa. The microwave power could be set "ON" and "OFF" for 0-10 s, where 10 s implies a continuous supply of microwave power. Sample was put in perforated cylindrical container (polypropylene cylinder having 3.8 mm diameter holes) which was placed in a roller (10 rpm) inside the drying chamber. The drying chamber was 35 cm long with a 45 cm diameter. Both lentil types were dried in pulsed-mode of 2 s, 5 s and 8 s of 2000 W microwave power (MW). To examine combined effect of microwave power and vacuum (MVD), two levels of constant vacuum pressure were selected, namely 15 kPa and 45 kPa. The MVD independent parameters were selected based on maximum capacity of the dryer. To compare with conventional hot air (HA) drying, germinated seed was dried in a hot air dryer at 65 °C and 1.5 m/s air velocity. The system had a wire mesh tray (24×27×0.5 cm) mounted on a weighing balance which allowed the recording of changes in weight during drying. The final moisture content of dried seed was in the range of 10±2% dry basis. The results were reported as the mean value of two repetitions.

3.4.2 Mathematical modeling of MVD and MW drying

Thin-layer models are divided into theoretical, semi-theoretical and empirical equations. Various assumptions were made during modeling of thin layer drying. The assumptions are as follows (Erbay and Icier, 2009): i) the particle is homogenous and isotropic; ii) the material characteristics are constant, and the shrinkage is neglected; iii) the pressure variations are neglected; (iv) evaporation occurs only at the surface; (v) initially moisture distribution is uniform and symmetrical during process; (vi) surface diffusion is ended, so the moisture equilibrium arises on the surface; (vii) temperature distribution is uniform and equals to the ambient drying air temperature, namely the lumped system; (viii) the heat transfer is done by conduction within the product, and by convection outside of the product; and (ix) effective moisture diffusivity is constant versus moisture content during drying. However, semi-theoretical models are more widely used as they need fewer assumptions and they incorporate experimental data. The semi-theoretical models are derived from Newton's law of cooling and Fick's second law of diffusion. In order to determine the moisture ratio as a function of drying time, five different thin-layer drying models were used. The moisture ratio (MR) was calculated using the following equation:

$$MR = \frac{M_t - M_e}{M_i - M_e} \tag{3.1}$$

Where, M_t is moisture content at time t, M_i is the initial moisture content and M_e is the equilibrium moisture content, which is equal to zero if vacuum is employed, or final moisture content for the rest. The equilibrium moisture content (M_e) was assumed to be zero for both MW and MVD drying (McMinn 2004). The drying curve for both microwave (MWD), microwave-vacuum (MVD) and hot air (HA) drying were fitted to five, thin-layer drying models which are presented in Table 3.1.

Table 3.1: Empirical and semi-empirical thin layer drying models

Model	Mathematical expression
Modified Page II (1978)	$MR = exp(-(kt)^n)$
Henderson and Pabis (1961)	$MR = a\ exp(-kt)$
Logarithmic (Asymptotic) (1995)	$MR = a\ exp(-kt)+c$
Medilli (2002)	$MR=a\ exp(-kt^n)+bt$
Wang and Singh (1978)	$MR=1+at+bt^2$

Regression analyses of these models were done for both drying methods and lentil types. The criteria adopted to evaluate the goodness of fit model were root mean square error (RMSE) and coefficient of determination (R^2). These statistical parameters were determined as follows:

$$RMSE = \left[\frac{1}{N}\sum_{i=1}^{N}(MR_{exp,i} - MR_{pred,i})^2\right]^{0.5} \tag{3.2}$$

$$R^2 = \frac{S_t - S_r}{S_t} \tag{3.3}$$

$$S_t = \sqrt{\sum_{i=1}^{n_{points}}(\bar{y} - y_i)^2} \tag{3.4}$$

$$S_r = \sum_{i=1}^{n_{points}}(y_i - y(x_i))^2 \tag{3.5}$$

$$\bar{y} = \frac{1}{n_{points}}\sum_{i=1}^{n_{points}} y_i \tag{3.6}$$

3.4.3 Determination of effective moisture diffusivity (D_{eff})

Drying comprises different diffusion mechanisms which can be combined together as one parameter, termed the effective moisture diffusivity (D_{eff}). The effective moisture diffusivity can be determined by following Fick's second law of diffusion, for sufficiently long drying:

$$MR = \frac{M_t - M_e}{M_i - M_e} = A_1 \exp\left(-\frac{\pi^2 D_{eff}}{A_2}\right) \tag{3.7}$$

If D_{eff} is assumed to be constant during drying, then equation 3.7 can be rearranged as:

$$MR = a\ exp(-kt)$$

$$\text{or } \ln MR = \ln a - kt \tag{3.8}$$

where, a is a model constant based on shape (dimensionless) and k is the drying constant (s^{-1}), in which the k term can be written as equation 3.9 (from equation 3.8):

$$k = -\frac{\pi D_{eff}}{A_2} \tag{3.9}$$

where, A_2 is a geometric constant. In the present study, as the samples were placed in a rotating drum, A_2 was calculated assuming spherical shape and calculated after determining the equivalent geometric diameter of lentil seeds.

3.4.4 Determination of energy consumption

3.4.4.1 Hot air drying

The amount of energy consumed during HA drying can be calculated from equation 3.10 (Motevali et al., 2011; Aghbashlo et al., 2008):

$$E = Av\rho_a C_a \Delta T t \tag{3.10}$$

where, E is energy consumed (kWh); A is the area of the sample container (m^2); v is air velocity (m/s); ρ_a is air density (kg/m^3); C_a is specific heat of air (kJ/kg °C); ΔT is temperature difference (°C) and t is drying time (h).

3.4.4.2 Microwave drying (MWD) and Microwave vacuum drying (MVD)

The energy consumed during MWD can be calculated from rated power output (P_{MW}) and time consumed (pulsed mode) as in equation 3.11:

$$E_{MWD} = P_{MW}\, t_1 \tag{3.11}$$

For MVD, energy consumed can be calculated with the addition of energy consumed by the vacuum pump (P_v), as in equation 3.12:

$$E_{MVD} = P_{MW}t_1 + P_v t_2 \qquad (3.12)$$

where, t_1 is the total time of pulsed mode microwave power during ON and t_2 is the total time consumed during drying at constant vacuum pressure.

3.5. Results and Discussion

3.5.1 Drying kinetics

The initial moisture content of the germinated lentil sample was 70±5% (wet basis). The moisture was reduced to 10±2% wet basis for all the drying experiments. The change in moisture ratio during drying different MWD and MVD are shown in Figure 3.1 and 3.2. It was observed that MVD had lower drying time than MWD. Drying time decreased with an increased in pulse-mode microwave pulse level and vacuum pressure level. The least drying time was observed at 8 s and 45 kPa for both green and red lentil, 25 and 26 min respectively. The longest drying time was observed for HA drying being 7.75 h for both lentil types. During microwave heating, heat is generated by ionic polarization and dipole mechanism. In ionic polarization, ions move at accelerated speed due to the electric field generated by the microwave, resulting in collisions between molecules which create heat in the system, while dipole movement is oscillation of the polar molecules according to the electric field applied, resulting in heat generation due to friction. The dipolar mechanism is more dominant the during microwave heating process. The advantage of using microwave energy is rapid volumetric heating, resulting in higher energy efficiency when compared to conventional hot air (HA) heating/drying. This leads to a significant reduction in drying time of germinated lentil using MWD and MVD as

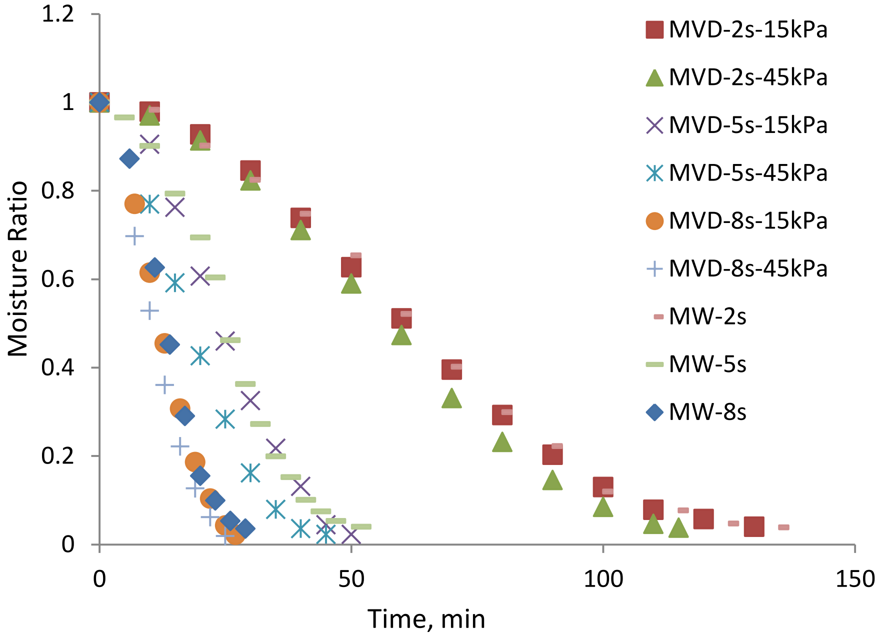

Fig 3.1: Drying kinetics of germinated green lentil at different drying conditions

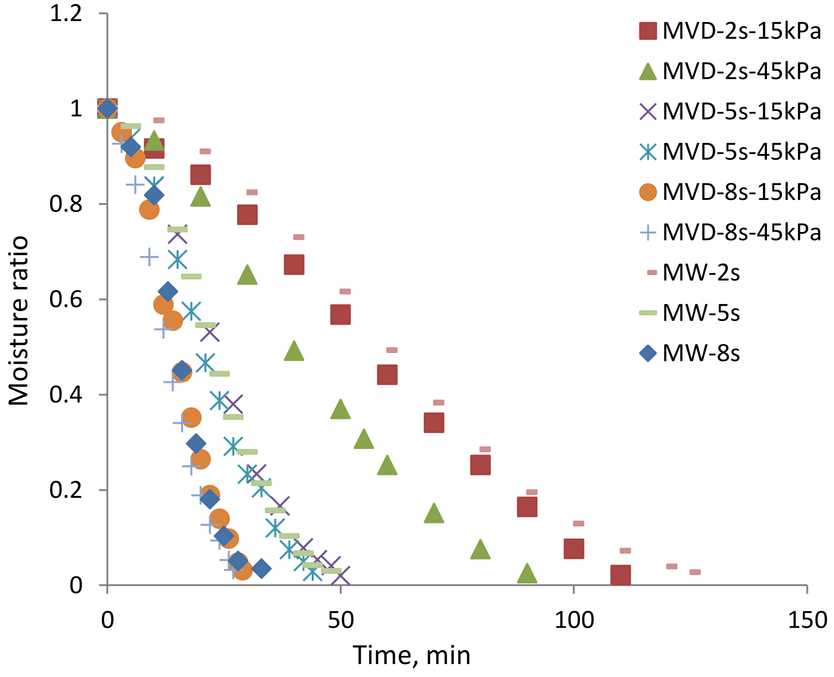

Fig 3.2: Drying kinetic of germinated red lentil at different drying conditions

Table 3.2: Effect of different drying conditions on drying kinetics and statistical analysis of green lentils

Models	Treatment	Coefficients			R^2	RMSE
Modified Page model	2s-15kPa	k=0.01388;	n=2.07522		0.9997	0.0058
	2s-45kPa	k=0.01528;	n=2.00638		0.9976	0.0174
	5s-15kPa	k=0.03642;	n=1.99044		0.9965	0.0202
	5s-45kPa	k=0.04846;	n=1.67368		0.9951	0.0231
	8s-15kPa	k=0.06895;	n=1.94677		0.9994	0.0072
	8s-45kPa	K=0.07872;	n=1.80425		0.9993	0.0072
	2s	k=0.01240;	n=2.01473		0.9977	0.0167
	5s	k=0.03479;	n=2.41787		0.9966	0.0199
	8s	K=0.06469;	n=2.16566		0.9992	0.0096
	Control	K=0.0060;	n=1.10189		0.9978	0.0145
Henderson	2s-15kPa	k=0.01367;	a=1		0.8839	0.1193
	2s-45kPa	k=0.01482;	a=1		0.8794	0.1237
	5s-15kPa	k=0.03559;	a=1		0.8679	0.1232
	5s-45kPa	k=0.04917;	a=1		0.9245	0.0905
	8s-15kPa	k=0.07286;	a=1		0.9199	0.1014
	8s-45kPa	K=0.08420;	a=1		0.9308	0.0847
	2s	k=0.01347;	a=1		0.8888	0.1163
	5s	k=0.03480;	a=1		0.8519	0.1316
	8s	K=0.06865;	a=1		0.8876	0.1147
	Control	K=0.0060;	a=1.015		0.9961	0.0192
Logarithmic	2s-15kPa	a=4.75695;	k=0.0018;	c=-3.75695	0.9688	0.0618
	2s-45kPa	a=1.53697;	k=0.008;	c=-0.53697	0.9407	0.0859
	5s-15kPa	a=1.81020;	k=0.015;	c=-0.81020	0.9503	0.0756
	5s-45kPa	a=1.15897;	k=0.039;	c=-0.15897	0.9566	0.0686
	8s-15kPa	a=1.22689;	k=0.053;	c=-0.22690	0.9564	0.0749
	8s-45kPa	a=1.10588;	k=0.073;	c=-0.10588	0.9525	0.0701
	2s	a=1.56834;	k=0.0069;	c=-0.56834	0.9507	0.0773
	5s	a=1.89055;	k=0.014;	c=-0.89554	0.9484	0.0777
	8s	a=1.53333;	k=0.035;	c=-0.53333	0.9506	0.0760
	Control	a=1.07313;	k=0.0051;	c=-0.074943	0.9988	0.1088
Medilli	2s-15kPa	a=1;	k=0.0041;	b=-0.0045	0.9142	0.0622
	2s-45kPa	a=1;	k=0.0091;	b=-0.0026	0.9513	0.0778
	5s-15kPa	a=1;	k=0.021;	b=-0.0065	0.9520	0.0743
	5s-45kPa	a=1;	k=0.044;	b=-0.0024	0.9552	0.0698
	8s-15kPa	a=1;	k=0.060;	b=-0.0055	0.9588	0.0728
	8s-45kPa	a=1;	k=0.080;	b=-0.0030	0.9509	0.0713
	2s	a=1;	k=0.005;	b=-0.0037	0.9676	0.0628
	5s	a=1;	k=0.011;	b=-0.0109	0.9475	0.0781
	8s	a=1;	k=0.045;	b=-0.0090	0.9513	0.0785
	Control	a=1;	k=0.0054;	b=-0.0001	0.9987	0.0110
Wang and Singh	2s-15kPa	b=-0.0088;	$a=7.590x10^{-6}$		0.9691	0.0615
	2s-45kPa	b=-0.0081;	$a=-7.435x10^{-6}$		0.9773	0.0531
	5s-15kPa	b=-0.0218;	$a=3.698x10^{-5}$		0.9700	0.0588
	5s-45kPa	b=-0.0345;	a=0.00027		0.9834	0.0425
	8s-15kPa	b=-0.0469;	a=0.00037		0.9867	0.0413
	8s-45kPa	b=-0.0571;	a=0.00068		0.9897	0.0326
	2s	b=-0.0088;	$a=1.053x10^{-5}$		0.9690	0.0615
	5s	b=-0.0220;	$a=5.263x10^{-5}$		0.9489	0.0773
	8s	b=-0.0451;	a=0.00036		0.9634	0.0654
	Control	b=-0.0050;	$a=7.208x10^{-6}$		0.9588	0.0072

Table 3.3: Effect of different drying conditions on drying kinetics and statistical analysis of red lentils

Models	**Treatment**	**Coefficients**	**R^2**	**RMSE**
Modified Page model	2s-15kPa	k=0.01510; n=1.67502	0.9893	0.0336
	2s-45kPa	k=0.02035; n=1.78079	0.9985	0.0126
	5s-15kPa	k=0.03703; n=1.99177	0.9982	0.0145
	5s-45kPa	k=0.04080; n=1.92179	0.9985	0.0125
	8s-15kPa	k=0.05752; n=2.10704	0.9973	0.0175
	8s-45kPa	K=0.06585; n=1.90534	0.9989	0.0106
	2s	k=0.01416; n=2.03970	0.9993	0.0095
	5s	k=0.03758; n=2.11800	0.9997	0.0055
	8s	k=0.05617; n=2.62182	0.9949	0.0240
	Control	k=0.00616; n=1.11559	0.9993	0.0083
Henderson	2s-15kPa	k=0.01495; a=1	0.9022	0.1020
	2s-45kPa	k=0.02594; a=1	0.9218	0.0918
	5s-15kPa	k=0.04003; a=1	0.8943	0.1096
	5s-45kPa	k=0.04212; a=1	0.9001	0.1025
	8s-15kPa	k=0.05839; a=1	0.8773	0.1170
	8s-45kPa	K=0.06863; a=1	0.9077	0.1004
	2s	k=0.01414; a=1	0.8878	0.1173
	5s	k=0.03838; a=1	0.8752	0.1178
	8s	K=0.05839; a=1	0.8251	0.1413
	Control	k=0.00636; a=1.03	0.9993	0.0083
Logarithmic	2s-15kPa	a=1.3758; k=0.0095; c=-0.3758	0.9509	0.0722
	2s-45kPa	a=1.1772; k=0.0163; c=-0.1772	0.9501	0.0733
	5s-15kPa	a=1.3356; k=0.025; c=-0.3356	0.9524	0.0736
	5s-45kPa	a=1.1277; k=0.029; c=-0.1590	0.9459	0.0755
	8s-15kPa	a=1.7885; k=0.025; c=-0.7885	0.9527	0.0727
	8s-45kPa	a=1.2775; k=0.047; c=-0.2775	0.9513	0.0730
	2s	a=1.5683; k=0.007; c=-0.5683	0.9507	0.0773
	5s	a=1.7266; k=0.017; c=-0.7266	0.9519	0.0732
	8s	a=1.9426; k=0.022; c=-0.9426	0.9172	0.0973
	Control	a=1.0210; k=0.0057; c=-0.0524	0.9990	0.0098
Medilli	2s-15kPa	a=1; k=0.0118; b=-0.0016	0.9500	0.0728
	2s-45kPa	a=1; k=0.0175; b=-0.0015	0.9557	0.0691
	5s-15kPa	a=1; k=0.031; b=-0.0035	0.9502	0.0752
	5s-45kPa	a=1; k=0.033; b=-0.0039	0.9503	0.0724
	8s-15kPa	a=1; k=0.037; b=-0.0095	0.9503	0.0745
	8s-45kPa	a=1; k=0.056; b=-0.0055	0.9503	0.0737
	2s	a=1; k=0.0075; b=-0.0019	0.9507	0.0777
	5s	a=1; k=0.024; b=-0.0061	0.9514	0.0736
	8s	a=1; k=0.029; b=-0.0124	0.9229	0.0939
	Control	a=1.02; k=0.0059; b=-8.9x10^{-5}	0.9989	0.0103
Wang and Singh	2s-15kPa	b=-0.0087; a=-3.258x10^{-6}	0.9939	0.0254
	2s-45kPa	b=-0.0136; a=2.759x10^{-5}	0.9870	0.0374
	5s-15kPa	b=-0.0249; a=9.777x10^{-5}	0.9798	0.0480
	5s-45kPa	b=-0.0266; a=8.707x10^{-5}	0.9815	0.0441
	8s-15kPa	b=-0.0327; a=-6.068x10^{-5}	0.9797	0.0475
	8s-45kPa	b=-0.0425; a=0.00020	0.9843	0.0414
	2s	b=-0.0087; a=5.433x10^{-5}	0.9782	0.0517
	5s	b=-0.0243; a=7.309x10^{-5}	0.9690	0.0593
	8s	b=-0.0365; a=0.00016	0.9298	0.0895
	Control	b=-0.0052; a=7.55x10^{-6}	0.9996	0.0063

compared to HA drying. The data were fitted to five, thin-layer drying models as shown in Tables 3.2 and 3.3 (green and red lentil respectively). The acceptance of the model was determined based on coefficient of determination (R^2) and root mean square error (RMSE). The Highest R^2 and RMSE were observed in the Modified Page II model for MWD and MVD. The R^2 value of 0.9997 were highest at 2 s and 15 kPa (MVD) for green and 5 s microwave pulse level (MWD) for red lentil. The corresponding lowest RMSE values were 0.0058. Highest R^2 and lowest RMSE for HA drying were observed in the Logarithmic model for green lentil, with an R^2 value of 0.9988, while the Wang and Singh model showed highest R^2 (0.9996) and lowest RMSE value for red lentil. It also can be observed that the drying constant k (min^{-1}) value increased with an increase in microwave pulse and vacuum level, which is responsible for lowering the drying time. It can be concluded that the Modified Page II model can be used to study the kinetics of during MWD and MVD of germinated lentil seed.

3.5.2 Effective moisture diffusivity

Effective moisture diffusivity (D_{eff}) represents overall mass transfer, properties such as molecular diffusion, vapour diffusion, liquid diffusion, hydrodynamic flow and all other possible forms of mass transfer during drying (Karathanos et al. 1990). D_{eff} was calculated using the method of slopes. Curves of ln (MR) against drying time (t) were plotted using the experimental data. The value of k (s^{-1}) was obtained from the curve and D_{eff} was calculated using equation (3.9).

Table 3.4. Effect of different drying conditions on *k* and D_{eff} of green lentil.

Drying conditions	k, s^{-1}	D_{eff}, m^2s^{-1}
2s-15kPa	0.000430	1.2091×10^{-07}
2s-45kPa	0.000486	1.3659×10^{-07}
5s-15kPa	0.001263	3.5496×10^{-07}
5s-45kPa	0.001400	3.9346×10^{-07}
8s-15kPa	0.002564	7.2059×10^{-07}
8s-45kPa	0.002564	7.2059×10^{-07}
2s	0.000428	1.2029×10^{-07}
5s	0.001127	3.1674×10^{-07}
8s	0.002070	5.8176×10^{-07}
Control	0.000120	3.0326×10^{-10}

Table 3.5. Effect of different drying conditions on *k* and D_{eff} of red lentil.

Drying conditions	k, s^{-1}	D_{eff}, m^2s^{-1}
2s-15kPa	0.000490	8.5658×10^{-08}
2s-45kPa	0.000619	1.0821×10^{-07}
5s-15kPa	0.001293	2.2603×10^{-07}
5s-45kPa	0.001299	2.2708×10^{-07}
8s-15kPa	0.001862	3.3774×10^{-07}
8s-45kPa	0.002029	3.5469×10^{-07}
2s	0.000469	8.1987×10^{-08}
5s	0.001259	2.2009×10^{-07}
8s	0.001875	3.2779×10^{-07}
Control	0.000119	3.0174×10^{-10}

The variation in D_{eff} during different drying parameters is shown in Tables 3.4 and 3.5 for green and red lentil, respectively. The highest D_{eff} was observed at 8 s microwave pulse and 45 kPa vacuum pressure level for both lentil types, 7.2054×10^{-7} m^2s^{-1} and 3.5469×10^{-7} m^2s^{-1} for green and red lentil, respectively. The corresponding values of *k* were 0.002564 s^{-1} and 0.002029 s^{-1} for green and red lentil, respectively. The D_{eff} value increased with an increase in microwave pulse and vacuum pressure level. The increase in microwave pulse level (2 s to 8 s) prominently increased its value up to 495% at 15 kPa vacuum pressure (MVD) for green lentil, and up to about 300% in red lentil for MWD drying. An increase in vacuum pressure level increased D_{eff} only up to 24% at 8 s for green lentil, and 31% at 2 s for red lentils during MVD drying of germinated lentil. Higher microwave pulse and vacuum pressure levels increases the driving force of heat and mass transfer, thereby increasing D_{eff} during drying (Dak and Pareek 2014). This phenomenon can be related to drying time, where an increased in D_{eff} resulted in a reducing drying time. Similar observations were made during MVD of spinach (Dadali et al. 2007), bamboo shoot slices (Bal et al. 2010) and pomegranate arils (Dak and Pareek 2014).

3.5.3 Energy consumption

The energy consumption during MWD and MVD is shown in Figure. 3.3. HA had the highest consumption of energy (up to 42.81 kWh and 43.12 kWh during drying of germinated green and red lentils seed, respectively), while the lowest was observed in

MWD. The highest energy consumption during MWD was observed at 2 s microwave pulse level, consuming 0.8267 kWh in green lentil and 0.8 kWh in red lentil, while the lowest was 0.6933 kWh for both lentil types at 8 s microwave pulse level. For MVD, the highest was observed at 2 s and 45 kPa, consuming 1.7238 kWh and 1.0859 kWh for green and red lentils respectively, while the lowest was observed at 8s and 15 kPa, consuming 0.7016 kWh for both lentil types. An increase in microwave pulse level leads to decreased energy consumption in MWD and MVD. The difference in energy consumption due to microwave pulse level at a particular vacuum pressure level decreased with an increase in microwave pulse level (2 s to 8 s). Variation in microwave pulse from 2 s to 8 s microwave pulse level reduced energy consumption in MWD up to about 16.1% in green and 13.3% in red lentil, while energy consumption in MVD was reduced up to 39.5% and 34.6% in green and red lentil, respectively.

Energy consumption increased with increased vacuum pressure in all MVD experiments. At 2 s microwave pulse level, the increment was about 42% for green lentils, while about 36% for red lentils. However, the increment decreased with an increase in microwave pulse, up to about 2.9% at 8 s and 45 Kpa for green lentil, while about 2.4% for red lentils. The decrease in energy consumption was mainly related to drying time. As D_{eff} and k value increases with an increase in microwave pulse level, the energy consumption decreases with an increase in the microwave pulse level. Moreover, volumetric heating during microwave drying led to higher efficiency in consumption of energy as compared to conventional HA drying. Similar observations were made in the drying of garlic cloves (Sharma and Prasad 2006), pomegranate arils (Motevali et al. 2011a) and mushroom slices (Motevali et al. 2011b).

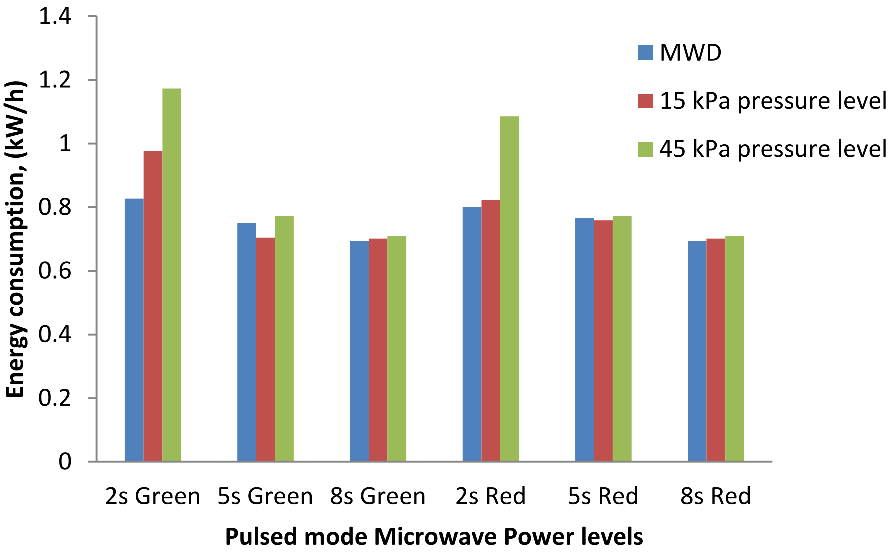

Fig 3.3: Energy consumption during pulsed mode MWD and MVD of germinated lentil

3.6 Conclusions

The study revealed that MWD and MVD were more efficient in drying germinated lentils as compare to HA drying. The drying time in MVD was less than MWD due to the addition of vacuum pressure. The drying kinetics can be best explained by the Modified Page II model for germinated lentil. The drying time decreased with an increase in microwave pulse level and vacuum pressure level as a result of the increase in value of *k* and D_{eff}. The increase in D_{eff} was more pronounced with an increased in microwave pulse level (495%) as compared to vacuum pressure (31%) level. Consumption of energy during drying decreased with an increase in microwave pulse level. The difference in energy consumption due to vacuum pressure was reduced with increasing microwave pulse. Overall, germinated lentil can be dried faster and with less energy consumption by MVD. The dried product can be utilized in development of new food products.

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CHAPTER 4

OPTIMIZATION OF MICROWAVE VACUUM DRYING PARAMETERS FOR GERMINATED LENTIL BASED ON STARCH DIGESTIBILITY, ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT

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Contribution of this paper to the overall study

Research on optimization of processing parameters for microwave vacuum drying of germinated lentil has not been reported. This paper focused on the effect of microwave vacuum drying of germinated lentil on starch digestibility, antioxidant activity and total phenolic compounds. The processing parameters were optimized based on these responses keeping microwave pulse and vacuum as independent parameters. Response surface methodology was employed to express the changes during drying. Optimally dried germinated lentil were ground and used for subsequent extrusion experiments. This paper is specific to objective 3. All the experiments were conducted and was drafted by myself.

4.1 Abstract

The aim of this study was to optimize the processing parameters for pulse-mode, microwave-vacuum drying of germinated green and red lentil (CDC Greenland and CDC Maxim) and investigate changes in the total phenolic content (TPC), total antioxidant activity (TAA) and *in vitro* starch digestibility (SD). The lentils were germinated for 5

days and dried using the pulse-mode microwave-vacuum method, using 2 s to 8 s out of 10 s pulsed mode 2000 W microwave power and varying constant vacuum pressure level of 15 to 45 kPa. *In vitro* starch digestibility increased significantly with increased microwave pulse level. The vacuum pressure levels did not significantly ($p>0.05$) affect any responses. Green lentil could be dried at 8 s microwave pulse and 45 kPa vacuum pressure, and red lentil at 5.5 s microwave pulse and 42.19 kPa vacuum pressure. The results show that there was reduction of about 16% in TPC and about 13% in TAA, of the lentil studied after processing at optimum condition. Microwave-vacuum drying showed great potential in drying of germinated lentils.

4.2 Nomenclature

A	Pulse mode microwave power (s)	NRS	Non-resistant starch
B	Vacuum pressure (kPa)	RS	Resistant starch
ANOVA	Analysis of variance	SD	Starch digestibility (%)
CCRD	Central composite rotate design	TAA	Total Antioxidant activity (Teq mg/g DM)
DM	Dry matter		
DPPH	2, 2-diphenyl-1-picrylhydrazyl	TPC	Total phenolic content (GAE mg/g DM)
GAE	Gallic Acid equivalent	TS	Total starch
MW	Microwave	Teq	Trolox equivalent
MV	Microwave vacuum	Y	Response

4.3. Introduction

Lentils (*Lens culinaris* Medikus), an important legume crop, is among the oldest food cultivated for human consumption (Iqbal et al. 2006). It is widely cultivated in the American and Asian subcontinents. They are consumed mainly in south-east Asian countries, the Middle-East, Africa and Latin America. Pulses, including lentil, are considered an inexpensive and affordable source of protein and carbohydrates, and other valuable nutrients (Giannakoula et al. 2012; Gharachorloo et al. 2013). However, there are reports indicating the presence of antinutritional compounds, such as trypsin inhibitor, α-amylase inhibitor, tannin, phytates, saponins and oligosaccharides (Bhatty 1988;

Alonso et al. 2000; Wang and Daun 2006.). These antinutrients can be significantly removed by germination and heat treatment (Mulimani and Paramjyothi 1994; Sampathkumar 2011). Germination (sprouting) is an inexpensive process for removal of antinutrients and increasing nutritional quality (Vidal-Valverde et al. 2002). Among germinated legumes, lentil contains various functional components which, upon consumption, may reduce or prevent risks of diabetes, cardiovascular diseases and inflammation (Chung et al. 2008; Caccialupi et al. 2010). In the context of living a healthy lifestyle, consumption of pulses has become a path for incorporating healthy and nutrient-rich foods into our daily diets. In addition, consumption after germination is proven as one of the most effective methods for increasing accessibility to bio-available nutrients (Świeca et al. 2013). Many trials have been made to modify the functional chemical composition of lentils using the germination process by varying time, illumination and elicitation (Świeca et al. 2013). During the germination process, seeds are first soaked in water before being placed under warm and humid conditions, which are also ideal and optimal conditions for bacterial proliferation. The germination step is the main source of contamination in sprouts; hence, consumers are recommended to cook (thermal treatment) sprouts thoroughly before consumption (FDA 2004). On the other hand, incorporation of germinated lentil in our daily diet can be made through breakfast cereals, snacks and extruded products, for example pasta and noodles. However, germinated lentil, having higher moisture content, should be dried to desirable moisture content before further processing and value addition. Among the electro-technology-based heating technologies, viz., radio frequency (RF), microwave (MW), ohmic and infrared (IR), microwave shows more promise for drying of food items (Sham et al. 2001). Kadlec et al. (2001) reported that germination followed by microwave and hot-air heat treatment reduced α-galactooligosaccharides, while improving nutritive value, during the processing of pea. With the combining of vacuum with microwave drying, materials can be dried in shorter time and at lower temperatures, allowing better retention of sensory attributes such as colour, texture and essential nutrients (Mitra and Meda 2009). Vacuum-assisted microwave drying has been applied to reduce the moisture content of various plant materials, such as cranberries (Sunjka et al. 2004), tomatoes

(Durance and Wang 2002), garlic (Figiel 2009), rosemary (Szumny et al. 2010; Calín-Sánchez et al. 2011), Saskatoon berries (Mitra and Meda 2009) and sour cherries (Wojdyło et al. 2014). Hence, microwave-assisted drying shows potential as a method for drying of germinated seeds, as the operation is rapid and more uniform and energy efficient as compared to conventional hot air drying.

So far, little has been reported regarding the effects of drying of germinated lentil on antioxidants and *in vitro* starch digestibility. Therefore, the objective of the current study was to optimize the processing parameters for microwave-vacuum drying of germinated lentil and investigate changes in total phenolic content, total antioxidant activity and *in vitro* starch digestibility.

4.4. Materials and Methods

Samples of green (CDC Greenland) and red (CDC Maxim) lentil were supplied by University of Saskatchewan, Crop Development Centre (CDC), Saskatoon, SK, , Canada. Both types were germinated in aluminium containers at room temperature (25 °C) for 5 days (120 h) under dark conditions after soaking in distilled water for 8 h. Sprouts were rinsed every 12 h. The number of days of germination was selected based on preliminary trials on antioxidant activity and starch digestibility (chapter 2 of the thesis).

4.4.1. Experimental design of microwave-vacuum drying

A statistical experimental design based on central composite rotatable design (CCRD) was employed to analyze the effect of microwave-vacuum drying of germinated lentil using Design expert 8.0.7.1 (Stat-Ease, Minneapolis, MN, USA). A microwave-vacuum (MV) dryer (Model: VMD 1.8, EnWave^{TM} Corp., Vancouver, BC, Canada) was employed for drying germinated lentil. The dryer was capable of producing 2000 W microwave power continuously; however it could also be used in pulsed mode by supplying microwave power for 0-10 s out of 10 s, where 10 s out of 10 s served as continuous mode. Pulsed-mode microwave power of 2-8 s out of 10 s and constant vacuum pressure of 15-45 kPa were independent parameters, each at 5 levels. Therefore, a total of 13 experiments were conducted for each lentil type. Samples of 300 g were dried to 10±2% moisture content (wet basis) using perforated polypropylene cylindrical containers rotating at 10 rpm. The actual and coded values of the experimental design are shown in Table 4.1. The input range was selected based on initial trials to avoid burning of samples. The samples were ground using a multipurpose grinding mill equipped with a 1 mm diameter sieve, and stored at -20 °C in a re-sealable polypropylene bag for chemical analysis. All the experiments were conducted in triplicate.

Table 4.1. Actual and coded values of the experimental design.

Microwave pulse , s	Vacuum pressure, kPa	Coded values
0.757	8.79	-1.41
2	15	-1
5	30	0
8	45	+1
9.243	51.21	+1.41

The following second order polynomial responses surface model was fitted to each of the response for analysis, as below:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1}{}_{i<j} \sum_{j=2}^{k} \beta_{ij} X_i X_j \tag{4.1}$$

where, Y is response, β_0, β_i, β_{ii}, and β_{ij} are regression coefficients of the constant, linear, quadratic and interaction terms, respectively, and X_i and X_j are codes of independent variables and k is the number of variables. X_i and X_j were replaced by A and B which signify microwave pulse and vacuum pressure, respectively. A sequential model sum of squares (type I) was carried out to select the final model, where the quadratic model was suggested to fit the responses as cubic and higher order polynomial models were considered aliased. The goodness-of-fit of the model was measured by the coefficient of determination (R^2). The adequacy of the quadratic models was confirmed by ANOVA using Fisher Test value (F-value) and lack of fit.

The optimization of process variables was based on maximum values of all of the responses, i.e. total phenolic content (TPC), total antioxidant activity (TAA) and *in vitro* starch digestibility (SD). In the software, responses can be assigned to different importance levels, where importance level varies from 1 to 5. Therefore, the process variables were optimized based on higher desirability of maximum TAA and SD (importance level: 5) as compared to maximum TPC (importance level: 3).

4.4.2. Total phenolic content and total antioxidant activity

Ground samples were extracted using 70% aqueous ethanol (1/10 w/v) at 25 °C. The supernatants were decanted to a glass vial after centrifuging at 3500 rpm for 10 min. This process was repeated three times to maximize the extraction. On the third repetition, the supernatant was a clear solution. The extracts were stored at -20 °C for further analysis.

Total phenolics content (TPC) were determined based on the colour reaction of Folin-Ciocolteu reagent with hydroxyl groups (Świeca et al. 2014). The absorbance was measured using a spectrophotometer (Model: 6305, Jenway, Bibby Scientific Limited, Staffordshire, UK) at 765 nm and total phenolics content were expressed in terms of gallic acid equivalents (GAE) in mg/g DM. For total antioxidant activity (TAA), a 2,2-diphenyl-1-picrylhydrazyl (DPPH) stock solution of 500 µM was used. TAA was determined according to the procedures described in Mitra et al. (2013). The absorbance was measured at 517 nm for both sample and blank. The spectrophotometer was calibrated using distilled water and 70% methanol for TPC and TAA, respectively. Percentage DPPH inhibition was determined using the following equation:

$$\%\, DPPH\ inhibition = \left(1 - \frac{Abs_{sample}}{Abs_{blank}}\right) x100 \qquad (4.2)$$

where,

Abs = absorbance for both sample and blank.

The level of DPPH radical scavenging capacity was expressed in terms of mg-trolox equivalents per g dry matter by plotting a standard-curve of trolox.

4.4.3. *In vitro* starch digestibility

In vitro starch digestibility (% SD) was determined using the Megazyme resistant starch assay kit (K-RSTAR, Megazyme International Ireland Limited, Wicklow, Ireland). Resistant starch (RS), non-resistant starch (NRS) and total starch (TS) content were determined by hydrolyzing the sample using pancreatic α-amylase and amyloglucosidase for 16 h at 37 °C, following the procedure described in the assay kit. Absorbance was measured using a spectrophotometer at 510 nm against a reagent bank (sodium acetate buffer sand glucose oxidase/peroxidase reagent only). Total starch and *in vitro* starch digestibility were calculated using the following equation (Świeca et al. 2013):

$$\text{Total starch (TS)} = \text{Resistant starch (RS)} + \text{Non resistant starch (NRS)} \tag{4.3}$$

$$\%\,\text{SD} = 100 - (\text{RS}/\text{TS}) \times 100 \tag{4.4}$$

4.5. Results and Discussion

4.5.1. Experimental results of microwave-vacuum drying of germinated lentils

Both lentil types (green and red) were germinated for 5 days and dried to 10±2% moisture content (wet basis) using a pulsed-mode, microwave-vacuum (MV) dryer, having an initial moisture content of 70±5% (wet basis). The experimental data for MV drying of both germinated green and red lentil are shown in Table 4.2. The drying time ranges from about 130 min for 2 s MW power to 30 min for 8 s MW power. The minimum drying time was about 25 min, as observed at 9.243 s MW power. Maximum TPC was observed at 8 s MW power and 15 kPa vacuum pressure for germinated green lentil, and at 5 s MW power level and 30 kPa vacuum pressure for germinated red lentil with 6.82 and 5.69 GAE mg/g DM, respectively. Conversely, minima were observed at 0.757 s MW power and 30 kPa vacuum pressure for both lentil types. There exists little information on the drying of germinated pulses. However, the increase in TPC with increased microwave pulse level is similar to MW drying of other food commodities such as sour cherries (Wojdyło et al. 2014), red pepper (Vega-Galvez et al. 2009) and kiwi (Kaya et al. 2009). For total antioxidant activity (TAA), minimum and maximum values were observed at similar processing parameters as for TPC for both lentil types. The maximum value of TAA for germinated green lentil was 10.12 Teq mg/g DM and the minimum was 3.67 Teq mg/g DM, while 11.57 Teq mg/g DM and 6.75 Teq mg/g DM were determined for red lentil. This shows that there is a good relationship between TPC and TAA. The reduction and relationship between TPC and TAA also were observed in drying of Thai red curry powder (Inchuen et al. 2010), goldenberry (İzli et al. 2014) and other fruits (Sultana et al. 2012).

Table 4.2. Experimental design and data obtained for microwave-vacuum drying of germinated lentils.

			Green lentil			Red Lentil		
Run	MW pulse, s	Vacuum pr., kPa	TPC, GAE mg/g DM	TAA, Teq mg/g DM	SD,%	TPC, GAE mg/g DM	TAA, Teq mg/g DM	SD,%
1	2	15	3.88	5.44	91.03	3.08	7.01	86.54
2	8	15	6.82	10.12	92.34	4.91	8.77	88.49
3	2	45	4.24	6.29	91.47	3.14	9.06	86.32
4	8	45	6.3	9.67	92.43	3.94	8.43	88.79
5	0.757	30	3.65	3.67	90.1	3.09	6.75	84.36
6	9.243	30	6.02	9.38	93.26	3.43	8.1	87.05
7	5	8.79	6.75	5.47	91.74	5.43	11.08	86.8
8	5	51.21	5.21	10.09	92.31	5.56	10.46	88.56
9	5	30	5.49	7.32	92.3	5.67	11.35	87.23
10	5	30	5.51	7.11	92.16	5.49	11.02	87.87
11	5	30	5.59	7.79	91.52	5.67	11.23	87.02
12	5	30	5.52	6.34	92.78	5.43	11.14	87.14
13	5	30	6.03	7.46	92.75	5.69	11.57	86.96

MW= microwave; TPC= total phenolic content; TAA= total antioxidant activity; DM= dry matter SD= starch digestibility; GAE= Gallic acid equivalent; Teq= Trolox equivalent.

It also can be noted that TAA of germinated red lentil was higher than for green lentil at the same processing conditions, except for runs 2, 4 and 6, while the TPC of green lentil were higher than for red, at the same processing conditions, except for runs 8, 9 and 11. In the case of *in vitro* starch digestibility (SD), the percent reduction from maximum to minimum in both lentil types were much less, 3.39% and 4.99% for germinated green and red lentil, respectively. Maximum SD was observed at 9.243 s MW power and 30 kPa vacuum pressure for green lentil, and 8 s MW power and 45 kPa vacuum pressure for red lentil. In contrast, minimum SD was observed at 0.757 s MW power and 30 kPa vacuum pressure for both lentil types. Similar effects of microwave power were reported after drying of germinated pea (Kadlec et al. 2001) and drying of barley (Emami et al. 2012).

In vitro SD of germinated green lentil was higher than for germinated red lentils after microwave drying.

4.5.2. Statistical analysis and model fitting using RSM

The values of responses (TPC, TAA and SD) at different microwave (MW) and vacuum pressure levels were fitted to a second-order polynomial response surface model and their significance was assessed using analysis of variance (ANOVA). Estimated coefficients of process parameters, R^2 and adjusted R^2 of the models developed are shown in Table 4.3.

Table 4.3. Estimated coefficients of process parameters, R2 and adjusted R2 of the models.

	Estimated coefficients					
	Green lentils			**Red lentils**		
Factor	TPC, Y_{11}	TAA, Y_{12}	SD, Y_{13}	TPC, Y_{21}	TAA, Y_{22}	SD, Y_{23}
Intercept	5.63	7.20	92.30	5.45	10.955	87.24
A-MW	1.04	2.02	0.84	0.39	0.38	1.03
B-Pressure	-0.29	0.87	0.17	-	-	0.32
AB	-0.22	-0.33	-0.09	-	-	0.13
A^2	-0.42	-0.16	-0.32	-1.29	-2.06	-0.56
B^2	0.15	0.47	-0.15	-	-	0.43
R^2	0.9119	0.8548	0.8003	0.8588	0.8572	0.8299
Adj. R^2	0.8490	0.7511	0.6576	0.8359	0.8287	0.7084

TPC= total phenolic content; TAA= total antioxidant activity; SD= starch digestibility.

Lack of fit, F-value and P-value of individual processing parameters of microwave vacuum drying of germinated green and red lentil are listed in Table 4.4 and 4.5, respectively. The models developed for all responses are significant ($p<0.05$). R^2 value, which determines the variation in fitting the model, was higher than 0.8 for all models. In addition, adjusted R^2 indicates the percentage of variation explained by only independent variables that actually affect the dependent variable. A minor difference between R^2 and adjusted R^2 is desirable since it means that the ability of the model to explain data is not diminished with inclusion of a number of covariates/factors. Since the models developed were significant with higher R^2 values, they can be used to predict and explained the changes made during pulsed-mode, microwave-vacuum drying of germinated lentil (within the range).

The F-value and P-value, which indicate the significance of the model developed, of TPC of green lentil (Y_{11}) was 14.49 with a P-value of 0.0014, which implies that the model is significant. Similarly, other responses, TAA (Y_{12}) and SD (Y_{13}) of germinated green lentils with F-values of 8.24 and 5.61, respectively, were significant ($p<0.05$). P-values less than 0.05 were considered significant. In Y_{11}, only microwave pulse level (A) and A^2 were significant. This shows that TPC is significantly ($p<0.05$) influenced by microwave pulse only. Similarly, for Y_{12} and Y_{13}, only term A was significant and vacuum pressure was less effective in altering total antioxidant activity and *in vitro* starch digestibility (SD) during pulsed-mode microwave vacuum drying of germinated lentil. The lack of fit for all of the models for green lentil have P-values greater than 0.05. Insignificant lack of fit implies that the model can be well fitted to describe the response. In the case of germinated red lentil, the lack of fit for TPC (Y_{21}) and TAA (Y_{22}) was significant when all the terms in the quadratic model were included. Removal of insignificant model terms improves the adequacy of a model. Unfortunately, even after application of backward elimination regression, lack of fit was significant for both Y_{21} and Y_{22}. However, the predicted R^2 for Y_{22} was 0.6827, and the difference in corresponding adjusted R^2 and predicted R^2 was within the limit of 0.2 which indicates that the model had sufficient capability to predict their responses (DeLoach and Ulbrich, 2007; Alshaibani et al. 2014). F-values of Y_{21}, Y_{22} and Y_{23} were 30.41, 30.02 and 6.83, respectively, with P-values less than 0.05, indicating that the models were significant. Model term A and A^2 terms were significant ($p<0.05$) for Y_{21} while only the A^2 and A term were significant for Y_{22} and Y_{23}, respectively, which are similar to the findings for green lentil. None of the interaction terms (AB) was significant which implies that microwave pulse level and vacuum pressure level did not have any significant interaction in altering TPC, TAA or SD.

Table 4.4. Lack of fit, F-value and P-value of individual processing parameters of green lentils.

	Y_{11}		Y_{12}		Y_{13}	
Source	F-value	P-value	F-value	P-value	F-vale	P-value
Model	14.49	0.0014*	8.24	0.0076*	5.61	0.0215*
A-MW	56.76	0.0001*	32.83	0.0007*	23.69	0.0018*
B-Pressure	4.45	0.0729	6.06	0.0433	0.93	0.3667
AB	1.26	0.2986	0.43	0.5347	0.13	0.7312
A^2	8.02	0.0253*	0.17	0.6888	2.97	0.1286
B^2	1.04	0.3415	1.55	0.2533	0.63	0.4536
Lack of Fit	5.57	0.0653	6.52	0.0509	0.77	0.5659

*Significant at $P \leq 0.05$

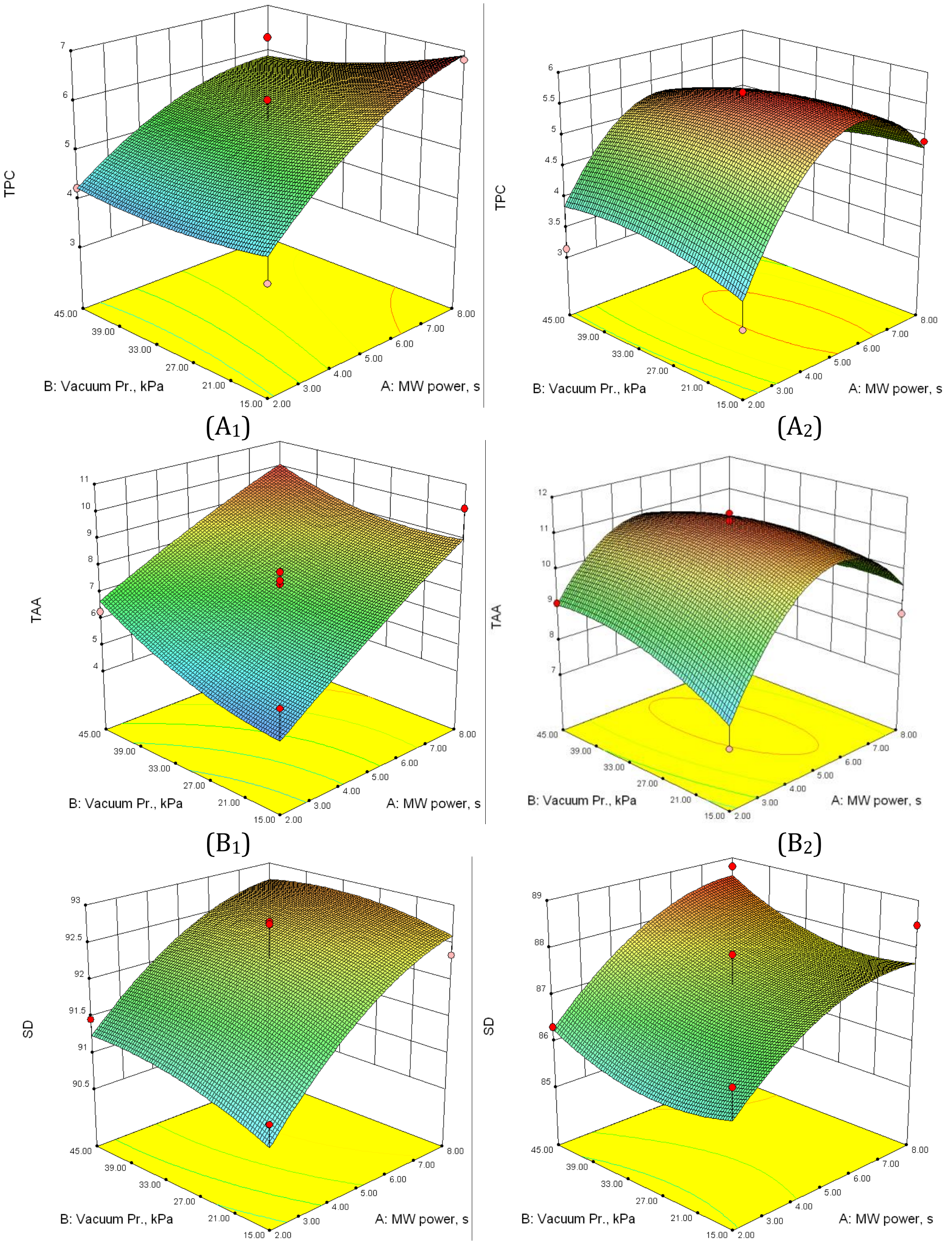
Table 4.5. Lack of fit, F-value and P-value of individual processing parameters of red lentils

	Y_{21}		Y_{22}		Y_{23}	
Source	F-value	P-value	F-value	P-value	F-vale	P-value
Model	30.41	<0.0001*	30.02	<0.0001*	6.83	0.0127*
A-MW	5.66	0.0387*	2.32	0.1601	21.71	0.0023*
B-Pressure	-	-	-	-	2.12	0.1889
AB	-	-	-	-	0.17	0.6894
A^2	55.18	<0.0001*	57.81	< 0.0001*	5.58	0.0502
B^2	-	-	4.11	-	3.28	0.1130
Lack of Fit	23.75	0.0043*	18.81	0.0067*	5.45	0.0670

*Significant at $P \leq 0.05$

4.5.3. Response surface analysis

Response surfaces, shown in Figure 4.1, were plotted to help visualize the effects of microwave pulse, vacuum pressure and their interactions on TPC, TAA and SD of germinated green and red lentil. It was observed that TPC, TAA and SD of germinated green lentil increased with an increase in microwave pulse and vacuum pressure, and microwave pulse level was more significant for changes in the responses. The interaction terms were not affecting significantly in all the responses. However, for germinated red lentil, the TPC and TAA increased with increasing microwave pulse up to 5 s microwave pulse level and started to descend with a further increase in microwave pulse level. There was a gradual rise in TPC and TAA with increasing vacuum pressure level, but only the squares of microwave pressure level were significant. Similar to the case of germinated green lentil, the interaction terms of microwave pulse and vacuum pressure level did not affect significantly ($p>0.05$) on TPC or TAA. The increase in TPC and TAA with increasing microwave pulse and vacuum pressure level may be due to the shorter drying time and lower temperature of drying. Microwave energy causes volumetric heating, resulting in heat transfer from inside to the surface, while higher vacuum pressure helps the lower evaporating temperature which ultimately results in faster drying. Prolonged exposure to this type of drying technique may result in loss of volatile materials and oxidative reduction of phenolic compounds (Mitra et al. 2013). Lower MW power drying resulted in longer drying time and lower temperature. At lower MW power, it is possible that oxidative enzymes such as polyphenoloxidases and peroxidases were not inactivated immediately, resulting in degradation of phenolic compounds during long exposure. Moreover, most of the antioxidants are phenolic compounds present in the seed coat of lentil, which are easily accessible to oxidative process (Khan et al. 1979). There are reports of positive relationships between phenolics and antioxidant activity in lentil (Cevallos-Casals and Cisneros-Zevallos, 2010; Świeca 2015) and thus, the reduction of antioxidant activity can be explained by oxidative processes and long exposure of lentils during drying process. The difference in trends of TPC and TAA among the lentil types studied may be due to differences in size, as surface area for oxidation would vary, and also due to the types and amount of phenolics present in them might be different.



(A1) (A2)

(B1) (B2)

(C1) (C2)

Fig. 4.1. Response surface plots showing the effects of pulse mode microwave (MW) power and vacuum pressure on Total phenolic content (A), Total antioxidant activity (B) and *in vitro* starch digestibility (C) of germinated lentils. ($_1$: green lentil; $_2$: red lentil).

In vitro starch digestibility of germinated lentil also increased with an increase in microwave pulse, similar to germinated red lentil, and the findings match those of Kadlec et al. (2001) in drying of germinated pea and Emami et al. (2012) in drying of barley. It has been reported that starch might degrade due to starch fragmentation (starch micronization) during irradiation, resulting in increasing sugar content of legumes and cereal crops (Khan et al. 1979; González and Pérez 2002). Emami et al. (2010) also reported that micronization increased the amount of readily digestible starch and decreased resistant starch and slowly digestible starch. This means the *in vitro* digestibility of starch will increase with an increased dose of microwave energy. In addition, higher microwave power may decrease the levels of antinutritional compounds, leading to higher digestibility of starch.

The optimum processing parameters generated based on maximizing TPC, TAA and SD with maximum importance given to TAA and SD are shown in Table 4.6. Samples were treated at optimum process conditions to validate the values of optimum processing parameters.

Table 4.6. Optimum processing parameters generated for MV drying of germinated lentils

Lentil Variety	**MW power, s**	**Vacuum pr., kPa**	**TPC, GAE mg/g DM**		**TAA, Teq mg/g DM**		**SD,%**		**Desirability**
			Pred	Expt	Pred	Expt	Pred	Expt	
Green	8	45.00	5.89	6.28	10.07	9.68	92.76	92.50	0.86
Red	5.507	42.19	5.38	5.51	10.98	11.13	87.96	87.61	0.85

Pred= Predicted ; Expt= Experimental (Validation); MW= microwave; TPC= total phenolic content; TAA= total antioxidant activity; DM= dry matter SD= starch digestibility; GAE= Gallic acid equivalent; Teq= Trolox equivalent.

The variations with the predicted and observed values was less than 10% (Table 4.6). Therefore, the models generated can be employed for determining changes during drying of germinated green lentil (CDC Greenland) and red lentil (CDC Maxim) in the given processing range. Raw samples (before germination) had TPC values of 7.48 and 6.42 GAE mg/g DM for green and red lentil, respectively, while TAA values were 10.44

and 12.87 Teq mg/g DM, respectively. These results show that the reduction in TPC and TAA of the lentil studied after processing at optimum condition was about 16% and 13%, respectively. While for SD (raw sample having SD of 64.95% and 62.18% for green and red lentil, respectively), there is an increase in starch digestibility up to about 40%, showing that microwave-vacuum drying after germination increases starch digestibility of lentil with low reduction in TPC and TAA. Aguilera et al. (2009) reported that starch digestibility may be improved by initial soaking and thermal treatment due to gelatinization of starch granules and reduction of antinutritional compounds from the raw seed.

4.6. Conclusions

In drying of germinated lentils using the pulsed-mode, microwave-vacuum drying technique, starch digestibility increased significantly with an increase in microwave pulse level. Vacuum pressure levels did not affect significantly ($p>0.05$) the responses studied. For optimum drying of 5-days germinated lentil in pulsed-mode, microwave-vacuum condition, green lentil can be dried at 8 s out of 10 s rated microwave power of 2000 W and 45 kPa constant vacuum pressure, whereas red lentil can be dried at 5.5 s out of 10 s rated microwave power of 2000 W and 42.19 kPa constant vacuum pressure. The results show that there was reduction of about 16% in TPC and about 13% in TAA, of the lentil studied after processing at optimum condition. The mathematical model generated for drying of germinated lentil can be used for scaling up the process. Germination followed by microwave vacuum drying ccould be a promising path to increase the digestibility of starch with minimum changes in phenolics and antioxidant activity. Moreover, this technology can also be employed for drying other pulses for further processing. The dried germinated lentils obtained can be treated as a raw material for further utilization as ingredients in bakery products, extruded snacks and other value-added healthy food products.

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CHAPTER 5

EFFECT OF EXTRUSION PROCESS ON GERMINATED LENTIL (*Lens culinaris* Medikus) BASED SNACK FOODS: IMPACT ON STARCH DIGESTIBILITY, PHYSIO-FUNCTIONAL, AND ANTIOXIDANT PROPERTIES

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Contribution of this paper to the overall study

Optimum processing parameters for the extrusion of a blend of germinated lentil flour and corn flour has not yet been developed. The aim of this study was to develop a new, healthy extruded product. The dried, germinated lentil flour obtained from Chapter 4 was utilized to produce extruded lentil-based products. Their physico-functional properties and nutrient contents were examined to identify optimum processing parameters for extrusion of germinated lentil. This paper is specific to objective 4. All of the experiments were conducted and the manuscript was drafted by myself.

5.1 Abstract

Formulations of germinated green and red lentil were extruded in blends with corn flour. Response surface methodology was employed to study the effects of moisture, percent lentil in the blend and screw speed at 150℃ barrel temperature on expansion ratio, extrudate density, hardness, water absorption index, water soluble index, total phenolics content, total antioxidant activity and *in vitro* starch digestibility. The percentage of germinated lentil flour in the blend demonstrated more significant effects on the response variables as compare to moisture and screw speed. Optimum process parameters were

identified as 20% lentil blend, 19.5% moisture and 147 rpm screw speed for germinated green lentil extrudates, and 20.7% of lentil in the blend, 19.7% moisture and 141 rpm screw speed for germinated red lentil extrudates.

5.2 Nomenclature

A	Blend percentage (%)	NRS	Non-resistant starch
B	Moisture (%)	RS	Resistant starch
C	Screw speed (rpm)	SD	Starch digestibility (%)
D	Diameter of extrudate, cm	TAA	Total Antioxidant activity (Teq mg/g DM)
DPPH	2, 2-diphenyl-1-picrylhydrazyl	Teq	Trolox equivalent
ED	Extrudate density (g/cm^3)	TPC	Total phenolic content (GAE mg/g DM)
ER	Expansion ratio	TS	Total Starch
GAE	Gallic Acid equivalent	WAI	Water absorption index
GOPOD	Glucose oxidase/peroxidase reagent	WSI	Water solubility index
l	Length of extrudate, (cm)		
m	Mass of extrudate, (g)		

5.3 Introduction

Extruded food products such as snack foods, baby foods, pet foods, noodles, pasta etc. have significant importance in daily life. Development of these extruded products is done using flours from a variety of commonly used food grains, including chickpea, kidney bean, lentil, barley, corn, soybean, pea etc., with or without pre-treatments such as heating, cooking, frying, roasting, germination, etc. (Morales et al. 2015; Camire et al 2007; Flores-Silva et al 2014). Lentil, which is one of the food legumes found globally, is an important staple food commodity and a good source of protein, carbohydrates and other vital macro and micronutrients (Joshi et al. 2017). However, it also contains antinutrients such as trypsin inhibitor, tannins, α-amylase inhibitor, and phytates (Wang and Daun 2006) which lower the nutritional availability of biomolecules. The common practices employed to improve nutritional quality, flavor and digestibility and reduce the activity of anti-nutrients (Nongmaithem and Meda 2017a) are germination and heat treatment. Germination improves food functional components (Caccialupi et al. 2010) whereas heat treatment converts micro- and macro-nutrients into more digestible forms, reduces water activity and enhances shelf life. It further leads to compositional changes and changes in the antioxidant and functional properties which increases consumer acceptance (Jogihalli et al. 2017).

Extrusion cooking is a high temperature and short time (HTST) process which combines several unit operations such as mixing, shearing, conveying, heating, puffing and partial drying, depending on the extruder design and process conditions. HTST leads to cooking of the food material as well as simultaneous gelatinization of starch, resulting in alterations in physic-chemical and functional properties, and nutrient composition (Alam et al. 2015). Starch which is the main component required in extrusion processing undergoes gelatinization on heat treatment and leads to expansion of the end product. However, other components such as fibre reduces the expansion of air bubbles by rupturing the cell walls of expanding extrudates (Perez-Navarrete et al. 2006), while excess moisture and protein impedes the expansion of the extrudate (Obatolu Veronica et al. 2006). Generally, starch found in legumes such as lentil are considered inferior to cereal starch by food processing industries (Gonzalez and Perez 2002). However, a nutrient-rich product can be developed from a combination of a cereal and a pulse such as lentils (Rathod and Annapure 2017).

Extrusion cooking for production of a novel, fibre-rich lentil product significantly ($P<0.05$) reduced the trypsin inhibitor content and completely inactivated lectin, which is very much desirable in increasing the digestibility of protein (Morales et al. 2015). Ghuman et al. (2016) reported that temperature exhibited positive influence in increasing the expansion ratio of lentil extrudates, whereas feed moisture had a negative impact. On the other hand, physical attributes such as the hardness, expansion and density of the extrudate were found to be negatively affected by the incorporation of legumes (Wani et al. 2016). Lentil starch can be greatly modified by germination, followed by microwave drying (Gonzalez and Perez 2002; Nongmaithem and Meda 2017a). They further suggested that the flour produce could also be utilized for development of extruded products. Studies in this area have focused on determination of functional properties (Lazou and Krokida 2010a), starch modification (González& Pérez 2002), structural and textural characteristics (Lazou and Krokida 2010b), thermal characteristics (Lazou and Krokida 2011), antinutritional factors, protein and starch digestibility (Rathod et al., 2016), and physico-chemical and antioxidant properties (Lv et al. 2018) of lentil based extrudates.

Among all the benefits from extrusion technology, some disadvantages and their remedy had also been reported. While quickly digestible starch is beneficial to newborns and the elderly, the sudden increase in blood sugar and insulin levels is regarded to be a risk factor for insulin insensitivity and type II diabetes. However, the addition of high amylose starch reduces digestibility of starch. Extrusion with high amylose rice noodles showed lower starch digestibility and decreased GI (Panlasigui et al. 1992). Excessive Millard reaction may reduce lysine contain up to 50% (Camire 2000). The maillard reaction is promoted by high barrel temperature, low moisture, and high shear. Lysine can be retained, but only if extruder working conditions and formulas are correctly adjusted (Konstance et al. 1998). Anderson and Hedlund (1991) reported reduction in vitamin C drops in wheat flour when extruded at high barrel temperature at relatively low moisture (10%). Concerns over low vitamin levels have prompted some producers to apply vitamins as a spray after extrusion. Extrusion lowered the phenolic content. Extrusion dramatically lowered anthocyanin levels in sweetened corn breakfast cereals thereby lowering antioxidant activity (Chavanalikit 1999). Also, due to loss of minerals during extrusion, mineral fortification has become customary, particularly in ready-to-eat morning cereals.

Based on the literature reviewed, it was observed that there is limited information on the effect of germination of lentil followed by extrusion processing on starch digestibility and its physico-functional and antioxidant properties. Therefore, the present study was aimed to produce a nutritious, extruded snack food from blends of germinated lentil flour and corn flour, and employing response surface methodology for optimization of the product and process parameters.

5.4 Materials and Methods

5.4.1 Germinated lentil flour preparation

Samples of green and red lentil (CDC Greenland and CDC Maxim, respectively) were supplied by the University of Saskatchewan, Saskatoon, SK, Crop Development Centre, (CDC). Germinated lentil flour was produced according to Nongmaithem and Meda (2017a and 2017b). Seeds was soaked in distilled water for 8 h and then transferred to aluminum trays for germination up to 5 days. During the germination period, seed was rinsed with distilled water every 12 h. The germinated seed was dried using pulse-mode microwave vacuum dryer (Model: VMD 1.8, EnWave^{TM} Corp., Vancouver, BC, Canada). Green lentil was dried at 8 s, 2000 W microwave power and 45 kPa vacuum pressure, and red lentil was dried at 5.5 s microwave power and 42 kPa vacuum pressure to 10±2 (% wet basis). The dried samples were ground with a multipurpose hammer mill (Ross hammer mill, Canada) equipped with a sieve having 1 mm aperture.

5.4.2 Experimental design

Response surface methodology was applied using a central composite rotatable design (CCRD) for designing the experiment. Three independent parameters were selected, viz. percent of germinated lentil flour in the blend, moisture and screw speed. The dependent variables were expansion ratio, extrudate density, hardness, water absorption and solubility index, total phenolics, antioxidant activity and *in vitro* starch digestibility. Five levels of the independent parameters were chosen having a total of 20 experiments with 6 central points as shown in Table 5.2(a,b) and 5.3(a,b).

The design was developed using Design expert 8.0.7.1 (Stat-Ease, Minneapolis, MN, USA). The following second order polynomial responses surface model was fitted to each of the response for analysis, as below:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1}{}_{i<j} \sum_{j=2}^{k} \beta_{ij} X_i X_j \qquad (5.1)$$

where, Y is response, β_0, β_i, β_{ii}, and β_{ij} are regression coefficients of the constant, linear, quadratic and interaction terms, respectively, and X_i and X_j are codes of independent variables and k is the number of variables. X were replaced by A, B and C which signify blend, moisture and screw speed. A sequential model sum of square (type I) was carried out to select the final model, where quadratic model was suggested to fit the responses as cubic and higher order polynomial models were considered aliased. The goodness-of-fit of the model was measured by the coefficient of determination (R^2). The optimization of process variables was based on maximum values of expansion ratio (ER), water absorption index (WAI) and *in vitro* starch digestibility (SD). The actual and coded values of the experimental design are shown in Table 5.1

Table 5.1. Actual and coded value of experimental design for extrusion.

Blend (%)	Moisture (%)	Screw speed (rpm)	Coded values
16.59	17.32	89.55	-1.68
20	18	110	-1
25	19	140	0
30	20	170	+1
33.41	20.68	190.45	+1.68

5.4.3 Extrusion

Different blends of germinated lentil flour and corn flour (20% to 30% of lentil flour) was extruded using a single screw extruder (KE19 Stand-Alone, Brabender GmbH and Co. KG, Duisburg, Germany) having a 19 mm barrel diameter; screw length of 47.5 cm and depth of die with 4 mm circular diameter. After various trial combination, the barrel temperature during extrusion was fixed at 150 °C and the temperature at the die was fixed at 180 °C. The other independent variables were moisture and screw speed. The moisture (18-20%) of the feed was adjusted using distilled water and was allowed to equilibrate for 24 h. Screw speed was maintained in the range of 110 to 170 rpm.

5.4.4 Expansion ratio

Expansion ratio (ER) was measured as per Rathod & Annapure (2016). It was determined by measuring the diameter of five random extrudate samples using a Vernier caliper. Expansion ratio (ER) was expressed as:

$$Expansion\ ratio(ER) = \frac{Diameter\ of\ the\ product}{Diameter\ of\ the\ hole} \tag{5.2}$$

5.4.5 Extrudate density

The extrudate density (ED) was determined from 10 random measurements on the diameter and length of the extrudate using digital calipers, and the weight was determined on an analytical balance. The extrudate density was obtained from the following formula (Ding et al. 2005):

$$extrudate\ density\ (ED) = \frac{4m}{\pi D^2 l} \tag{5.3}$$

Where, m is mass of the extrudate, D is the average diameter of the extrudate and l is the length of the extrudate.

5.4.6 Hardness

The textural characteristics were measured using a texture analyzer (Model: TA-XT2, Stable micro systems, UK;) equipped with a 25 kg load cell. Extruded samples were compressed to 20% using a 5 mm diameter cylindrical probe. The studies were conducted at a pre-test speed of 1.0 mm/s, test speed of 0.5 mm/s and post-test speed of 10 mm/s. Hardness was determined and expressed in grams (g).

5.4.7 Water absorption and solubility indices

The water absorption index (WAI) was determined according to the method prescribed by Jogihalli et al. (2017) with some modifications. Ground extrudate (0.2 g) was mixed with 5 mL of distilled water and vortexed for 2 min. The mixture then was centrifuged for 20 mins at 4500 rpm. The supernatant liquid was collected in a Petri dish. The remaining gel in the centrifuge tube was weighed and the WAI was calculated as:

$$WAI = \frac{m_g}{m_s} \tag{5.4}$$

Where m_g is the weight of hydrated gel (g) and m_s is the weight of the sample (g).

The water solubility index (WSI) was determined as the amount of dry solids remaining after evaporation of water from the Petri dish (dehydrated in hot air over).

$$\mathrm{WSI} = \frac{\mathrm{m_{ds}}}{\mathrm{m_s}}.100 \tag{5.5}$$

Where $\mathrm{m_{ds}}$ is the weight of the dry solids.

5.4.8 Extraction

Ground samples were extracted using 70% aqueous ethanol (1/10 w/v) at 25 °C for 1 h. The supernatants were decanted into a glass vial following centrifuging at 3500 rpm for 10 min. This process was repeated three times to maximize the extraction. On the third repetition, the supernatant was a clear solution. The extracts were stored at -20 °C for further analysis.

5.4.9 Total phenolic content

Total phenolics content was determined based on the colour reaction of Folin-Ciocolteu reagent with hydroxyl groups (Swieca et al., 2014). To 0.5 mL of extract, 0.5 mL of water and 2 ml of Folin-Ciocalteau regent (the reagent is diluted to 1:5 with distilled water) were added. After 3 mins, 10 ml of 10% sodium carbonate solution was added and the mixture was allowed to stand for 30 min. Absorbance was measured using a UV-visible spectrophotometer (Model: UV-2450, Shimadzu, Tokyo, Japan) at 725 nm. A standard curve (calibration) of gallic acid (50, 100, 150, 250 and 500 mg in 1 litre) was plotted. Total phenolics content was calculated as gallic acid equivalents (GAE) in mg per g of dry matter.

5.4.10 Total antioxidant activity

A DPPH (2, 2-diphenyl-1-picrylhydrazyl) stock solution of 500 µM was prepared by dissolving 19.6 mg of DPPH in 100 ml of 70% aqueous methanol and allowed to stand for 20 minutes at 25°C (Mitra et al. 2013). Using 70% aqueous methanol, three sample solution of crude extract, 5 fold and 10 fold, were produced. Aliquot of 0.25 ml was added to 2 ml DPPH solution and vortex for 15 s and held at room temperature for 15 minutes. A blank solution was prepared by adding an equal amount of 70% aqueous methanol to DPPH. The absorbance was measured at 517 nm for the sample and blank, which was calibrated with 70% methanol. Percentage DPPH inhibition was determined using following equation:

$$\%\ \mathrm{DPPH\ inhibition} = \left(1 - \frac{\mathrm{Abs_{sample}}}{\mathrm{Abs_{blank}}}\right) \mathrm{x}100 \qquad (5.6)$$

where, Abs is absorbance for both sample and blank.

The level of DPPH radical scavenging capacity was expressed in terms of mg -Trolox equivalents per g dry matter by plotting a stand curve of Trolox.

5.4.11 *In vitro* starch digestibility

In vitro starch digestibility (% SD) was determined using the Megazyme^{TM} resistant starch assay kit (K-RSTAR, Megazyme International Ireland Limited, Wicklow, Ireland). One hundred mg samples were hydrolysed using pancreatic α-amylase and amyloglucosidase for 16 h at 37 °C for determination of resistant starch (RS), non-resistant starch (NRS) and total Starch (TS) content. With addition of ethanol, the reaction was terminated and the supernatants were collected after centrifugation of 10 min at 3000 rpm for determination. Supernatants were removed by decantation. RS (remaining pellets in glass vials) was dissolved in 2 M KOH by vigorously stirring in an ice water bath over a magnetic stirrer for 20 min; the solution then was neutralised with acetate buffer and hydrolysed to glucose with amyloglucosidase. Using glucose oxidase/peroxidase reagent (GOPOD) D-glucose was quantified as the RS content of the sample. Non-resistant starch was determined using supernatant collected previously. Absorbance were measured using a spectrophotometer at 510 nm against a reagent bank. Total starch and *in vitro* starch digestibility were measured using following equation (Swieca et al. 2013):

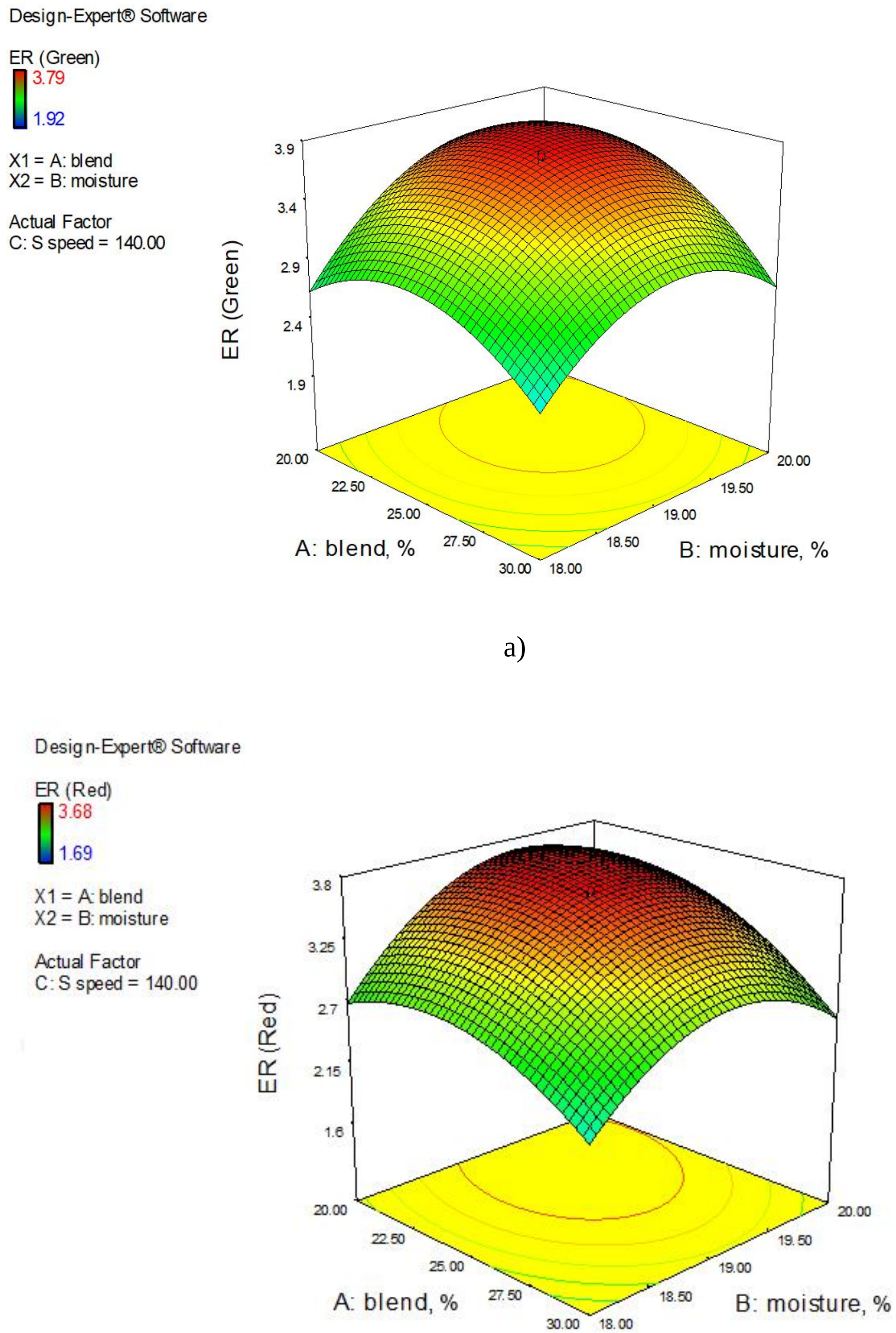
$$\text{Total starch (TS)} = \text{Resistant starch (RS)} + \text{Non-resistant starch (NRS)} \qquad (5.7)$$

$$\%SD = 100 - \left(\frac{RS}{TS}\right) * 100 \qquad (5.8)$$

5.5 Results and Discussion

5.5.1 Expansion ratio (ER)

The ER illustrates the degree of puffing during extrusion of a material, perpendicular to the direction of material flow. The expansion ratio of extruded samples was determined using a Vernier caliper. The highest ER was observed in germinated green lentil extrudate with a maximum value of 3.79 whereas the highest value for red lentil was 3.68, as shown in Tables Table 5.2a and 5.3a, respectively. In both the lentil types, the highest ER was observed at 25% blend, 19% moisture content and 140 rpm screw speed. Higher ER was observed in germinated green lentil blends.



a)

b)

Fig 5.1 Response surface plots showing the effects of extrusion on expansion ratio of extruded green (a) and red (b) germinated lentils

The ER was influenced more by moisture content as compared to blend ratio and screw speed. All the single and squared terms were significant ($p<0.05$). AB interaction terms were significant for green lentil, where as both AB and BC were significant for red lentil varity. The ER increased up to a certain level with an increase in moisture and blend ratio, before dropping. The expansion of extrudates occurs when a portion of moisture quickly flash off from the blend during the exit of the extrudates from the die due to rise in temperature (above boiling point) during extrusion. Similar characteristics have been observed with extrusion of lentil (Rathod and Annapure 2017). The coded coefficients of corresponding response surface model ($p<0.005$) for green and red lentil are shown in Table 5.4. The R^2 for ER of germinated green lentil was 0.9948, while for red lentil it was 0.9991.

Table 5.2a Central composite rotatable design: experimental and predicted values of response variable of green lentils.

SL	Blend (A) %	Moisture (B) %	Screw speed (C) rpm	ER (%)		ED (g/cm^3)		H (g)		WAI (g/g)	
				Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.
1	25	19	190.45	2.98±0.02	2.94	0.39±0.001	0.3987	1676.30±42.5	1593.06	3.99±0.04	3.95
2	20	18	170	2.34±0.01	2.41	0.42±0.001	0.3999	2462.30±32.5	2242.41	3.38±0.01	3.49
3	25	17.32	140	1.92±0.01	1.86	0.43±0.005	0.4423	3931.20±45.6	4116.8	2.09±0.01	2.07
4	30	18	110	2.03±0.02	2.05	0.42±0.003	0.4232	3899.10±65.5	3961.57	2.28±0.03	2.27
5	30	20	170	2.45±0.03	2.45	0.41±0.002	0.4162	1934.50±12.3	2223.04	3.26±0.02	3.27
6	25	19	140	3.79±0.02	3.74	0.32±0.001	0.3446	924.30±15.6	1173.6	4.92±0.02	4.83
7	16.59	19	140	3.05±0.02	2.94	0.31±0.002	0.3431	827.80±17.5	1491.2	4.42±0.03	4.19
8	20	18	110	2.12±0.03	2.19	0.420.003	0.4038	3313.00±36.5	2851.67	2.88±0.02	2.97
9	33.41	19	140	2.06±0.03	2.08	0.42±0.001	0.401	3659.80±56.6	3240.77	2.48±0.01	2.58
10	20	20	110	2.87±0.04	2.93	0.39±0.001	0.3757	1772.10±23.5	1432.8	3.68±0.01	3.85
11	25	19	140	3.75±0.03	3.74	0.34±0.001	0.3446	1008.80±10.3	1173.6	4.84±0.03	4.83
12	25	19	140	3.68±0.05	3.74	0.37±0.00	0.3446	1395.20±56.6	1173.6	4.71±0.02	4.83
13	30	18	170	2.06±0.01	2.07	0.42±0.00	0.4243	3379.90±66.5	3546.41	2.33±0.01	2.26
14	25	19	140	3.71±0.01	3.74	0.36±0.002	0.3446	1398.30±45.3	1173.6	4.76±0.02	4.83
15	30	20	110	2.25±0.02	2.25	0.41±0.001	0.4201	2162.30±23.5	2209.4	3.18±0.01	3.16
16	20	20	170	3.29±0.05	3.34	0.38±0.003	0.3668	1487.60±47.3	1252.34	4.38±0.03	4.48
17	25	19	140	3.75±0.06	3.74	0.35±0.002	0.3446	1374.20±36.9	1173.6	4.83±0.02	4.83
18	25	20.68	140	2.84±0.06	2.81	0.41±0.002	0.4118	1752.10±34.8	1810.86	3.76±0.01	3.65
19	25	19	89.55	2.65±0.02	2.59	0.4±0.005	0.4054	1766.30±32.1	2093.91	3.52±0.02	3.43
20	25	19	140	3.77±0.01	3.74	0.33±0.006	0.3446	982.70±30.2	1173.6	4.89±0.03	4.83

ER= expansion ratio; ED= extrudate density; H= hardness; WAI= water absorption index; GAE=Gallic acid equivalent; Teq=Trolox equivalent; Exp.=experiment values: Pred.=Predicted values

Table 5.2b Central composite rotatable design: experimental and predicted values of responses variable of green lentils.

SL	Blend (A) %	Moisture (B) %	Screw speed (C) rpm	WSI (g/g)		TPC (GAE mg/g dm)		TAA (mg Teq/ mg dm)		SD (%)	
				Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.
1	25	19	190.45	17.69±0.2	17.56	3.17±0.01	3.09	17.69±0.2	17.56	3.17±0.01	3.09
2	20	18	170	16.65±0.5	16.83	2.56±0.10	2.63	16.65±0.5	16.83	2.56±0.10	2.63
3	25	17.32	140	13.89±0.8	13.74	3.19±0.03	3.19	13.89±0.8	13.74	3.19±0.03	3.19
4	30	18	110	14.57±0.7	14.54	3.88±0.02	3.84	14.57±0.7	14.54	3.88±0.02	3.84
5	30	20	170	16.36±0.5	16.32	3.50±0.02	3.52	16.36±0.5	16.32	3.50±0.02	3.52
6	25	19	140	20.18±0.3	19.76	3.02±0.06	3.04	20.18±0.3	19.76	3.02±0.06	3.04
7	16.59	19	140	19.54±0.5	19.04	2.30±0.05	2.2	19.54±0.5	19.04	2.30±0.05	2.2
8	20	18	110	15.29±0.5	15.61	2.70±0.06	2.71	15.29±0.5	15.61	2.70±0.06	2.71
9	33.41	19	140	15.9±0.2	16.01	3.97±0.05	4.02	15.9±0.2	16.01	3.97±0.05	4.02
10	20	20	110	17.66±0.6	17.93	2.52±0.05	2.56	17.66±0.6	17.93	2.52±0.05	2.56
11	25	19	140	19.68±0.7	19.76	2.99±0.12	3.04	19.68±0.7	19.76	2.99±0.12	3.04
12	25	19	140	19.47±0.5	19.76	3.07±0.03	3.04	19.47±0.5	19.76	3.07±0.03	3.04
13	30	18	170	14.98±0.4	14.98	3.64±0.03	3.64	14.98±0.4	14.98	3.64±0.03	3.64
14	25	19	140	19.54±0.4	19.76	3.08±0.05	3.04	19.54±0.4	19.76	3.08±0.05	3.04
15	30	20	110	16.08±0.3	16.18	3.75±0.07	3.71	16.08±0.3	16.18	3.75±0.07	3.71
16	20	20	170	18.55±0.5	18.85	2.41±0.06	2.49	18.55±0.5	18.85	2.41±0.06	2.49
17	25	19	140	19.62±0.8	19.76	3.01±0.05	3.04	19.62±0.8	19.76	3.01±0.05	3.04
18	25	20.68	140	17.06±0.8	16.82	3.01±0.03	2.97	17.06±0.8	16.82	3.01±0.03	2.97
19	25	19	89.55	16.68±0.5	16.42	3.28±0.01	3.32	16.68±0.5	16.42	3.28±0.01	3.32
20	25	19	140	20.01±0.3	19.76	3.09±0.02	3.04	20.01±0.3	19.76	3.09±0.02	3.04

WSI= water solubility index; TPC=total phenolic content; TAA=total antioxidant activity; SD=*in vitro* starch digestibility; GAE=Gallic acid equivalent; Teq=Trolox equivalent; Exp.=experiment values: Pred.=Predicted values

Table 5.3a Central composite rotatable design: experimental and predicted values of responses variable of red lentils.

SL	Blend (A)	Moisture	Screw speed	ER		Extrudate Density		Hardness		WAI	
	%	(B)	(C)	(%)		(g/cm^3)		(g)		(g/g)	
		%	rpm	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.
1	25	19	190.45	2.75±0.01	2.75	0.42±0.01	0.4197	1212.92±32.6	1138.97	3.46±0.03	3.4
2	20	18	170	2.34±0.015	2.37	0.45±0.01	0.4538	2558.2±45.6	2553.09	2.92±0.02	2.97
3	25	17.32	140	1.69±0.00	1.68	0.47±0.02	0.4728	4030±63.56	3766.2	1.6±0.03	1.54
4	30	18	110	1.97±0.00	1.97	0.45±0.01	0.4476	4075.5±32.3	3920.75	1.78±0.01	1.8
5	30	20	170	2.35±0.005	2.34	0.44±0.03	0.4401	2483.23±35.6	2168.83	2.78±0.04	2.79
6	25	19	140	3.65±0.02	3.65	0.38±0.01	0.3901	1098.5±32.5	1074.18	4.41±0.05	4.34
7	16.59	19	140	3.26±0.01	3.21	0.45±0.04	0.4389	1838.7±36.5	1898	3.91±0.07	3.78
8	20	18	110	2.27±0.00	2.29	0.46±0.03	0.4627	2530±45.6	2728.92	2.39±0.02	2.48
9	33.41	19	140	2.09±0.012	2.12	0.44±0.02	0.4471	3300±12.3	3404.01	2.24±0.01	2.22
10	20	20	110	2.87±0.002	2.89	0.42±0.01	0.4292	1956.3±25.3	1529.86	3.37±0.6	3.44
11	25	19	140	3.65±0.021	3.65	0.37±0.01	0.3901	1014.3±24.6	1074.18	4.35±0.06	4.34
12	25	19	140	3.64±0.014	3.65	0.4±0.002	0.3901	1194.8±45.1	1074.18	4.23±0.04	4.34
13	30	18	170	1.9±0.010	1.89	0.46±0.03	0.4536	2327±26.5	2637.97	1.78±0.05	1.81
14	25	19	140	3.67±0.021	3.65	0.39±0.03	0.3901	986.5±16.5	1074.18	4.27±0.02	4.34
15	30	20	110	2.09±0.018	2.08	0.44±0.02	0.4391	3346.3±14.5	3235.93	2.69±0.02	2.74
16	20	20	170	3.29±0.013	3.31	0.41±0.01	0.4153	1530.43±36.5	1569.7	3.89±0.4	3.97
17	25	19	140	3.63±0.015	3.65	0.4±0.02	0.3901	1206.4±56.5	1074.18	4.34±0.02	4.34
18	25	20.68	140	2.56±0.012	2.56	0.44±0.01	0.4332	1936.3±12.5	2363.41	3.25±0.02	3.17
19	25	19	89.55	2.48±0.017	2.47	0.43±0.01	0.4263	1946.9±35.6	2184.15	3.02±0.01	2.94
20	25	19	140	3.68±0.017	3.65	0.4±0.04	0.3901	972.6±52.6	1074.18	4.39±0.02	4.34

ER= expansion ratio; ED= extrudate density; H= hardness; WAI= water absorption index

Exp.=experiment values: Pred.=Predicted values

Table 5.3b Central composite rotatable design: experimental and predicted values of responses variable of red lentils.

SL	Blend (A) %	Moisture (B) %	Screw speed (C) rpm	WSI (g/g)		TPC (GAE mg/g dm)		TAA (mg Teq/ mg dm)		SD (%)	
				Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.
1	25	19	190.45	17.69±0.2	17.56	3.17±0.01	3.09	17.69±0.2	17.56	3.17±0.01	3.09
2	20	18	170	16.65±0.5	16.83	2.56±0.10	2.63	16.65±0.5	16.83	2.56±0.10	2.63
3	25	17.32	140	13.89±0.8	13.74	3.19±0.03	3.19	13.89±0.8	13.74	3.19±0.03	3.19
4	30	18	110	14.57±0.7	14.54	3.88±0.02	3.84	14.57±0.7	14.54	3.88±0.02	3.84
5	30	20	170	16.36±0.5	16.32	3.50±0.02	3.52	16.36±0.5	16.32	3.50±0.02	3.52
6	25	19	140	20.18±0.3	19.76	3.02±0.06	3.04	20.18±0.3	19.76	3.02±0.06	3.04
7	16.59	19	140	19.54±0.5	19.04	2.30±0.05	2.2	19.54±0.5	19.04	2.30±0.05	2.2
8	20	18	110	15.29±0.5	15.61	2.70±0.06	2.71	15.29±0.5	15.61	2.70±0.06	2.71
9	33.41	19	140	15.9±0.2	16.01	3.97±0.05	4.02	15.9±0.2	16.01	3.97±0.05	4.02
10	20	20	110	17.66±0.6	17.93	2.52±0.05	2.56	17.66±0.6	17.93	2.52±0.05	2.56
11	25	19	140	19.68±0.7	19.76	2.99±0.12	3.04	19.68±0.7	19.76	2.99±0.12	3.04
12	25	19	140	19.47±0.5	19.76	3.07±0.03	3.04	19.47±0.5	19.76	3.07±0.03	3.04
13	30	18	170	14.98±0.4	14.98	3.64±0.03	3.64	14.98±0.4	14.98	3.64±0.03	3.64
14	25	19	140	19.54±0.4	19.76	3.08±0.05	3.04	19.54±0.4	19.76	3.08±0.05	3.04
15	30	20	110	16.08±0.3	16.18	3.75±0.07	3.71	16.08±0.3	16.18	3.75±0.07	3.71
16	20	20	170	18.55±0.5	18.85	2.41±0.06	2.49	18.55±0.5	18.85	2.41±0.06	2.49
17	25	19	140	19.62±0.8	19.76	3.01±0.05	3.04	19.62±0.8	19.76	3.01±0.05	3.04
18	25	20.68	140	17.06±0.8	16.82	3.01±0.03	2.97	17.06±0.8	16.82	3.01±0.03	2.97
19	25	19	89.55	16.68±0.5	16.42	3.28±0.01	3.32	16.68±0.5	16.42	3.28±0.01	3.32
20	25	19	140	20.01±0.3	19.76	3.09±0.02	3.04	20.01±0.3	19.76	3.09±0.02	3.04

WSI= water solubility index; TPC=total phenolic content; TAA=total antioxidant activity; SD=*in vitro* starch digestibility; GAE=Gallic acid equivalent; Teq=Trolox equivalent; Exp.=experiment values: Pred.=Predicted values

Table 5.4 Coded coefficients of regression equation for the responses.

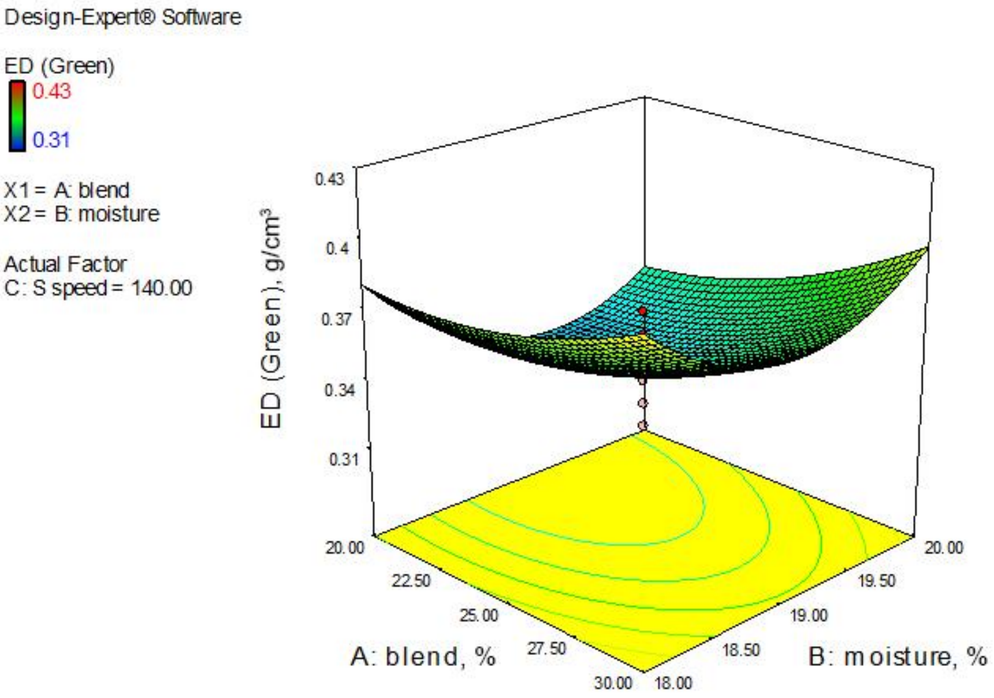
Factor	ER		ED		H		WAI		WSI		TPC		TAA		SD	
	G	**R**	**G**	**R**	**G**	**R**	**G**	**R**	**G**	**R**	**G**	**R**	**G**	**R**	**G**	**R**
A	3.74*	3.65*	0.34*	0.39	1173.60*	1074.18*	4.83*	4.34*	21.36*	19.76*	2.72*	3.04*	3.93*	4.31*	94.89	90.85*
B	-0.26*	-0.32*	0.02	0.002*	520.15*	447.74*	-0.48*	-0.46*	-0.84*	-0.90*	0.54*	0.54*	0.68*	0.80	-0.53*	-0.32*
C	0.28*	0.26*	-0.01	-0.01	-685.56	-417.05*	0.47*	0.49*	0.92*	0.91*	-0.07*	-0.07*	-0.08*	0.02	0.13	0.12*
AB	0.10*	0.08*	-0.0020	-0.0020	-148.90	-	0.16	0.14	0.34	0.34	-0.07	-0.07	-0.07	0.02*	-0.02	-0.08*
AC	-0.14	-0.12	0.01	0.01	-83.32	-310.73*	0.0038*	-0.0050*	-0.18	-0.17	0.02	0.01	0.03	-0.29*	-0.14	-0.22*
BC	-0.05	-0.04*	0.0013	0.0038	48.53	-	-0.13	-0.12	-0.19	-0.20	-0.03	-0.03	-0.02	-0.34*	0.05	-0.03*
A^2	0.05*	0.09*	-0.0012	-0.001*	107.20*	-276.74*	0.03*	0.01*	0.14*	-0.07*	0.0025	0.0025	-0.01	-0.34	0.10	-0.04*
B^2	-0.44*	-0.35*	0.01*	0.02*	421.57*	557.49*	-0.51*	-0.47*	-1.01*	-0.79*	0.02	0.02	0.01*	-0.01*	0.15	-0.21*
C^2	-0.50*	-0.54*	0.03*	0.02*	632.94*	703.79*	-0.70*	-0.70*	-1.58*	-1.58*	0.02*	0.01*	0.07*	0.06*	-0.07*	-0.12*
R^2	0.9948	0.9991	0.8285	0.9221	0.9213	0.9471	0.9899	0.9950	0.9684	0.9851	0.9910	0.9890	0.9829	0.9924	0.8104	0.9951
Lack of fit (F-value)	4.90	3.67	1.68	0.53	4.96	12.39*	4.84	2.63	4.91	1.87	5.03	4.33	4.76	4.68	4.94	4.04

*Significant ($p<0.05$)

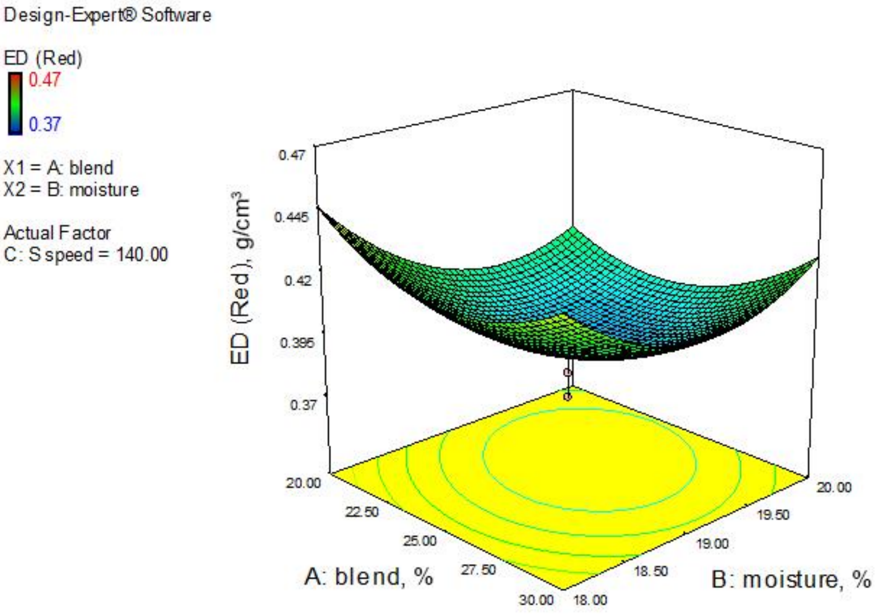
Where, A=Blend (%), B=Moisture (%), C= Screw speed (rpm), G= Green lentil, R= Red lentil, ER= Expansion ration (%), ED= Extrudate density (g/cm^3), H=Hardness (g), WAI= Water absorption indices (g/g), WSI=Water solubility indices (%), TPC= Total phenolic content (GAE mg/g of dm), TAA= Total antioxidant activity (Teq mg/g of dm) and SD= starch digestibility (%)

5.5.2 Extrudate Density

The extrudate density measures the degree of expansion in all direction and describes the amount of air present in the extrudate. The extrudate density varied with lentil type and higher extrudate density was recorded for germinated red lentil as compared to green lentil. A significant effect ($P<0.05$) of blend ratio (%) was observed in green lentil extrudates whereas both blend% and moisture content had significant effect on red lentil extrudates. The highest value for the extrudate densities of green and red lentil extrudated were 0.43 g/cm^3 and 0.47 g/cm^3, respectively. Response surface plots showed that the ED had variable effects with blend% and moisture content, i.e. initially decreased and further increased (Figure 5.2). This can be further correlated to the ER of the extrudate. Higher ER generates more voids which reduce the density of extrudate. Similar results have been reported by Rathod and Annapure (2017) for extrudates of lentil based noodles and Kumar et al. (2013) for extrudates developed from honey and barley. The response surface model developed was significant ($p<0.05$) and the R^2 value for germinated green and red lentil extrudate were 0.8285 and 0.9213, respectively. A and B single terms were significant for green and red lentil, respectively. None of the interaction terms was significant ($p>0.05$). All of the squared terms were significant, except for A in green lentil. The coded coefficients of the responses are shown in Table 5.4.



a)



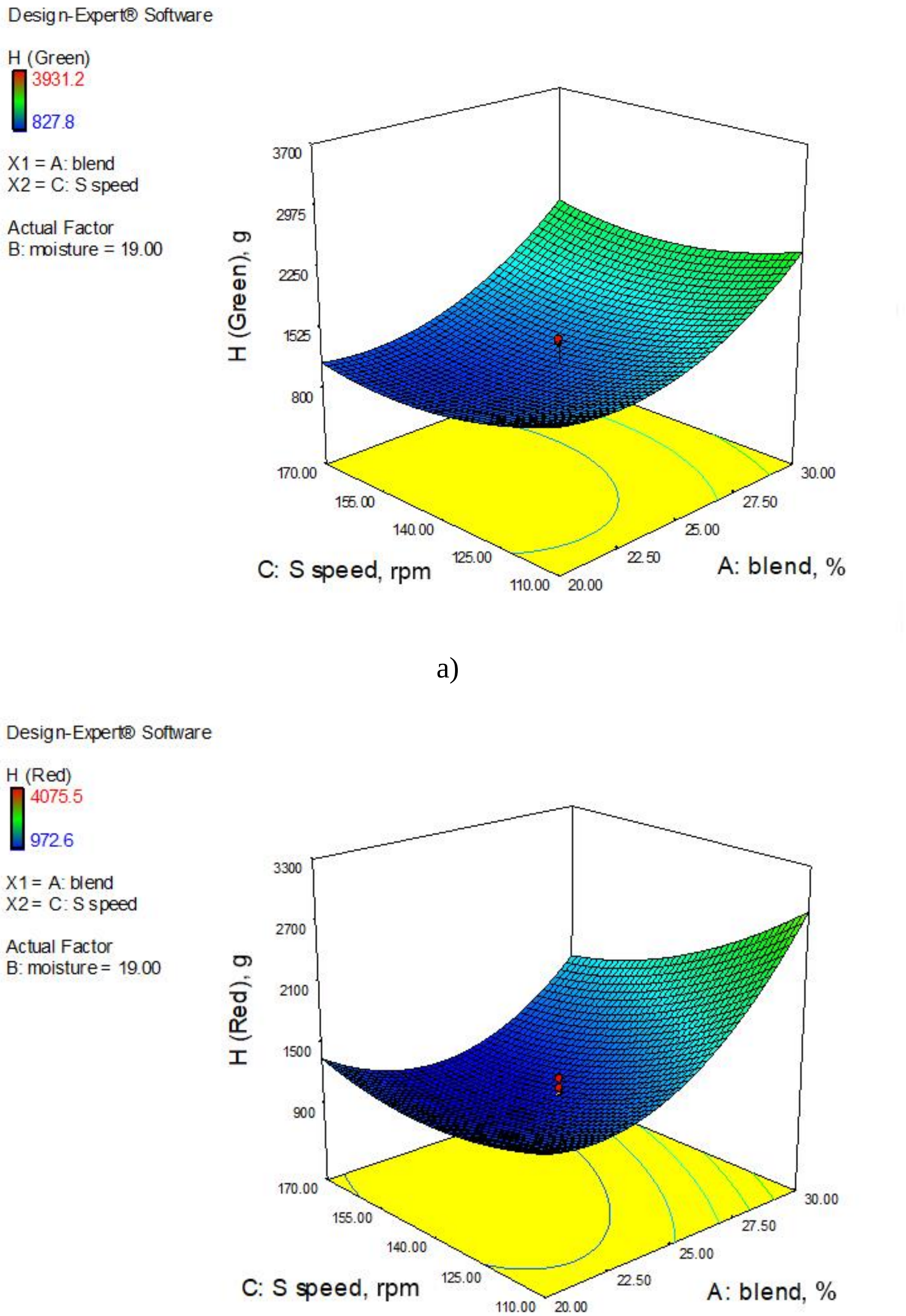
b)

Fig 5.2. Response surface plots showing the effects of extrusion on extrudate density (g/cm^3) of the green (a) and red (b) germinated lentils.

5.5.3 Hardness

Texture profile analysis was conducted and the force required to develop the first peak was recorded as the hardness of the extrudate. The highest value recorded was 3931.2 g for a 25% blend, 17.32% moisture content and, 140 rpm screw speed for green lentil whereas 4075.5 g was the highest value forced lentil (30% blend, 18% moisture content and 110 rpm). Hardness increased with decreasing moisture content and increasing percentage of lentil blend ratio. Lower ER and higher extrudate density increased the hardness of the sample. This may be due to smaller air pockets resulting in a thicker layer of the other portion of extrudates. Lentils contains a higher amount of protein as compared to corn. Protein has the capability to influence the water distribution matrix to change macromoleclar structure and conformation, resulting in the reduction of expansion. The increase in lentil percentage in the blend led to a decrease in expansion, hence an increase in extrudate density and hardness. The interaction between percent blend and moisture at 140 rpm is shown in Figure 5.3.

Blend% was more significant ($p<0.05$) than moisture and screw speed in influencing hardness. All the single terms were significant for both lentil types except for C for green lentil (Table 5.4). The Backward method was employed to quadratic regression model for red lentil. Only the AC term of red lentil was significant. The quadratic model developed was significant ($p<0.05$) with R^2 value of 0.9231 and 0.9471 for green and red lentil, respectively (Table 5.4). The lack of fit was significant for red lentil, however, the predicted $R^2$of 0.7859 was in reasonable agreement with the adjusted R^2 of 0.9163. The adequacy precision measures the signal to noise ratio and a ratio greater than four is desirable.



a)

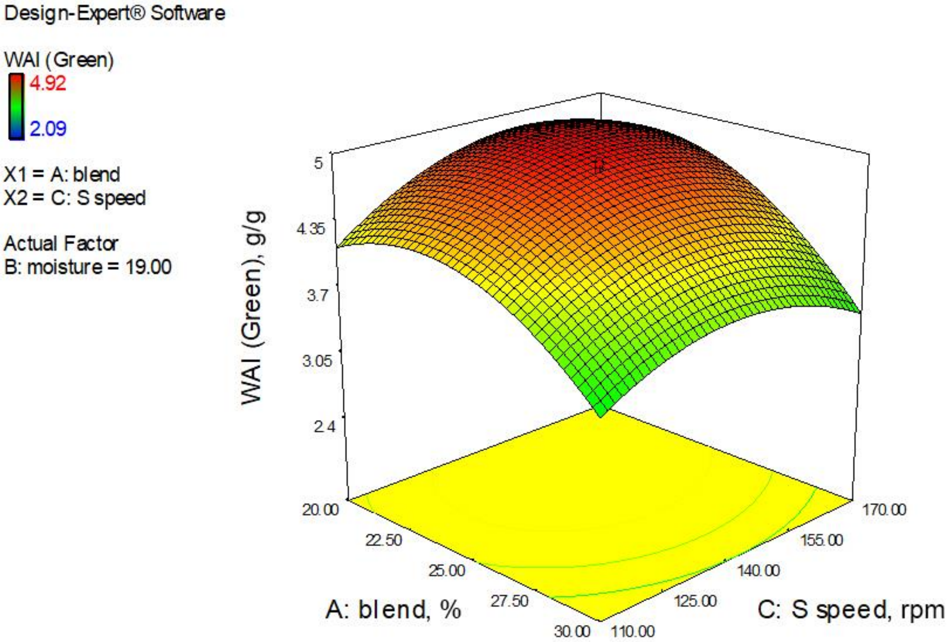
b)

Fig 5.3: Response surface plots showing the effects of extrusion on hardness (g) of the green (a) and red (b) germinated lentils.

5.5.4 Water Absorption Index

WAI was higher for green lentil extrudate as compare to red lentil extrudate. This may be due to more exposed area to water, as the ER for green lentil was higher than red lentil. WAI increased to a maximum level and then dropped with an increased in blend ratio and moisture content (Fig 5.4). Highest WAI was observed for a 25% blend ratio, 19% moisture and 140 rpm screw speed for lentil types. The WAI also can be used as an index for gelatinization, as it measures the amount of water absorbed by the starch. The reduction in WAI after a peak value with an increase in the percentage of blend can be explained by different molecular transformation of starch and protein matrix, resulting in unavailability of sites for water absorption. A similar trend of increasing to a peak and decrease with an increase in moisture also was observed in extrusion of lentil, bean and chickpea (Lazou and Krokida 2010; Gujska and Khan 1991; Singh et al. 2007). The decrease in WAI after the peak value may be due to damage to the polymer chain at a higher shear rate, which reduces hydrophilic groups, resulting in reduction of WAI (Guha et al. 1997).

The corresponding quadratic models developed were significant ($p<0.05$) for all first and second order terms (Table 5.4). The model developed had R^2 value of 0.9899 and 0.9950, for green and red lentil, respectively. Blend ratio had a more significant influence on WAI as compared to screw speed and moisture . Only the AC interaction terms were significant ($p<0.05$). The change in WAI of extrudates may be due to variation in lentil composition and the processing parameters (Abu-Ghoush et al. 2015). Hence, it will affect the physical and chemical properties of extruded products (Gajula et al. 2009).



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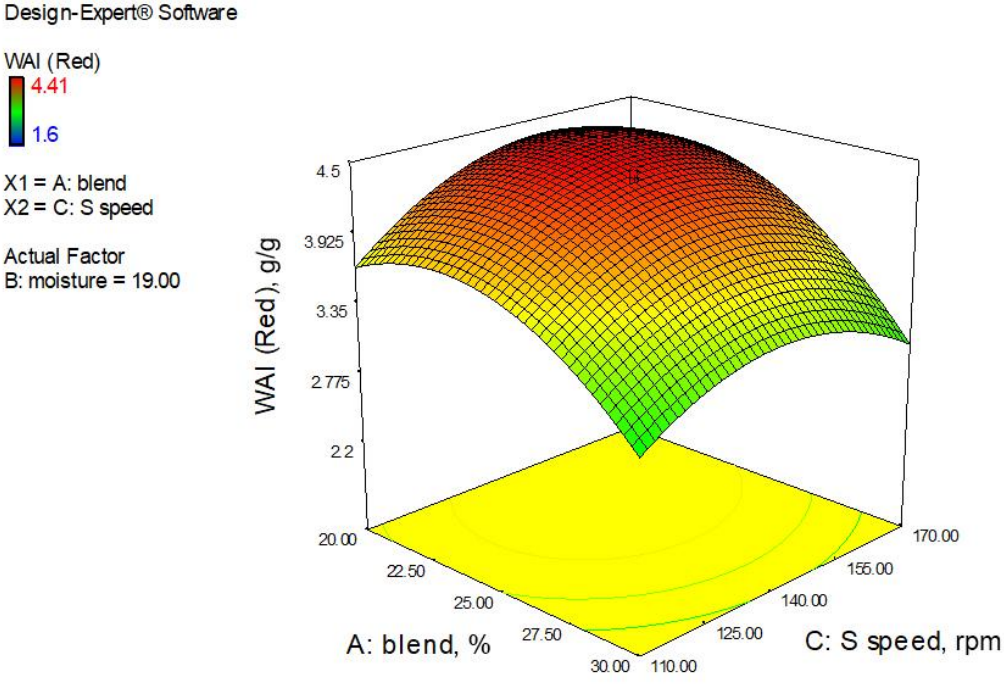


b)

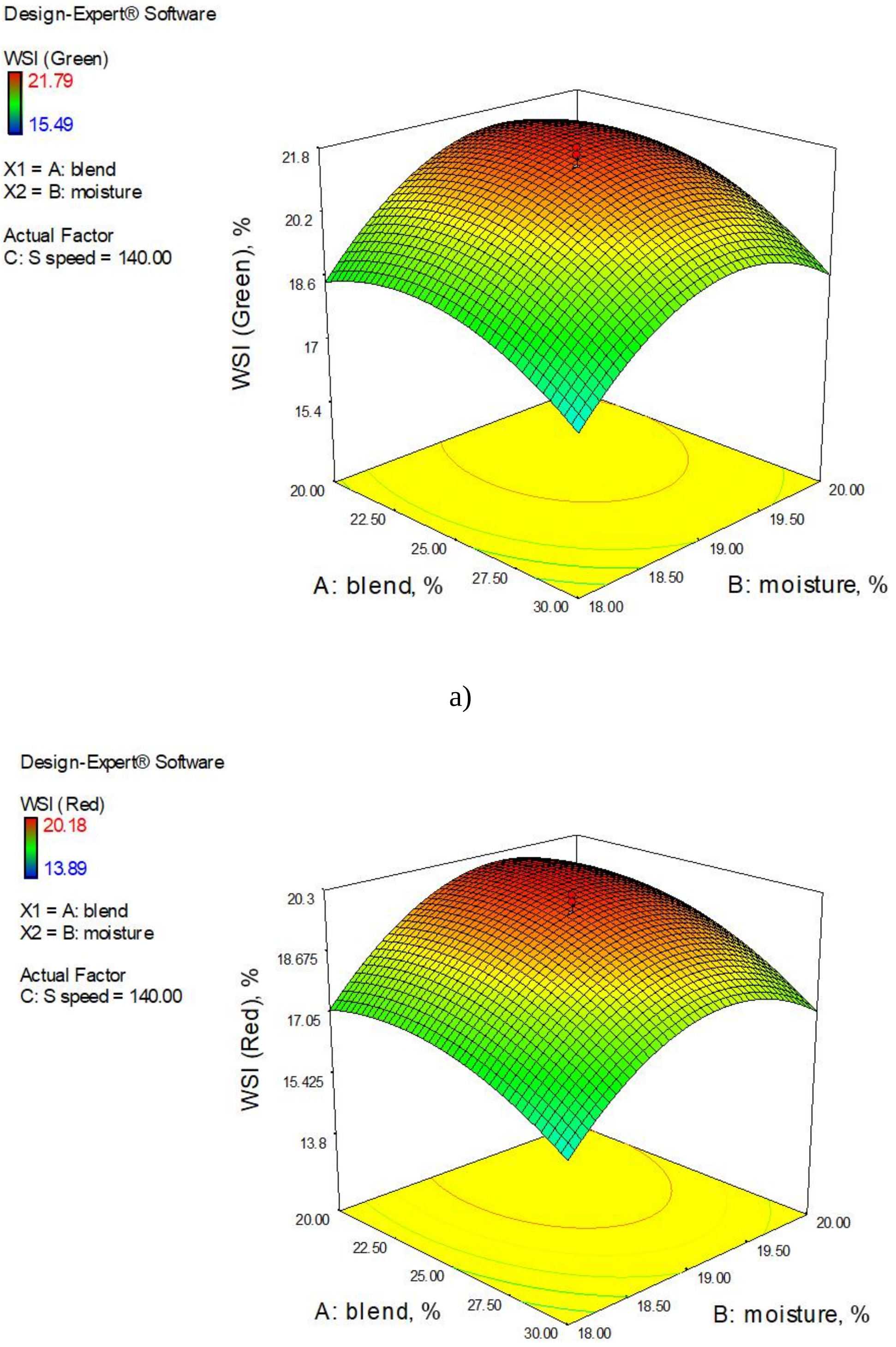
Fig 5.4. Response surface plots showing the effects of extrusion on WAI of the green (a) and red (b) germinated lentils

5.5.5 Water solubility index

WSI was influenced by all the independent terms; however, percent the blend ratio had more influence. The highest and lowest WSI were observed at the treatment where highest and lowest WAI was recorded in both the lentil types (Tables 5.2b and 5.3b). Similarly, a higher ER had a marked influence on increasing the interaction of the interior part of the extrudates, as higher ER extrudates provided macro and micro tunnels for water to enter, thereby increasing the WSI. Dextrinization is related to the quantity of soluble molecules; therefore, it indicates the degree of starch conversion during extrusion due to degradation of molecular compounds at lower moisture content (Gomez and Aguilera 1983; Colonna et al. 1989; Ding et al. 2005). Therefore, an increase in WSI with feed moisture content is expected. The quadratic model developed had R^2 values of 0.684 and 0.9851 for green and red lentil, respectively (Table 5.4). First and second order terms were significant. However, none of the interaction terms was significant.

5.5.6 Total phenolic content

The optimally microwave-vacuum dried lentil flour had 6.28 GAE mg/g dry matter and 5.51 GAE mg/g dry matter for germinated green and red lentil, respectively. However, extrusion cooking reduced the TPC in both the lentil types. The highest TPCs were recorded as 3.64 GAE mg/g dry matter and 3.97 GAE mg/g dry matter for green and red lentil, respectively, both at 33.41% blend ratio, 19% moisture and 140 rpm screw speed (Tables 5.2b and 5.3b). The TPC was reduced with a reduction in lentil flour blend and an increase in moisture content and screw speed. The increase in the TPC value may have been due to higher the TPC in lentil flour as compared to corn flour. The reduction may be caused by a decrease in free phenolics and an increase in bound phenolics (Sarawong et al. 2014). Reduction in TPC during extrusion cooking also was reported in extrusion cooking of other food commodities, such as barley and banana flour (Sharma et al., 2012; Sarawong et al. 2014). High temperature also causes degradation or alteration of phenolic molecular structure, which may reduce the chemical reactivity leading to a greater degree of polymerization and thus reducing TPC (Altan et al. 2009; Nayak et al. 2011 and Sharma et al. 2012).



a)

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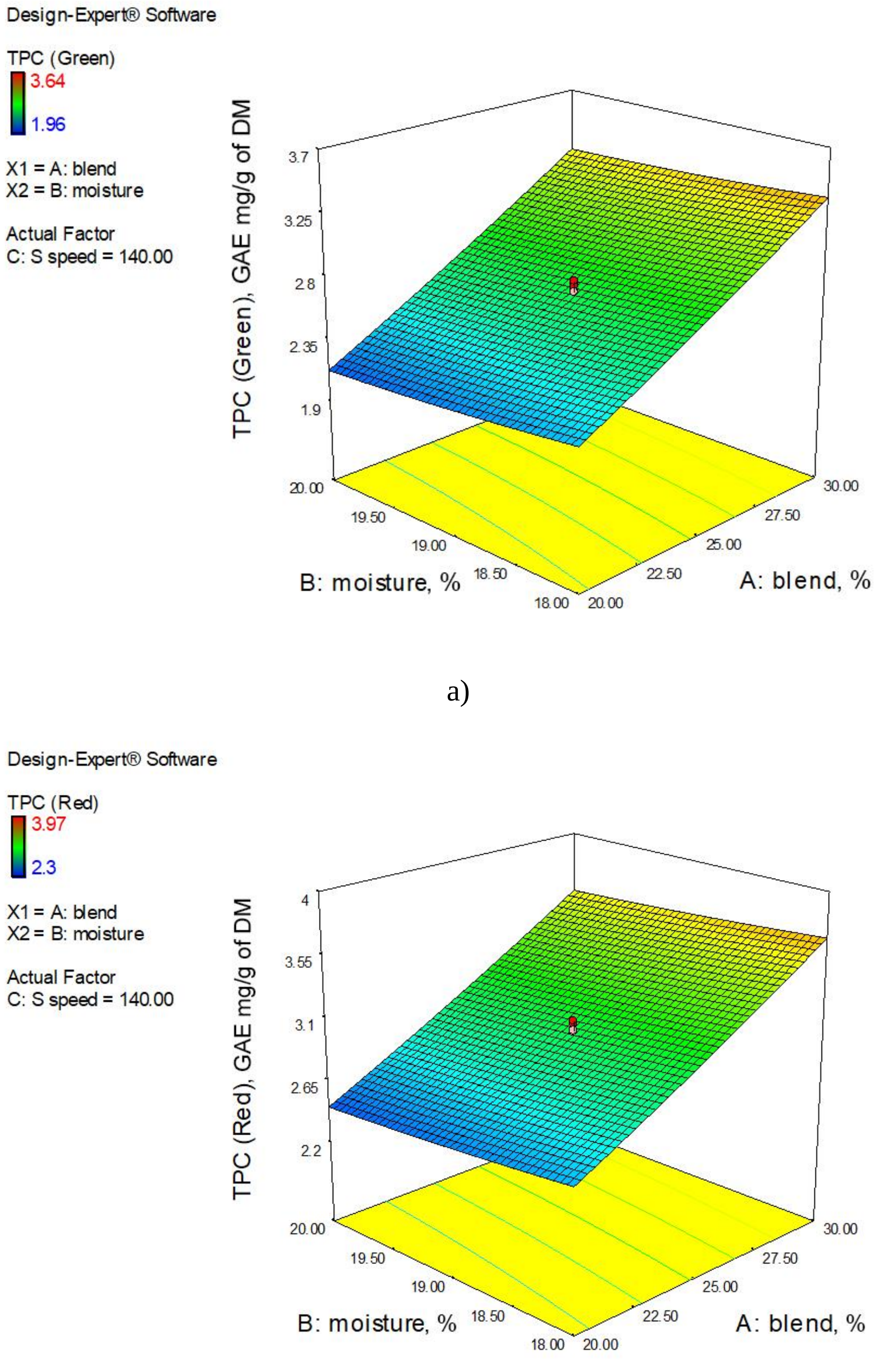
Fig 5.5: Response surface plots showing the effects of extrusion on WSI of the green (a) and red (b) germinated lentils



a)

b)

Fig 5.6: Response surface plots showing the effects of extrusion on TPC of the green (a) and red (b) germinated lentils

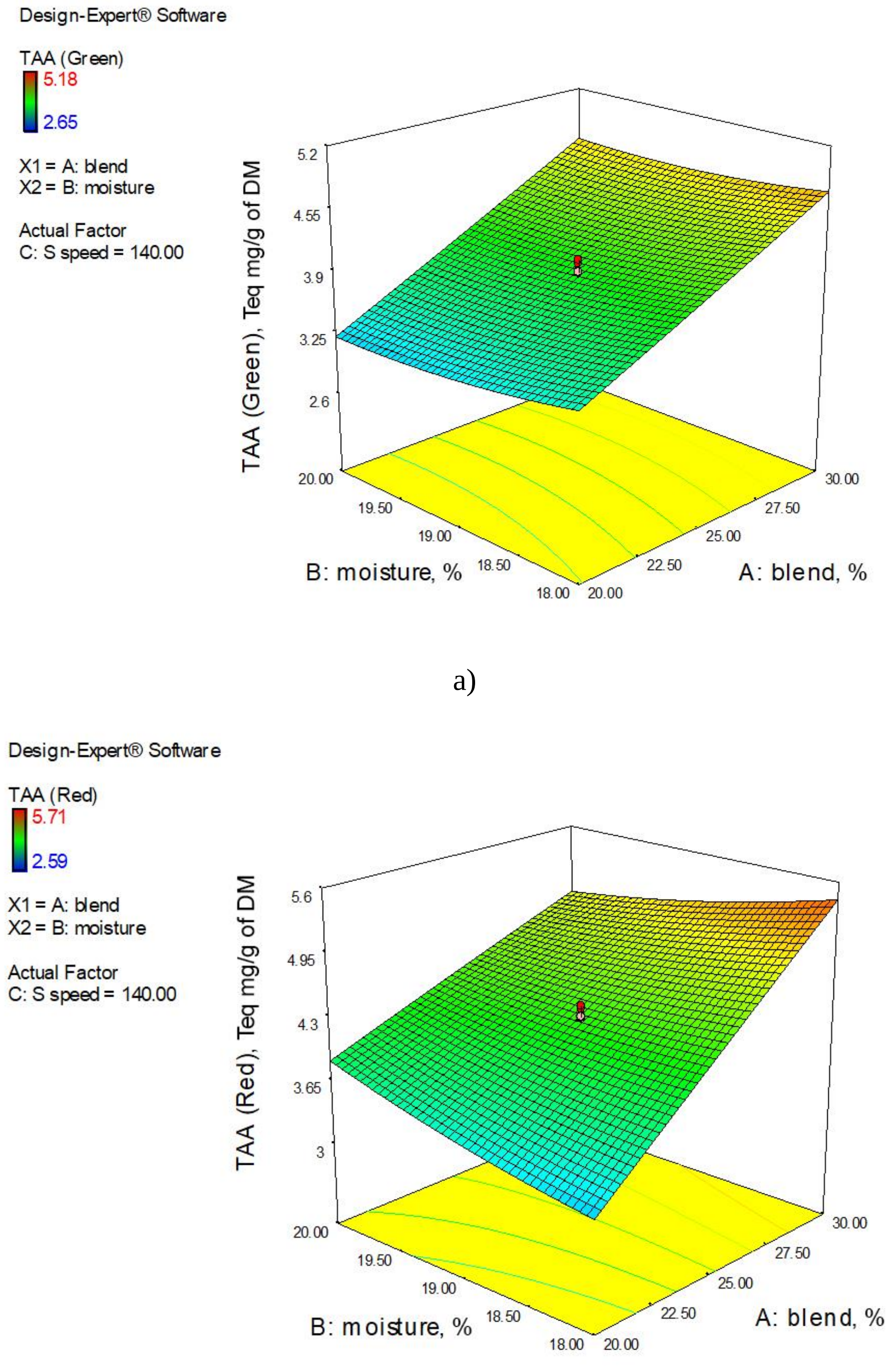
The response surface quadratic models developed were significant ($P<0.05$) with R^2 value of 0.9910 and 0.9829 for green and red lentil, respectively. All the first order independent terms and C^2 were significant (Table 5.4). The blend ratio had more influence as compared to moisture and screw speed. None of the interaction terms was significant. Similar tends were observed for extrusion with a chickpea, oat, carrot hazelnut and corn flour mix (Ozer et al., 2006).

5.5.7 Total antioxidant activity

TAA exhibited a trend similar to that of TPC. TAA varied from 5.18-2.65 mg Teq/mg DM for green lentil, and 5.71-2.59 mg Teq/ mg DM for red lentil. As most of the antioxidant present in pulses are phenolics, TAA was reduces with a reduction in the blend ratio and moisture for both lentil types. However, the reduction due to moisture was more pronounced for red lentil. This reduction was attributed to reduction and polymerization of free phenolic compounds (Sarawong et al. 2014). The models developed had R^2 value of 0.9828 and 0.9924 for green and red lentil, respectively. All of the first order independent terms for green lentil were significant ($p<0.05$) whereas only the A term (blend ratio) was significant ($p<0.05$) for red lentil. All of the interaction terms were significant ($p<0.05$) for red lentil, whereas none of the interaction terms were significant ($p>0.05$) for green lentil. The coded estimated coefficient values are presented in Table 5.4.

5.5.8 *In vitro* Starch digestibility

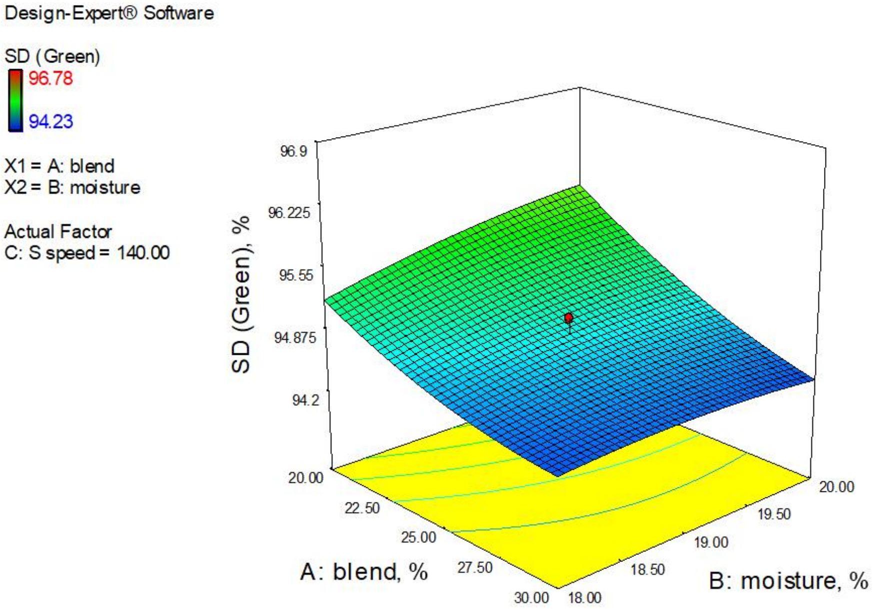
The *in vitro* starch digestibilities of microwave-vacuum-dried lentil flour were 92.5% and 87.6% for green and red lentil, respectively. The starch digestibilities of the extrudates were higher as compared to optimally microwave-vacuum-dried germinated lentil flour. Although there was an increase in digestibility with an increase in moisture, the digestibility increase due to moisture was more pronounced in red lentil (Tables 5.2b and 5.3b). Highest SDs recorded were 96.8% and 91.3% for green and red lentil, respectively. The increases in digestibility after extrusion were similar to previous research work of various researchers (Rathod and Annapure 2017). Starch gelatinization during extrusion process directly affects the digestibility of starch due to modification in the quality of starch by thermo-mechanical treatment (Dust et al. 2004, Lankhorst et al. 2007). During extrusion, the amorphous regions of the starch granules may be disrupted followed by bonding of free hydroxyl group from water. This may induce new crystallization and recrystallization of starch molecules resulting in higher gelatinization and significant improvement in starch digestibility (Ljokjel et al. 2004, Rathod and Annapure 2017). It also was reported that the increase in digestibility was mainly due to reduction in antinutritional components present in lentil by high temperature treatment (Reddy et al. 1985).The corresponding quadratic models developed were significant ($p<0.05$) with R^2 value of 0.8104 and 0.9951 for green and red lentil, respectively. The B and C^2 terms were significant for green lentil, whereas all the terms were significant for red lentil (Table 5.4).



a)

b)

Fig 5.7: Response surface plots showing the effects of extrusion on TAA of the green (a) and red (b) germinated lentils



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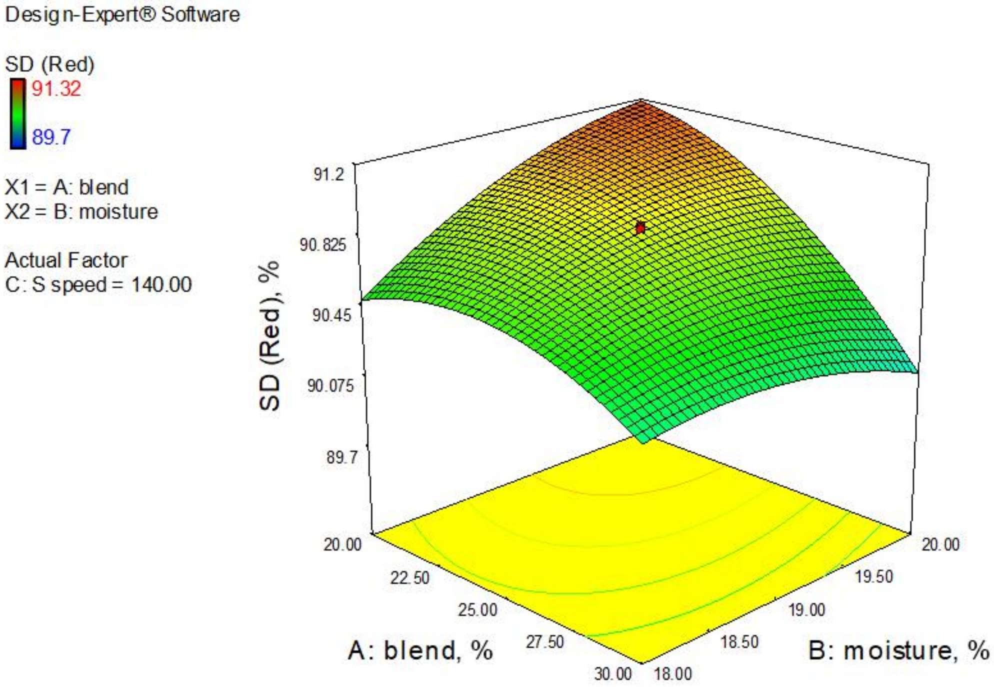

b)

Fig 5.8: Response surface plots showing the effects of extrusion on SD of the green (a) and red (b) germinated lentils

5.5.9 Optimization of process parameters

The optimization of process parameters were done based on maximum ER, WAI and *in vitro* starch digestibility, with an importance level of 5, for each and maintaining the independent parameters in the range of selection (Table 5.5). The highest desirability was selected as the optimum level and cross-check experiments were conducted. The values exhibited less than 10% error. Therefore, the germinated green lentil flour blend with corn flour can be extruded at 20% blend, 19.5% moisture and 147 rpm screw speed, whereas a 20.7% blend ratio, 19.7% moisture and 141 rpm screw speed was obtained for red lentil. These values can be set as independent parameters for producing optimum extruded products for green and red lentils/ corn flour blends. (The temperature of the barrel should be maintained at 150℃ and 180℃ for the die). The retention time during extrusion was about 45 s.

Table 5.5: Optimized parameters for extrusion of germinated green and red lentils.

Lentil variety	Blend	Moisture	Screw speed	expansion ratio	WAI	SD	Desirability
green	20	19.53	147.20	3.67	4.90	95.68	0.807
red	20.73	19.67	140.96	3.68	4.41	91.13	0.959

WAI= water absorption ratio; SD= in-vitro starch digestibility

5.6 Conclusion

The germinated lentil flours could be utilized as a raw ingredient in developing new extruded healthy food products. Germination and microwave-vacuum drying reduced level of antinutritional compounds, thereby increasing the digestibility of starch. The expansion ratio (ER) of germinated lentil extrudates was comparable with those of cereal extrudates. Extrudate density and hardness were inversely correlated to the ER of the extrudates, whereas WAI and WSI were directly related to ER. Increases in TPC and TAA were observed with an increased in amount of germinated lentil flour in the blend. The optimized process conditions for the germinated green and red lentil flour blend with corn flour was at 20% blend ratio, 19.5% moisture and 147 rpm screw speed, whereas a 20.7% blend ratio, 19.7% moisture and 141 rpm screw speed was obtained for red lentil. The current work will provide a reference for future researchers to venture into the utilization of germinated legumes and the development of new pulsed-base products.

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CHAPTER 6

TECHNOECONOMIC EVALUATION FOR THE PRODUCTION OF EXTRUDED PRODUCTS FROM GERMINATED LENTIL

Partially reproduced with permission. Nongmaithem R. and V. Meda. 2018. Technoeconomic evaluation for the production of extruded products of germinated lentil. *CSBE/SCGAB Annual General Meeting and Technical Conference, Guelph, ON, Canada, July 22-25.*

Contribution of this paper to the overall study

The evaluation of technoeconomic and break-even analysis are important for starting a new project for production of any food materials. This chapter helps in understanding the feasibility of producing an extruded product from germinated green and red lentil. This chapter is specific to objective 5. All the assumptions and analysis were conducted and the conference manuscript was drafted by myself.

6.1 Abstract

In this study, a technoeconomical analysis (TEA) of germinated, lentil-based extruded products was carried out. Consumer awareness toward the healthy snack foods adds to the importance of germinated extruded products. Lentil is a pulse having high amount of protein and other macro- and micro-nutrients, and germination proved to increase the bioavailability of nutrients with less investment as compared other food processing treatments. The germination of samples, two lentil types (green and red lentil), was carried out for the development of extruded products and the technoeconomical analysis and break-even analysis were performed. The assumptions for TEA were CAD $360,000 per tonne of raw material for equipment cost, 20% of the equipment cost for installation and electricity, 5% of equipment cost for piping and 2.5 times the equipment cost for civil construction. Extrusion of germinated red lentil was more profitable (21.2%) and had a

shorter break-even point (BEP) (BEP of 949.3 tonne) as compared to germinated green lentil. Red lentil type had higher net present value (NPV) and internal rate of return (IRR). Extrusion of germinated lentil flours with corn flour may be a viable option for small-scale industries.

6.2 Nomenclature

CDC	Crop Development Centre	BEP	Break- even point
NPV	Net present value	IRR	Internal rate of return
TEA	Technoeconomic Analysis	d.b.	Dry basis
t	Tonne	h	Hour

6.3 Introduction

The demand for extruded snacks and breakfast cereals with improved nutritional value has been increasing in recent years. Extrusion cooking of food is an established technology for producing a wide variety of expanded products having different shapes and sizes with higher nutritional value (Ernoult et al. 2002; Kaur et al. 2007). Extrusion technology in food processing is used for producing healthy and ready-to-eat expanded products (Lazou and Krokida 2010). Moisture content, temperature, screw speed and percentage of different ingredients are the main process parameters to be optimized for the production of extruded food products (Meng et al. 2010).

Lentils (*Lens culinaris* Medikus) is known for good nutritive value. It provides protein, carbohydrates and both macro- and micro-nutrients (Rathod and Annapure, 2015). Germination is an inexpensive unit operation which increases the bioavailability of nutrients by lowering the levels of antinutritionl compounds present in lentil seed. Nongmaithem and Meda (2017a) optimized the drying parameters of microwave-vacuum drying for germinated lentils (both CDC Greenland and CDC Maxim variety) and suggested for production of extruded products using the germinated lentil flours. Today, consumers are aware of the nutrient content and ingredients present in their food products sold in the supermarkets.

For production of any new product, technoeconomic analysis (TEA) is very important. TEA is defined as a systematic analysis used for evaluating the economic feasibility and to recognize opportunities and threat of projects, with consideration of capital, variable (operational) and fixed costs (Simba et al. 2012), as well as benefits. The critical parameters in TEA are fixed and operating cost, which play a key role for cost estimation, project evaluation and process optimization (Marouli and Maroulis 2005). Upon appropriate assumptions based on capital, variable and fixed cost, TEA is calculated. In the food processing industry, energy consumption, such as in drying and extrusion in this case, is one of the most important operational costs. For drying, microwave assisted drying has been widely reported as an efficient drying technique. Combination of microwave and vacuum during drying improves in retention of nutrients, colour and texture as the products are dried at lower temperature in short time (Mitra et al. 2013). The addition of vacuum pressure in microwave drying certainly increases the energy consumption but it is minimal (less than 3% in total energy consumption) as reported by Nongmaithem and Meda (2017b). Therefore, microwave vacuum drying can be employed for drying germinated lentils while retaining nutrients. Reports on TAE of extrusion suggested that extrusion cooking is feasible economically for large scale production only, where twin-screw extruder are generally employed and requiring high capital investment.

In this study, technoeconomic analysis (TEA) and break-even point (BEP) for small-scale production of germinated lentil-based extruded product was analyzed.

6.4 Materials and methods

6.4.1 Process for production of germinated lentil-based extruded product

According to Nongmaithem and Meda (2017, 2024), green (CDC Greenland) and red lentil (CDC Maxim) was utilized for production germinated lentil-based extruded product. In the production process, a number of unit operations were employed viz. germination, drying, grinding, blending, extrusion and packaging. Five days of germination were performed at room temperature (25°C) followed by the drying in a microwave-vacuum dryer. The germinated lentils was dried to 10±2 (% wet basis) moisture content and ground in a multipurpose hammer mill having 1 mm diameter sieve. The germinated lentil flour were blended with corn flour and extruded by maintaining barrel and die temperature at 150 °C and 180 °C, respectively. The green lentil extruder feed was maintained at 19.5% moisture and 20% lentil flour blend-ratio, while red lentil feed was maintained at 19.7% moisture and 20.7% lentil flour blend-ratio to attain optimum and good quality extrudates (Chapter 5). The production process of extrusion and products based on germinated green and red lentil floor are shown in Figure 6.1 and Figure 6.2.

6.4.2 Technoeconomic analysis (TEA)

During TEA, calculation of total capital cost is the most important parameter, as the remaining parameters are assumed based on it. The assumption of total capital cost was based on the cost of equipment available today (Table 6.1). The cost was calculated based on extrudate production rate of 0.5 t/h. For better understanding of the TEA, two additional scenario (production rates) were assumed i.e 1 t/h and 5 t/h.

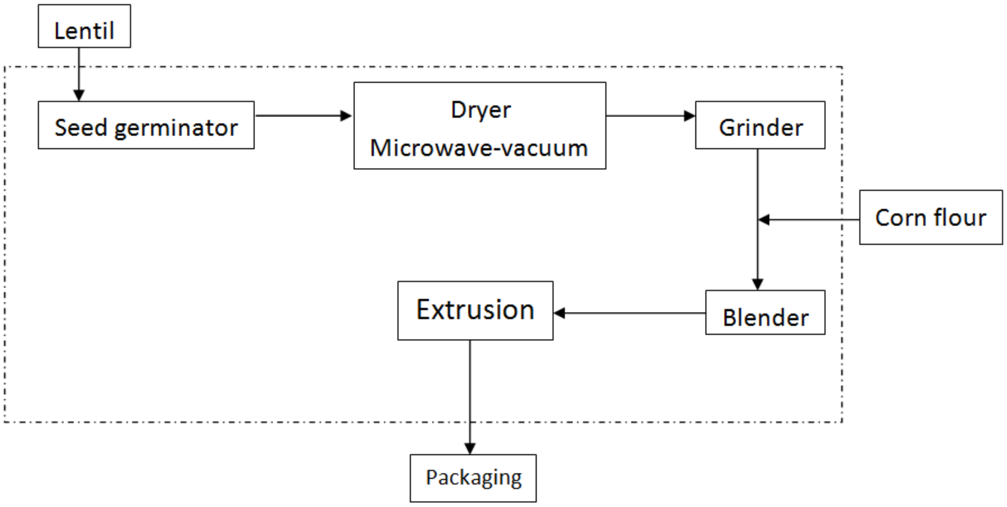

Fig 6.1 Flow diagram of the extruded product development.

Fig 6.2 Extrudates from germinated green and lentil.

Table 6.1 Cost of equipment.

Item	Capacity, kg/h	Power, kW	Cost, CAD
germinator	500	5	10000
MVD	500	500	50000
hammer mill	500	10	10000
ribbon blender	500	10	10000
extruder	500	200	50000
packaging	500	5	20000
conveyor belt	500	5	20000
Silo	-	-	10000
Total		735	180000

Price are quoted based on appendix II.

6.4.2.1 Total capital cost analysis

Total capital cost could be divided into equipment cost and construction cost. Equipment cost consisted of the equipment cost including transportation, installation cost, piping cost and electrical work. Construction cost is mainly the cost of engineering work including other direct and indirect expenses.

$$\begin{aligned} Capital\ cost &= Equipment\ cost + installation\ and\ electrical\ work + \\ &\quad Piping\ cost + \ construction\ cost \end{aligned} \tag{6.1}$$

6.4.2.2 Variable cost

Variable cost is the major component of production. It consisted of utilities cost, i.e. electricity and water, feed ingredients, labour, repair and maintenance costs and other miscellaneous costs to run the plant. The cost of corn per tonne ranges between CAD \$300 to CAD \$450 based on corn types however, the cost of corn flour per tonne ranges between CAD \$ 1,500 to CAD \$4,500 based on types and starch percentage (Bulkmart 2024; Umamishop 2024). In the present study, it is assumed that corn flour is directly purchased from the market, costing CAD \$3000. The variable cost is calculated as follows:

$$\begin{aligned} Variable\ cost &= Utility\ (electricity\ and\ water)\ costs + feed\ ingredients\ cost + \\ &\quad labour\ cost + maintenance\ and\ repair\ costs + \\ &\quad other\ miscellaneous\ supply\ cost \end{aligned} \tag{6.2}$$

6.4.2.3 Fixed and total costs

The fixed cost includes the depreciation, insurance, interest on loan amount, taxes to government and overhead charges required to run the plant. Depreciation was calculated by the straight line method with an estimated useful life of 15 years with 250 working days and having 10% salvage value. Insurance cost was estimated as the sum of 0.462% of the equipment and building cost (Davis et al., 2011). 5% interest rate was assumed for calculation as per Suleiman et al. (2013) and 0.35% of total capital cost as taxes. The overhead and taxes to the government may vary, while depreciation, insurance and interest will remain fixed.

$$Fixed\ cost = Depreciation\ cost + insurance\ cost + interest\ cost + taxes\ cost \tag{6.3}$$

Total production cost is the sum of all the investment done for production. It is the sum of fixed cost, capital cost and variable cost.

$$Total\ cost = Fixed\ cost + Capital\ cost + Variable\ cost \tag{6.4}$$

6.4.3 Break-even point analysis

Break-even point (BEP) analysis was employed to study the minimum extrudate production required to achieve a point where net investment and net revenue are equal. BEP is calculated as:

$$BEP = Net\ fixed\ cost \div (Sales\ price - variable\ cost) \tag{6.5}$$

Where,

$$Net\ fixed\ price = Total\ capital\ cost + Fixed\ cost \tag{6.6}$$

The sales price per tonne was assumed as CAD $4,500 (as per cost of breakfast/snacks available in the market, (appendix I))

6.4.4 Net present value and internal rate of return

The difference between current value of ash inflows and outflows over a given period of time is known as net present value (NPV). NPV assess the profitability and capital budgeting of a planned investment or project. It was being calculated as follows:

$$\mathrm{NPV} = \sum_{t=0}^{n} \left(\frac{C_t}{(1+r)^t}\right) \tag{6.7}$$

Where,

Ct=Expected net cash flow at time t.

n=projected life of the investment

r=discounted rate

In a discounted cash flow analysis, the internal rate of return (IRR) is the discounted rate that sets the NPV of all cash flows to zero. It is used in financial research to estimate the profitability of potential investment.

6.5 Results and Discussions

6.5.1 Analysis of total capital cost

The initial investment is the most crucial component in construction and plant establishment. Capital cost comprises equipment, installation, electrical and construction costs involved in plant development. The cost of equipment was assumed at CAD $180,000 for 0.5 t/h capacity (Table 6.1). The installation/electrical work, piping and construction costs were assumed to be 20%, 5% and 2.5 times of the equipment cost, respectively. The capital costs of the three scenarios 0.5 t/h, 1 t/h and 5 t/h were CAD $675,000; CAD $1,350,000 and CAD $6,750,000, respectively (Table 6.2 and 6.3). The capital cost varied with the plant capacity, and an increase in the capacity of the plant led to an increase in capital cost; however, the ratio of production to capital cost is the same for the three scenarios. There was no variation in the capital cost of green and red lentil extrudes production. During fractionalization of the capital cost it was observed that the 26.7% of the total capital cost was the equipment cost, and 66.7% was for construction and other engineering works, and 6.6% was for installation, piping and electrical work. The construction and engineering cost was the main component of the capital cost for the plant followed by equipment and other cost.

6.5.2 Analysis of total production cost

Production costs mainly varied with the cost of raw material. Production cost is the sum of variable cost and fix cost. Variable cost consists of the costs associated with the operation of the plant. All the values were assumed based on market value as on January, 2024. The utility (electricity and water), feed ingredients, labour, repair and maintenance, and other miscellaneous costs involved in plant operation are variable cost. The cost of feed ingredients was observed to be higher compared to other costs.

Table 6.2 Economic analysis of germinated green lentil extrudates.

Capital cost	0.5 t/day	1 t/day	5 t/day
Equipment (assumed)	180000	360000	1800000
Installation/electrical work	36000	72000	360000
Process spouting/piping cost	9000	18000	90000
Engineering/construction	450000	900000	4500000
Total capital cost	675000	1350000	6750000
Variable cost/ t			
Electricity	140	280	1400
Water	6.25	12.5	62.5
Lentil @ CAD $1400	140	280	1400
Corn @ CAD $3000	1200	2400	12000
Labor	46.9	93.8	469
Repair and maintenance	2.2	4.4	22
sub total	1535.35	3070.7	15353.5
other miscellaneous cost 5% of sub total	76.7675	153.535	767.675
total variable cost	1612.12	3224.24	16121.2
Fixed cost			
Depreciation	162	324	1620
Insurance	11.6424	23.2848	116.424
Interest @5%	135	270	1350
Tax	9.45	18.9	94.5
Total Fixed cost	318.092	636.185	3180.92
Total cost of production	1930.21	3860.42	19302.1

Values are in CAD.

For green lentil extrudates, ingredient cost comprises of 69.4% whereas for red lentil it was 68.4% of the total production cost. The variation is due to different costs for green and red lentil, Suleiman et al., (2014) also reported the highest cost for the production of aqua feed using extrusion techniques was cost of raw material. The total variable cost also varied with the capacity of the plant and an increase in plant capacity increased the cost of production, i.e. CAD $1,612.12, CAD $3,224.24 and CAD

$1,6121.2 for green lentil extrudates, and CAD $1,538.62, CAD $3,077.24 and CAD $1,5386.2 for red lentil extrudates, respectively, for 0.5 t/h, 1 t/h and 5 t/h of production. The calculation of labour costs was based on the assumption that 1 t extrudate could be produced by employing 5 person. If the wages is assumed at CAD $150 per day (8 h), then the labour cost would be CAD $93.75 for handling 1 t/h. The maintenance costs were estimated as 3% of the total capital cost.

Table 6.3 Economic analysis of germinated red lentil extrudates.

Capital cost	0.5 t/day	1 t/day	5 t/day
Equipment (assumed)	180000	360000	1800000
Installation/electrical work	36000	72000	360000
Process spouting/piping cost	9000	18000	90000
Engineering/construction	450000	900000	4500000
Total capital cost	675000	1350000	6750000
Variable cost/ t			
Electricity	140	280	1400
Water	6.25	12.5	62.5
Lentil @ CAD $700	70	140	700
Corn @ CAD $3000	1200	2400	12000
Labor	46.9	93.8	469
Repair and maintenance	2.2	4.4	22
sub total	1465.35	2930.7	14653.5
other miscellaneous cost 5% of sub total	73.2675	146.535	732.675
total variable cost	1538.62	3077.24	15386.2
Fixed cost			
Depreciation	162	324	1620
Insurance	11.6424	23.2848	116.424
Interest @5%	135	270	1350
Tax	9.45	18.9	94.5
Total Fixed cost	318.092	636.185	3180.92
Total cost of production	1856.71	3713.42	18567.1

Values are in CAD.

The fixed cost comprises depreciation of the plant, insurance, interest, overhead and taxes. The highest cost was observed to be the depreciation of the plant of 34.9% of the total fixed cost. The total production cost of 0.5 t/h, 1 t/h and 5 t/h production plant was CAD $1,930.21, CAD $3,860.42 and CAD $1,9302.1 for green lentil, and CAD $1,856.71, CAD $3,713.42 and CAD $1,8567.1 for red lentil, respectively.

6.5.3 Break-even point analysis

The break-even point (BEP) was calculated based on the assumption that the selling price of the product is above 15% profitable (as per Canadian standard for food manufacturing industry, (appendix I)). The BEP for green lentil extrudates was 1058.7 tonne whereas 949.3 tonne for red lentil extrudates (Fig 6.3 and 6.4). The difference in BEP between lentil types was due to the difference in the price of raw materials and keeping the same selling price. The selling price was fixed at CAD $4,500 per tonne (at above 15% profitability), in accordance with the higher production cost of green lentil types. The red lentil had higher profit (21.8%) as compared to green lentil.

The BEP could also be interpreted as time required to achieving a point where net investment and net revenue are equal. Assuming 8 working hour per day and 5 days a week, the BEP would be achieved in about 26.5 weeks (about 6.6 months) for green lentil type whereas about 23.7 weeks (about 5.9 months) in red lentil type, at net revenue of CAD $4,764,092 and CAD $4,271,850 for green and red lentil, respectively. For a successful business plan, lower BEP is desirable, which would have higher profitablility. Therefore, red lentil are more suitable than green lentil for production of extrudates, assuming the same selling price and similar texture and nutritional value.

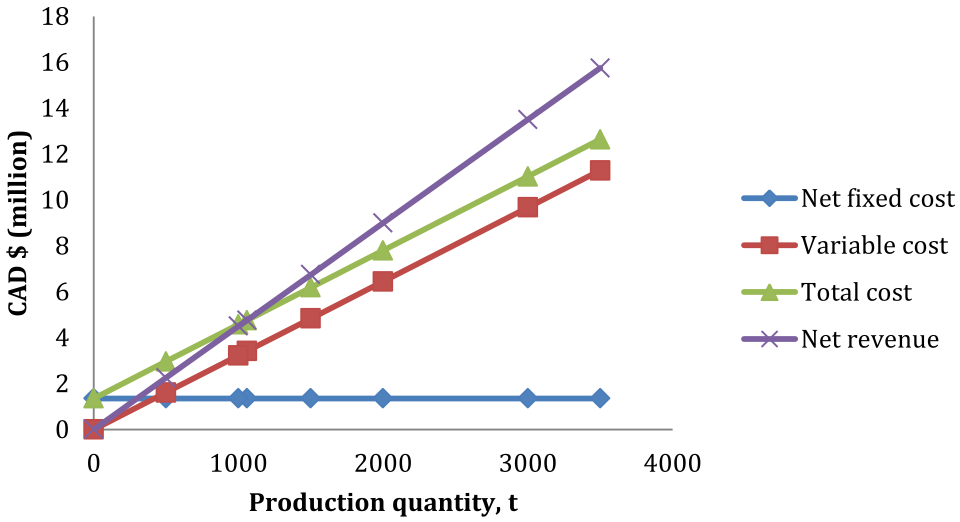

Fig 6.3 Break-even analysis of germinated green lentil extrudates.

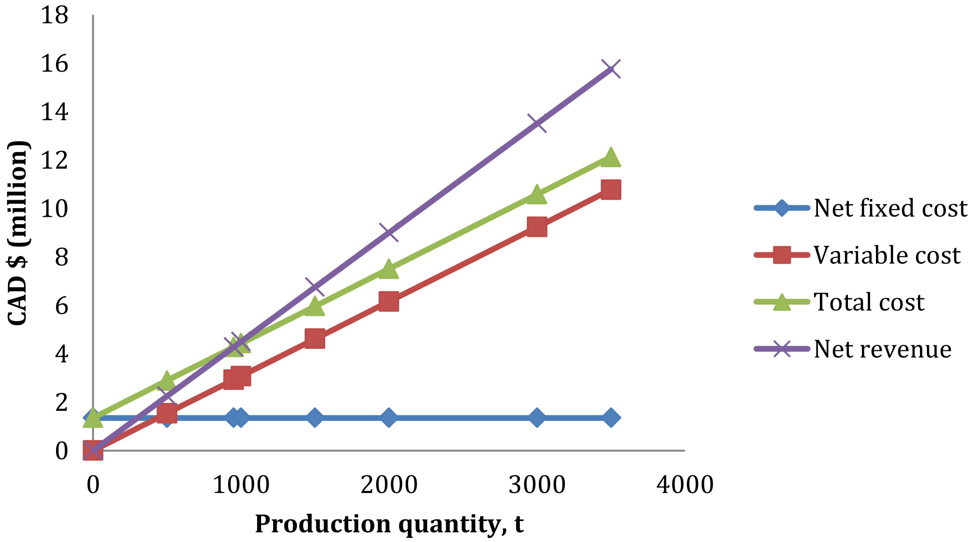

Fig 6.4 Break-even analysis of germinated red lentil extrudates.

6.5.4 Net present value and internal rate of return

The net present value was calculated for capacity of 0.5 t/day and 250 working days per year for 5 years. The discounted rate was considered as 10%. The cash inflow was assumed to be 70%, 75%, 80%, 90%, and 95% of the full production capacity for 1, 2, 3, 4, and 5 year, respectively. The NPV for green lentil type were CAD $ 310,914.5

whereas CAD $ 392,612.9 for red lentil type (Table 6.4 and 6.5). Positive NPV value shows that the planned investment is profitable. The NPV for red lentil type was 20.8% higher than green lentil type. Internal rate for return (IRR) was higher in red lentil type with 21% where as 19% for green lentil type. Both lentil types had higher IRR than discounted rate of 10%, meaning the project is profitable.

Table 6.4 Net present value and internal rate of return for green lentil (0.5 t/day)

Year	**0**	**1**	**2**	**3**	**4**	**5**
Cash inflow	0	787500	843750	900000	1012500	1068750
Cash outflow	1157552.5	482552.5	482552.5	482552.5	482552.5	482552.5
Net cash flow	-1157552.5	304947.5	361197.5	417447.5	529947.5	586197.5
DR @10%	-1157552.5	277225	328361.4	379497.7	481770.5	532906.8
NPV	310,914.5					
IRR	19%					

DR=Discounted rate; NPV=Net present value; IRR= Internal rate of return; All the values are in CAD

Table 6.5 Net present value and internal rate of return for red lentil (0.5 t/day)

Year	**0**	**1**	**2**	**3**	**4**	**5**
Cash inflow	0	787500	843750	900000	1012500	1068750
Cash outflow	1139177.5	464177.5	464177.5	464177.5	464177.5	464177.5
Net Cash Flow	-1139177.5	323322.5	379572.5	435822.5	548322.5	604572.5
DR @10%	-1139177.5	293929.6	345065.9	396202.3	498475	549611.4
NPV	392,612.90					
IRR	21%					

DR= Discounted rate; NPV=Net present value; IRR= Internal rate of return; All the values are in CAD

6.6 Conclusions

Extrusion technology has been used extensively in the production of breakfast cereals, snacks and other expanded food products. The technoeconomic analysis of the production of green and red lentil extrudate was performed and it was observed that at same capital cost, the total production cost of green lentil was 4.1% higher than red lentil. The difference in production cost was due to difference in the cost of raw material. At the same selling price, BEP can be achieved at 1058.7 tonne for green lentil whereas 949.3 tonne for red lentil extrudates or BEP would be achieved in about 26.5 weeks (about 6.6 months) for green lentil type whereas about 23.7 weeks (about 5.9 months) for red lentil type. Considering lower BEP of the analysis, red lentil was more profitable assuming the same selling price equal and similar texture and nutritional value. Both NPV and IRR for red lentils were higher than green lentil type, CAD $ 392,612.9 and 21% respectively. Extrusion of germinated lentil flours with corn flour may be a viable option for small-scale industries.

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7. GENERAL DISCUSSION AND CONCLUSIONS

Lentil (*Lens culinaris* Medikus) is an inexpensive source of protein, carbohydrate, vitamin, minerals and dietary fibre, but it also contain antinutritional compounds. Germination of seeds can improve nutritive value, and reduce or remove anti-nutritional factors (Vidal-Valverde et al. 2002). Apart from reduced level of antinutritional compounds, germinated seed shows significant increase in the phenolic antioxidant content of lentil when compared to non-germinated seeds. While lentil output has increased significantly globally, some countries still consume relatively little of it. By blending lentil flours in the optimum amounts for pasta, breakfast snacks, confections, etc., it can be used more effectively. To improve starch digestibility and antioxidant activity, an extruded product produced with germinated lentils can also be attempted (Nongmaithem and Meda (2016)).

In the first stage of this research (Chapter 2), the effect of germination on total phenolic compound (TPC), total antioxidant activity (TAA) and *in vitro* starch Digestibility (SD) of green lentil (CDC Greenland) and red lentil (CDC Maxim) were studied. The TPC, TAA and *in vitro* SD increases significantly ($p<0.05$) with days of germination, regardless of the lentil types. The findings are similar to the results reported by Gharachorloo et al. (2013), where all samples (including lentil) had higher phenolic contents as compared to non-germinated samples. The highest level of TPC and TAA was observed for 5 day germinated lentil. There are reports of positive relationships between phenolics and antioxidant activity in lentil (Cevallos-Casals & Cisneros-Zevallos 2010; Świeca 2015). The highest increment in SD was observed on 6 day germination, however the increment was much less after 5 day of germination. Comparable findings can be observed in Swieca et al. (2013), during the germination of lentils at various conditions. Therefore, based on the highest TPC and TAA, 5 days of germination was selected for further studies and utilization.

In the second stage of this research (Chapter 3), the effect of microwave and vacuum on the drying kinetics of pulsed mode microwave-vacuum drying (MVD) of germinated lentil was studied. The pulsed-mode, microwave-vacuum technique for 2 s, 5 s and 8 s of 2000 W microwave power at 0, 15 and 45 kPa vacuum pressure was employed for drying both germinated lentil types. MVD had less drying time as compare to conventional hot air (HA) drying. This study supports the theory that the ionic polarization and dipole movement mechanism during microwave drying reduce the drying time as compared to HA drying system. Among the thin-layer drying models studied, the Modified page model II showed the highest R^2 and lowest RMSE values. The drying rate constant k and effective moisture diffusivity (D_{eff}) increased with an increase in microwave pulse and vacuum pressure level, resulting in a reduction in drying time. The increase in vacuum pressure level lowers the partial pressure of water inside the drying chamber, and allows the water to evaporate at lower temperate and at faster rate. This reduction in drying time reduced energy consumption in MVD as compared to conventional HA drying. The study suggests that MVD is an energy efficient and promising technique in drying of germinated lentil.

In the third stage of this research (Chapter 4), optimization of the processing parameters for pulse-mode, microwave-vacuum drying (MVD) was conducted based on total phenolic content (TPC), total antioxidant activity (TAA) and *in vitro* starch digestibility (SD) for germinated green and red lentil (CDC Greenland and CDC Maxim). The lentils were germinated for 5 days and dried using the pulse-mode microwave-vacuum method. The results indicate that there was a reduction in TPC and TAA during MVD of both germinated lentil type. This reduction may be due to loss of volatile phenolic compounds. *In vitro* starch digestibility increased significantly with increased microwave pulse level. The vacuum pressure levels did not significantly ($p>0.05$) affect any responses. Optimum microwave power for pulse-mode MVD was 8 s and 5.5 s for green lentil and red lentil respectively, and at vacuum pressure level at 45 kPa and 42.19 kPa for green and red lentil types, respectively. The analysis supports the theory that Microwave-vacuum drying has a great potential in drying of germinated lentils.

In the fourth stage of this research (Chapter 5), formulations of germinated green and red lentil were extruded in blends with corn flour and investigated to determine an optimum process parameter to produce extrudates of germinated lentil and corn flour blend. The optimum process parameters were selected based on expansion ratio (ER), extrudate density (ED), hardness (H), water absorption index (WAI), water soluble index

(WSI), total phenolics content (TPC), total antioxidant activity (TAA) and *in vitro* starch digestibility (SD). The results indicate that changes in ER affected ED, H, WAI amd WSI. The data supports the theory that due to increase expansion the density and hardness of the extrudate decreases. Also, the WAI and WSI increases with an increased in ER due increase in porosity and surface area for interaction between water and extrudate. The TPC and TAA increases with increase in blend of germinated lentil flour. This demonstrate a correlation between the higher phenolic content of germinated lentil flour over the corn flour. The *in vitro* starch digestibility (SD) decreases with increase in blend of germinated lentil flour. This result suggest that the corn flour starch had more SD as compared to lentil flour starch. Optimum process parameters were identified as 20% lentil blend, 19.5% moisture and 147 rpm screw speed for germinated green lentil extrudates, and 20.7% of lentil in the blend, 19.7% moisture and 141 rpm screw speed for germinated red lentil extrudates. Also, the extrusion of germinated lentil extrudate blended with corn flour can be used as snacks or breakfast cereals.

In the last stage of this research (Chapter 6), technoeconomic analysis was conducted for the production of germinated lentil based extruded product. Three scenarios were assumed i.e 0.5 tonne/h, 1 tonne/h and 5 tonne/h production of extrudate, and break-even point (BEP) was determined. All the assumptions during calculation was based on cost of the equipment used during production. The engineering and construction cost incurred was highest while calculating total capital cost. Considering all the findings, the extrudate produced with germinated red lentil type was more profitable as the BEP was lower than green lentil type. Due to lower BEP, the time required to achieve a point where net investment is equal to net revenue is shorten. Red lentil type had higher net present value (NPV) and internal rate of return (IRR). This result is due the lower cost of red lentil in current market.

Germination increases the total phenolic content, total antioxidant acticity and starch digestibility. Microwave vacuum drying reduces the drying time and reduce energy consumption as compared to convention hot air drying. Extrusion increases the starch digestibility of the germinated lentil flour. Hence, Germination followed by microwave vacuum drying and extrusion showed a promising process for utilization of lentil.

Overall, red lentil was more promising in producing extrudates from germinated lentil as breakfast cereal or snacks, from economic perspective.

8. RECOMMENDATIONS AND FUTURE STUDIES

As lentil production is on increase, gaining further foundation knowledge on processing of lentils for further utilization is of utmost importance. Utilization of lentil is limited to few regions due to its composition and aged old consumption style (sold as a whole seed). The lentils can be utilized as an ingredient to our daily consumed food products. The knowledge on physic-chemical and *in vitro* properties of lentils, along with market strategy and economics would provide a great information to the decision makers in food industry. The addition of an ingredient in our current food system will provide a wide range of research in production of new food product.

Building on from the current study, various lentil types could be explored as a way to find the most suitable types for production of lentil-based extruded product. The germination method employed was limited to certain processing conditions, this could be explore to find the optimal condition to produce highest phenolic content and antioxidant activity. For instance, a) a wider consideration of germination temperature b) elicitation with different chemical compounds during germination, and c) different pretreatment conditions before germination of lentil types.

In terms of drying, although microwave vacuum drying was a great choice over conventional hot air drying, the levels of processing parameters and mode of application can be studies. Other drying methods such as freeze drying (if applicable for commercial production) can also be studied. In the current study, microwave power was limited to 2000 W, a wider range of microwave power would provide a better understanding of drying kinetics and energy consumption. The chemical analysis was limited to total phenolic content and total antioxidant. For better understanding of nutritional profile, different chemical components such as a) total flavonoid contain b) total tannin content c) changes in different profiles of protein and starch could be studied. The *in vitro* studies of protein will give clearer picture of protein utilization by our body for germinated lentils.

During extrusion, the die temperature was fixed, and level of moisture did not have much impact on physic-chemical properties studied. The methodological choices were constrained by literature available; however the scope of the study can be increased by widening the level of parameters and types of flour considered. For instance, a) use of wheat flour b) increasing the moisture level c) different barrel and die temperature. And finally, storage study of lentil-based germinated lentil extrudate could be conducted to investigate the shelf-life of the product.

The technoeconomic analysis was conducted based on the assumption that cost of equipment increases proportionally with increase in capacity. This analysis can be further explored with the assumption that the total capital cost (including cost of equipment) remains same with varying in production capacity viz. 30% capacity, 50% capacity, 80% capacity and 100% of the equipment used.

Appendix I

Table 9.1 ANOVA table of expansion ratio response during extrusion of green lentil.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	9.11	9	1.01	215.19	< 0.0001	significant
A-Blend	0.8944	1	0.8944	190.05	< 0.0001	
B-Moisture	1.09	1	1.09	231.50	< 0.0001	
C-Screw speed	0.1487	1	0.1487	31.59	0.0002	
AB	0.1485	1	0.1485	31.56	0.0002	
AC	0.0210	1	0.0210	4.46	0.0607	
BC	0.0171	1	0.0171	3.64	0.0856	
A^2	2.76	1	2.76	586.54	< 0.0001	
B^2	3.60	1	3.60	764.92	< 0.0001	
C^2	1.72	1	1.72	366.10	< 0.0001	
Residual	0.0471	10	0.0047			
Lack of Fit	0.0390	5	0.0078	4.82	0.0546	not significant
Pure Error	0.0081	5	0.0016			
Cor Total	9.16	19				

A=blend ration (%), B=moisture (%) and C=screw speed (rpm)

Table 9.2 ANOVA table of water absorption ratio response during extrusion of green lentil.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	17.74	9	1.97	109.74	< 0.0001	significant
A-Blend	3.12	1	3.12	173.95	< 0.0001	
B-Moisture	3.04	1	3.04	168.97	< 0.0001	
C-Screw speed	0.3292	1	0.3292	18.33	0.0016	
AB	0.0001	1	0.0001	0.0063	0.9385	
AC	0.1431	1	0.1431	7.97	0.0181	
BC	0.0066	1	0.0066	0.3681	0.5576	
A^2	3.76	1	3.76	209.18	< 0.0001	
B^2	6.99	1	6.99	389.31	< 0.0001	
C^2	2.34	1	2.34	130.19	< 0.0001	
Residual	0.1796	10	0.0180			
Lack of Fit	0.1487	5	0.0297	4.80	0.0550	not significant
Pure Error	0.0309	5	0.0062			
Cor Total	17.92	19				

A=blend ration (%), B=moisture (%) and C=screw speed (rpm)

Table 9.3 ANOVA table of starch digestibility response during extrusion of green lentil.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	4.86	9	0.5403	4.75	0.0115	significant
A-Blend	3.78	1	3.78	33.28	0.0002	
B-Moisture	0.2250	1	0.2250	1.98	0.1899	
C-Screw speed	0.0065	1	0.0065	0.0567	0.8165	
AB	0.1625	1	0.1625	1.43	0.2596	
AC	0.0220	1	0.0220	0.1939	0.6691	
BC	0.0800	1	0.0800	0.7035	0.4212	
A^2	0.3163	1	0.3163	2.78	0.1263	
B^2	0.0768	1	0.0768	0.6757	0.4302	
C^2	0.1324	1	0.1324	1.16	0.3060	
Residual	1.14	10	0.1137			
Lack of Fit	0.9457	5	0.1891	4.94	0.0522	not significant
Pure Error	0.1915	5	0.0383			
Cor Total	6.00	19				

A=blend ration (%), B=moisture (%) and C=screw speed (rpm)

Table 9.4 ANOVA table of expansion ratio response during extrusion of red lentil.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	9.41	9	1.05	1311.68	< 0.0001	significant
A-Blend	1.44	1	1.44	1800.01	< 0.0001	
B-Moisture	0.9401	1	0.9401	1178.83	< 0.0001	
C-Screw speed	0.0942	1	0.0942	118.09	< 0.0001	
AB	0.1201	1	0.1201	150.53	< 0.0001	
AC	0.0113	1	0.0113	14.11	0.0037	
BC	0.0578	1	0.0578	72.48	< 0.0001	
A^2	1.75	1	1.75	2198.67	< 0.0001	
B^2	4.26	1	4.26	5338.30	< 0.0001	
C^2	1.97	1	1.97	2474.62	< 0.0001	
Residual	0.0080	10	0.0008			
Lack of Fit	0.0062	5	0.0012	3.60	0.0930	not significant
Pure Error	0.0017	5	0.0003			
Cor Total	9.42	19				

A=blend ration (%), B=moisture (%) and C=screw speed (rpm)

Table 9.5 ANOVA table of water absorption index response during extrusion of red lentil.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	17.36	9	1.93	223.65	< 0.0001	significant
A-Blend	2.95	1	2.95	342.15	< 0.0001	
B-Moisture	3.22	1	3.22	373.71	< 0.0001	
C-Screw speed	0.2588	1	0.2588	30.00	0.0003	
AB	0.0002	1	0.0002	0.0232	0.8820	
AC	0.1152	1	0.1152	13.36	0.0044	
BC	0.0008	1	0.0008	0.0927	0.7670	
A^2	3.20	1	3.20	370.42	< 0.0001	
B^2	7.08	1	7.08	821.11	< 0.0001	
C^2	2.45	1	2.45	284.37	< 0.0001	
Residual	0.0863	10	0.0086			
Lack of Fit	0.0622	5	0.0124	2.58	0.1606	not significant
Pure Error	0.0241	5	0.0048			
Cor Total	17.45	19				

A=blend ration (%), B=moisture (%) and C=screw speed (rpm)

Table 9.6 ANOVA table of starch digestibility response during extrusion of red lentil.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3.00	9	0.3338	223.10	< 0.0001	significant
A-Blend	1.41	1	1.41	944.31	< 0.0001	
B-Moisture	0.2125	1	0.2125	142.02	< 0.0001	
C-Screw speed	0.0824	1	0.0824	55.08	< 0.0001	
AB	0.3916	1	0.3916	261.73	< 0.0001	
AC	0.0078	1	0.0078	5.22	0.0454	
BC	0.0120	1	0.0120	8.03	0.0177	
A^2	0.6389	1	0.6389	427.02	< 0.0001	
B^2	0.2152	1	0.2152	143.83	< 0.0001	
C^2	0.0376	1	0.0376	25.13	0.0005	
Residual	0.0150	10	0.0015			
Lack of Fit	0.0120	5	0.0024	4.07	0.0747	not significant
Pure Error	0.0029	5	0.0006			
Cor Total	3.02	19				

A=blend ration (%), B=moisture (%) and C=screw speed (rpm)

Appendix II

Table 10.1 References of prices assumed during Technoeconomic analysis

Microwave Vacuum dryer	https://www.nicolemachine.com/sale-31978589-ss304-food-grade-microwave-vacuum-dryer-220-440v-low-temperature.html
Hammer mill	https://pleasanthillgrain.com/commercial-hammer-mill-for-sale
Blender	https://fair888.en.made-in-china.com/product/hxrpvYBcamUO/China-Powder-Chemical-Food-Spiral-Horizontal-Ribbon-Mixer-Machine-Blender-Mixer.html
Extruder	https://nicolemachine.en.made-in-china.com/product/uwyfrcsjGCYO/China-Industrial-Puffed-Snack-Corn-Making-Machine-Food-Production-Line-Extruder-for-Sale.html
Packaging machine	https://www.alibaba.com/product-detail/Automatic-puffed-food-weighting-vertical-ffs_1600901199066.html?spm=a2700.7724857.0.0.75cd3169uvOYmv
Conveyor belt	https://theconveyorshop.co.uk/RCL-Conveyor-belt-1000mm-x-10m
Storage	https://www.alibaba.com/showroom/soybean-silos-cost.html
Enginnering/construction cost	http://economicdevelopmentbrandon.com/commercial-construction-costs
Water	https://www.saskatoon.ca/services-residents/power-water-sewer/drinking-water/water-wastewater-utility-rates
electricity	https://www.statista.com/statistics/516279/electricity-costs-for-end-users-canada-by-province/#:~:text=End%2Dusers%20in%20Canada%20face,stands%20at%2041%20Canadian%20cents
lentil	https://dashboard.saskatchewan.ca/agriculture/grain-and-specialty-crop-prices/lentils#lentils-small-redlessbrgreater(dollar-per-cwt)-tab
corn	https://bulkmart.ca/products/click-yellow-corn-flour-600-5-kg

	https://umamishop.ca/products/corn-flour-yellow
Breakfast cereal price	https://www.indexbox.io/search/breakfast-cereal-price-canada

*accessed on January, 2024

Table 10.2 Table of break-even point analysis for green lentil extrudate

Lentil (Tonne)	Net fixed cost CAD $	Variable cost CAD $	Total cost CAD $	Net revenue CAD $
0	1.350636	0	1.350636	0
500	1.350636	1.612118	2.962754	2.25
1058.687	1.350636	3.413456	4.764092	4.764092
1000	1.350636	3.224235	4.574871	4.5
1500	1.350636	4.836353	6.186989	6.75
2000	1.350636	6.44847	7.799106	9
3000	1.350636	9.672705	11.02334	13.5
3500	1.350636	11.28482	12.63546	15.75

CAD $ in million

Table 10.3 Table of break-even point analysis for red lentil extrudate

Lentil (Tonne)	Net fixed cost CAD $	Variable cost CAD $	Total cost CAD $	Net revenue CAD $
0	1.350636	0	1.350636	0
500	1.350636	1.538618	2.889254	2.25
949.3	1.350636	2.921219	4.271855	4.27185
1000	1.350636	3.077235	4.427871	4.5
1500	1.350636	4.615853	5.966489	6.75
2000	1.350636	6.15447	7.505106	9
3000	1.350636	9.231705	10.58234	13.5
3500	1.350636	10.77032	12.12096	15.75

CAD $ in million

Appendix III

Figure 11.1 CDC Greenland and CDC Maxim lentil types

Figure 11.2 Freeze dried germinated green lentils.

Figure 11.3 Freeze dried germinated red lentils

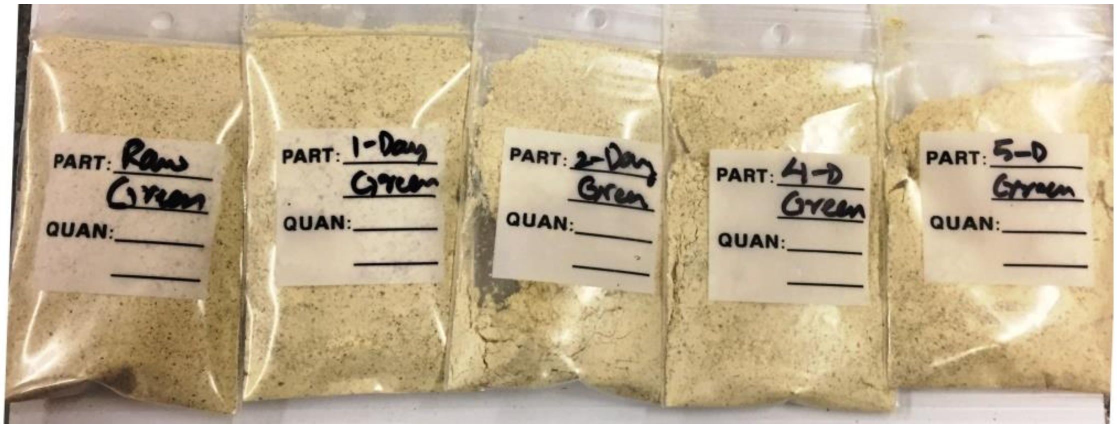

Figure 11.4 Ground germinated green lentils

Figure 11.5 Ground germinated red lentils


Extrudate of 100% corn
Extrudate of optimized green lentil
Extrudate of optimized red lentil

Figure 11.6 Extrudate product of germinated lentils

Microwave Vacuum dryer

Perforated polypropylene cylindrical container

Set up of perforated polypropylene cylindrical container inside microwave vacuum dryer

Figure 11.7 Microwave vacuum drying system