

EXPLORING EXTRUSION TECHNOLOGY IN DEVELOPING VALUE-ADDED
PRODUCTS FROM MONTANA LENTILS

by

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DEDICATION

To my family, to my girlfriend Gifty Osei, to my dear friends Ralph Klake and David Sewell, to my lab mates, to my PhD committee, to my professors, to my mentors, to my industry collaborations, to my friends and to the GOAT advisor Dr. Wan-Yuan Kuo, my heart beats loudly with echoes of gratitude. We did it!

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ABSTRACT

This study seeks to demonstrate that lentil flour and its fractionates are viable ingredients for use in the development of lentil puffs, lentil couscous and lentil texturized vegetable protein with a twin-screw pilot-scale extruder (Buhler-30 mm). Regarding the development of lentil puffs, an experimental design with three formulation factors of lentil flour (x1), lentil starch concentrate (x2) and lentil protein concentrate (x3) was created. The overall acceptance and texture liking score of lentil puff formulations was found to be dependent on all three formulation factors. An optimum lentil puff formulation was therefore predicted at a 50% ratio of lentil starch concentrate to 25% ratios of both lentil flour and lentil protein concentrate to be produced at an extrusion feed rate of 20 kg/h, a moisture rate of 10% and a screw speed of 350 rpm.

For the development of lentil couscous, an experimental design with factors of the ratio of lentil protein concentrate to lentil flour (x1) and the extruder screw speed (x2) was created. Increasing the ratio of lentil protein concentrate to lentil flour decreased the water absorption index, cohesiveness, chewiness, and resilience texture of lentil couscous formulations. Higher sensory acceptance, appearance, flavor and texture scores for lentil couscous samples were observed for formulations with lentil protein concentrate levels at 20% and a screw speed of 250 rpm. Clustering sensory results revealed that participants who issued higher sensory score were willing to buy the presented lentil couscous at \$3.37 for 10 oz of lentil couscous with lentil protein concentrate levels at 20% and produced at a screw speed of 250 rpm.

The development of a lentil TVP with lentil protein concentrate revealed that the techno functional properties of lentil TVPs were dependent on the total protein content, extrusion conditions and pH. Adding pea protein isolate to lentil protein concentrate in formulations increased the protein content to levels ideal for texturization. Increasing the extrusion dry feed rate and screw speed also increased the degree of texturization of ingredient mixes. Despite exploring the addition of gums, salts, transglutaminase as well as cross-linking agents, a change in pH, with additives of citric acid and calcium hydroxide, was the only strategy observed to influence the techno functional properties of lentil TVP samples. In comparison to the reference soy TVP produced from soy protein concentrate, lentil TVP samples produced from trials with higher contents of pea protein isolate ($\geq 30\%$, w/w, d.b. in the dry feed) presented higher instrumental springiness, cohesiveness, and chewiness texture.

CHAPTER ONE

INTRODUCTION

The global food extrusion market is projected to reach \$99.7 billion, with a compound annual growth rate of 6.4% by 2026 (Markets and Markets, 2021). The high versatility for product development and the high throughput of extrusion are the main reasons behind the promising market. Food extrusion according to Berrios et al., (2022) is described as a mechanical process used to create products of desired dimensions and texture by forcing mixed flour ingredients (starchy or proteinaceous) through an opening. Occurring at a high temperature-short time through a combination of heat, pressure and mechanical shear, food extrusion leads to molecular interactions and chemical changes that can modify the nutritional, physiochemical and functional properties in the final product (Ek et al., 2021; Morales et al., 2015; Yadav, 2022). Commercial products like pasta, puffed snacks, breakfast cereals, biscuits, crackers, baby foods, snack foods, alternative meat, confectioneries, and pet foods can be made through extrusion with high manufacturing yield and quality consistency (Berrios et al., 2022; T. Kim, Miller, et al., 2021; Morales et al., 2015).

Traditionally, cereal and grains with high starch contents (Jatav & Bhatt, 2020) or soy and whey with high protein contents (Riaz, 2011) have been used in developing extruded products. Cereals and grains as ingredients are however lower sources of protein (Jatav & Bhatt, 2020), soy and whey are known allergens (USDA, 2023), and cereals like wheat contain gluten which causes metabolic problems for certain individuals (I. S. Dogan et al., 2015). Additionally, the modern cultivation of cereals and grains as well as soy typically utilizes intensive land cropping and farming practices damaging to the environment (Rabès et al., 2020) which are also

concerns for the modern consumer. The increasing appreciation and cultivation of pulses which are more nutritious and environmentally sustainable (Warne et al., 2019) than traditional cereals have resulted in research (Berrios et al., 2022; Ek et al., 2021; Pasqualone et al., 2020) on the creation of extruded value-added pulse products (VAPPs). Value-added pulse products created from food processing operations reduce cooking constraints (Pasqualone et al., 2020) and provide products that have shorter cooking times and/or are ready-to-eat (Bresciani et al., 2021; Lazou et al., 2010), which can encourage pulse consumption by US consumers.

Pulse crops are defined as the dry edible seeds of plants in the Fabaceae or Leguminosae family which include field peas, dry beans, lentils, chickpeas and Faba beans (Asif et al., 2013), and are gluten-free with very few allergens and genetically modified organisms (Kim et al., 2021). Pulses are an excellent source of food to various cultures owing to their nutritional profile including high protein, fiber, and micronutrients (Asif, Rooney, Ali, Riaz, et al., 2013). The beneficial nutrition profile of pulses promotes good health by reducing insulin resistance, aiding weight management, and encouraging proper metabolic functioning (Morales et al., 2015; Singh et al., 2020) as well as preventing heart disease and diabetes (Asif et al., 2013). The nutritive and functional properties of pulses address the concerns of modern food consumers and make pulses a suitable alternative ingredient for food extrusion. Research on pulse extrusion has therefore begun to replace soy and wheat proteins (both allergens) in meat alternatives (Anggraini & Oliver, 2019; T. Kim, Miller, et al., 2021), and refined cereals and grains in pasta and puffed snacks with pulses like lentils (Bresciani et al., 2021; Ek et al., 2021).

Lentils (*Lens culinaris*) are a widely consumed pulse in the Middle East and South Asia (Joshi et al., 2017) with a high fiber content and a low glycemic response ideal for making

nutritious value-added pulse products (M. Singh et al., 2020). Lentils as a pulse are rich in starch, protein, minerals, and fiber, constituting a nutritious alternative to cereal and grain ingredients in food extrusion (Asif, Rooney, Ali, Riaz, et al., 2013). Despite not being recognized and consumed as much as chickpeas, lentils have 3% fat content compared to chickpeas with 7% (Bresciani et al., 2021), making lentils more preferred in creating extruded VAPPs as high-fat materials reduce friction and heat generation needed for gelatinization, expansion and texturization in extrusion (Moreno et al., 2018). Lentil flour has therefore been used in the development of products like puffed snacks (Ek et al., 2021; Lazou et al., 2010) and pasta (Bresciani et al., 2021) which sell for premium prices on the market as VAPPs (S.-H. Kim & Kuo, 2022).

Nevertheless, the pulse industry in the US is not fully benefitting from the market for VAPPs and 75% of the 2.6 million pounds of lentils harvested each year are exported without processing (Asif et al., 2013). Montana is the top producer of lentils in the US (USDA, 2021), however, only 1% of Montana farms are adding value to raw commodities (C. Lee, 2011). An estimate in 2010 by Lee (2011) revealed that the Montana pulse industry may have lost an annual \$15 million in profit from not processing and adding value to pulses. An extruder allows the development and manufacture of various products (Rizvi et al., 1995) and can adapt to the production and innovation needs based on the nature of different specialty crops. Creating extruded lentil products could generate additional income for Montana's agriculture industry.

Albeit the potential of lentils in the development of extruded products, refined grain, and cereal flours still dominate in extrusion because of their higher starch content which results in a crispy or crunchy snack product (Jatav & Bhatt, 2020a; Lazou et al., 2010) or presence of gluten

resulting in firmer and more cohesive pasta product (da Silva et al., 2016). Soy, wheat, and whey proteins are also preferred in the development of extruded texturized products due to their higher content of sulfur-containing amino acids which can crosslink to create meat-like fibrous texture (Riaz, 2011; Taylor & Walsh, 2002; Yeater et al., 2017). Creating products from lentil flour with commercial quality has typically required blending with high starch ingredients like corn starch (for puff and pasta products) (Dogan et al., 2013) or high protein ingredients like pea protein isolate (for texturized vegetable protein) (Li et al., 2022; Pasqualone et al., 2020) which add to the ingredient list. The recent introduction of lentil starch concentrate and lentil protein concentrate presents an opportunity to create protein-rich value-added pulse products from a single food ingredient.

Lentil starch concentrate and protein concentrate can be obtained from the mechanical separation of lentil flour (González & Pérez, 2002) and possess functional qualities comparable to traditional high starch and protein flours used in industry (Jarpa-Parra, 2018; Joshi et al., 2017). Lentil starch concentrate with its 70% starch content (Joshi et al., 2017) is especially suitable for ready-to-eat products and is currently being introduced in the production of pasta and breakfast cereals (Ren et al., 2021). Lentil protein concentrate with 50-55% protein content replaces animal and milk protein in baking cakes, soy protein in soy milk and tofu (Jarpa-Parra, 2018).

Research has been done on the extrusion of lentil flour in making VAPPs (Bresciani et al., 2021; Ek et al., 2021; Li et al., 2022), however, there are no published studies on the combination of lentil flour and its fractionates in developing extruded products. Evaluating the function and quality of lentil flour and its fractionates (lentil starch concentrate and lentil protein

concentrate) in creating extruded VAPPs can promote value addition to the abundant lentils produced in the US. From the extrusion literature reviewed (Anggraini & Oliver, 2019; Ek et al., 2021; Moussa et al., 2022), three products, representing the three common categories of extruded products, puffs (puffed snacks), couscous (compound/pressed products), and TVP (texturized vegetable protein) would showcase the extrusion potential of lentil flour, lentil starch concentrate and lentil protein concentrate.

The objectives of this study are therefore to examine the use of a twin-screw pilot scale extruder to (1) develop extruded lentil puffs from varying levels of lentil flour, lentil starch concentrate, and lentil protein concentrate, (2) create a 100% lentil couscous from lentil flour and lentil protein concentrate to, and (3) texturize lentil protein concentrate.

CHAPTER TWO

EXPLORING THE USE OF LENTIL FLOUR, LENTIL STARCH CONCENTRATE, AND LENTIL PROTEIN CONCENTRATE IN CREATING EXTRUDED LENTIL PUFFS.

Contribution of Authors and Co-Authors

Manuscript in Chapter [2]

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Contribution: Edwin Allan developed the recipes and process parameters for all lentil puff formulations, performed the analysis, interpreted results, and wrote the manuscript.

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Contribution: Dr. Wan-Yuan Kuo provided expertise in recipe development and analysis and edited the manuscript, figures, and tables.

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Abstract

Lentil puffs were developed from varying ratios of lentil flour, lentil starch concentrate, and lentil protein concentrate using a twin-screw pilot scale extruder at a dry feed rate of 20 kg/h, a water feed rate of 2kg/h and an extruder screw speed of 350 rpm. A thorough evaluation of the extrudate properties (expansion ratio, unit density), instrumental texture (hardness, crunchiness, and crispiness), and sensory properties (overall acceptance, check all that apply and just about right) were conducted to determine the optimum lentil puff formulation. Formulations with higher lentil flour and lentil starch concentrate presented lower bulk densities and hardness texture but exhibited higher expansion ratios and crispiness texture. Formulations with higher lentil protein concentrate exhibited lower expansion ratios and crispiness texture but higher bulk densities, hardness, and crunchiness texture. Extreme ratios of lentil protein concentrate resulted in reduced sensory scores whilst higher ratios of lentil starch concentrate were observed to increase sensory scores. Just about right tests revealed that all lentil puff formulations were penalized for not having the right level of hardness texture whilst the crispy texture attribute was identified in check all that apply tests as essential to increase overall acceptance and texture scores. The optimum lentil puff formulation was therefore predicted from a maximum overall acceptance score of 6.03 and texture score of 6.70, to be a 50% ratio of lentil starch concentrate to 25% ratios of both lentil flour and lentil protein concentrate to provide a high crispiness texture of 205.24 Ns.

Introduction

In the realm of food technology research, there is a growing global interest among consumers in obtaining high-quality plant-based protein (Nosworthy et al., 2018). This heightened focus is directing attention towards pulses as a nutritious dietary option. Pulse seeds, being devoid of gluten, boast substantial levels of protein, fiber, essential micronutrients, and slow-digesting carbohydrates (Kim et al., 2021; Singh et al., 2020). The nutrient-dense nature of pulses may be beneficial to health by reducing insulin resistance, aiding weight management, and encouraging proper metabolic functioning (Asif, Rooney, Ali, Riaz, et al., 2013; Fujiwara et al., 2017) as well as preventing heart disease and diabetes (Asif, Rooney, Ali, Riaz, et al., 2013). Nonetheless, it is noteworthy that in the United States, fewer than 5% of adults incorporate pulses into their daily diets (Perera et al., 2020). This low consumption of pulses in the U.S. (S.-H. Kim & Kuo, 2022; Perera et al., 2020; Warne et al., 2019) can mainly be attributed to longer cooking times, unpleasant flavor, poor protein digestibility, and intestinal discomfort following consumption (Joshi et al., 2017).

In order to encourage pulse consumption by US consumers, researchers (Bresciani et al., 2021; Lazou et al., 2010; Morales et al., 2015) are employing extrusion processing to develop value-added pulse products that feature shorter cooking times and/or are convenient as ready-to-eat options (Bresciani et al., 2021; Lazou et al., 2010). Extrusion cooking is a thermal food operation that can convert milled food ingredients into ready-to-eat foods such as puffed snacks and breakfast cereals (Berrios et al., 2022; Pasqualone et al., 2020). Extrusion stands out as an effective method for pulse processing because the elevated temperature, pressure, and

mechanical shear reduce the cooking time, and deactivates undesirable compounds. This leads to improved nutrition, flavor and texture of the final extruded pulse product (Berrios et al., 2022)

Refined grain and cereal flours are predominantly used for snack food extrusion because of their high starch content which yields a crispy or crunchy product (Ek et al., 2021a). Over the last decade, puffed snacks made from extrusion cooking have included lentil flour in ingredient formulations. This is driven by lentil flour's noteworthy attributes of high protein, fiber content, and a low glycemic index to create nutritionally enhanced products (Li et al., 2022; Romano et al., 2021; Wilson et al., 2022). Lentils as a widely cultivated pulse in the US (C. Lee, 2011), are in consequence ideal for making healthier versions of noodles, pasta, breakfast cereal, puffed snacks, and baked products (Ek et al., 2021).

While lentils are an excellent choice for creating more nutritious food products, relying solely on lentil flour as an ingredient can pose some challenges due to its lower starch content and higher levels of fiber and protein, which may hinder the desired expansion during puffed snack extrusion (Kaisangsri et al., 2016). As a solution, lentil flour is usually combined with corn, potato and tapioca flours all of which contain higher starch levels than lentils. This blending strategy bolsters the starch content necessary for achieving the desired expansion (Dogan et al., 2013; Li et al., 2022). To compensate for the dilution of protein by starch in the puff formulation, rice or pea proteins are commonly added to comply with the regulations on protein content labeling claims (FDA, n.d.).

Starch and protein concentrate have recently been developed from the physical separation of lentil flour (González & Pérez, 2002) and hence can be applied in lentil puff extrusion. These lentil-based starch and protein concentrates (González & Pérez, 2002) possess unique functional

and nutritional qualities comparable to traditional cereal and grain flours. Notably, lentil starch concentrate with its 70% starch content (Joshi et al., 2017) is especially suitable as an alternative to ready-to-eat products made from cereal starches (Manickavasagan & Thirunathan, 2020). The advent of lentil starch and protein concentrate presents a promising opportunity to create protein-rich lentil puffed snacks all derived from a single food ingredient.

Despite the progress made in lentil research, there is an absence of published studies on the use of lentil starch and lentil protein concentrate in puffed snack extrusion. Therefore, the objectives of this study are twofold (1) evaluate the feasibility of lentil starch and lentil protein concentrate in making protein-rich extruded snack puffs with lentil flour (2) determine the physical properties (bulk density, expansion ratio), nutrition profile, texture properties, and consumer sensory acceptance of the protein enriched extruded lentil puffs.

Hypothesis:

Increasing the ratio of lentil starch concentrate to lentil flour blend (lentil flour, lentil starch, and lentil protein concentrate) is predicted to increase the expansion ratio, instrumental crispiness and the overall liking to where the crispiness is perceived to be just about right whilst decreasing extrudate density.

Increasing the ratio of lentil protein concentrate to lentil flour blend (lentil flour, lentil starch, and lentil protein concentrate) is expected to increase the extrudate density, instrumental hardness, instrumental crunchiness, and the overall liking to where the hardness and crunchiness are perceived to be just about right, whilst decreasing the expansion ratio of lentil puffs.

Materials and Methods

Materials

Organic Richlea lentil flour obtained from milled dehulled lentils, lentil starch concentrate, and lentil protein concentrate obtained from the mechanical separation of milled lentil flour were purchased from Montana Pure Protein (Billings, MT, USA). Formulations were developed from Richlea lentil flour, lentil starch concentrate, and lentil protein concentrate using a simplex centroid mixed design (Table 2.1) and were blended using 40 quartz planetary floor mixer (Avantco, Idaho), Corn meal was purchased from Martha White Yellow Corn Meal (Jackson, TN, USA) to make corn puffs for comparison with the newly developed lentil puff formulations.

Proximate Analysis Richlea lentil flour, Richlea lentil starch and Richlea lentil protein were sent to Nestle Purina Analytical Laboratories (Nestle Purina PetCare, St. Louis, Missouri, USA) for chemical analyses. This analysis encompassed the assessment of various parameters, including ash, crude fat, crude fiber, crude protein, moisture, fatty acid profile (linoleic acid, capric acid, caprylic acid). The measurements adhered to established protocols outlined in the Association of Official Agricultural Chemists (AOAC) Methods 942.05, 922.06, 962.09, 990.03, 930.15, and 996.06, respectively.

Methods

Mixed Model Design for Lentil Puff Formulations Preliminary trials were conducted to choose suitable ranges of formulations for making the lentil puffs. We employed a simplex-centroid mixed model design using RS Version 1.2.1335 (RStudio Inc., Boston, MA, USA) to evaluate the effects of the three ingredients, lentil flour (X1), lentils starch concentrate (X2), and

lentil protein concentrate (X3), on the physical and sensory properties the lentil puffs. The component proportions were expressed as fractions of the mixture (X1:X2:X3) with a sum of 1. A total of nine combinations of the ingredients were prepared with the central point of the model replicated 3 times (Table 2.1). For each formulation, the extruder was loaded with 10 kg (w.b.) of dry feed (flour blend) with moisture content ranging from 7.55% to 8.70% (Table 2.1).

Extrusion cooking Extrusion cooking was performed with a pilot scale twin-screw extruder (BCTM-30, Buhler Inc., St. Paul, MN) with a screw diameter of 30 mm and a 20L:D process length. A feed rate of 20 kg/h was adjusted for each formulation based on the moisture content to account for differences in water absorption, and a screw speed of 350 rpm was kept constant. A circular extruder die with a diameter of 3.75 mm and a feed moisture level of 10% (w/w) was used. Cutting of the extrudates was performed using a variable-speed die face cutter equipped with three blades. Collection of the extrudates occurred when the system achieved stability, characterized by constant torque and back pressure at the die. Subsequently, the extrudates underwent a 2-hour drying process at room temperature immediately after extrusion. These dried extrudates were then stored in airtight plastic bags at room temperature for subsequent analysis.

Physical Properties The determination of the unit densities for the extruded lentil puffs involved the application of a glass bead displacement method (Ganjyal et al., 2004). Sample of known weight was randomly taken from each formulation and placed in a 50 mL measuring cylinder. Hollow glass beads with a known volume were then added to the 50 mL measuring cylinder. The volume of the sample was calculated from the difference between the total volume of the sample and the glass beads and only the glass beads.

$$\text{Unit Density} = \frac{w_e}{v_t - v_b},$$

where w_e is the weight of extrudate, v_t is the total volume and v_b is the volume of the glass beads. The expansion ratio of extruded lentil puffs was calculated by dividing the extrudate diameter after cooling by the diameter of the die. Ten measurements were performed on each sample using vernier calipers.

Instrumental Texture Texture analysis was conducted on the extruded lentil puffs with the TA.XT Plus C texture analyzer and software (Texture Technologies, La Crescenta, CA) following the procedure outlined by (Technologies, 2020). The texture analyzer was configured with a TA-30c probe and a 50 kg load cell. Each puff was compressed at 1.0 mm/sec at room temperature until reaching 80% strain, after which the probe was returned to starting position. For each batch, six texture measurements were obtained. These measurements encompassed hardness (N), denoting the peak force following the first compression, crunchiness (Ns), signifying the linear distance along the test curve, and crispiness, representing the total count of positive peaks (Li et al., 2022).

Sensory Evaluations Sensory evaluations were performed according to (Arueya et al., 2017) with modifications. The texture (1. Hardness, 2. Crispiness, 3. Crunchiness), and overall acceptance of the extruded lentil puffs were evaluated using a 9-point hedonic scale with extruded corn puffs as a reference. We recruited a total of 120 participants, all of whom were aged 18 years and older and did not have any dietary restrictions related to lentils and corn. These participants were given samples to taste and were asked to rate using a 9-point hedonic scale to express their overall liking, ranging from "Dislike extremely" (1) to "Neither like nor

dislike" (5) and up to "Like extremely" (9). Additionally, participants were asked to rate hardness, crunchiness, and crispiness attributes on a just about right scale (1=Much too little, 3=Just about right, and 5=Much too hard/crunchy/crispy) (Johnson, 2021). Furthermore, a Check-All-That-Apply (CATA) test was administered to the participants. They were presented with a list of terms, derived from a sensory panel, and instructed to select all the terms that they deemed appropriate to describe the texture of the samples (Chávez et al., 2021). The participants were instructed to rinse their palate with room temperature water between samples to ensure a neutral palate.

Statistical Analyses The data in this study were processed using R Version 1.2.1335 (R Core Team., Boston, MA). A multilinear regression model was fit to determine the effects of formulation factors x1, x2 and x3 on the physical properties, instrumental texture and the overall and texture liking of lentil puff formulations. We applied a one-way analysis of variance (ANOVA) to determine the differences in means among different samples, and to the reference corn puffs. A Fisher's least significant difference test ($\alpha=0.05$) was used to compare means. Mean, standard deviation and Pearson's correlation coefficients for the relationships between all properties were calculated using XLSTAT (XLSTAT 2020.1, Addinsoft Inc, New York, NY, USA).

Results and Discussion

Physical Properties

Unit Density Consumer preferences tend to favor extruded snack products characterized by lower unit densities (Ek et al., 2021). The unit density of extruded lentil puff formulations (Figure 2.1) exhibited a range from $0.09 \pm 0.01\text{g/cm}^3$ to $0.51 \pm 0.04\text{g/cm}^3$. Notably, factors x1 (lentil flour) and x2 (lentil starch concentrate) yielded the lowest unit densities whereas factor x3 (lentil protein concentrate) stood out with the highest unit density individually (Table 2.3). Our

finding aligns with the observations made by Devi et al., (2013) who reported an increase in extrudate density with the addition of protein or fiber sources. Increase in density during protein extrusion has been attributed to hydrophobic interactions which cause peptide chains to form tightly packed structural domains at high temperature and are stabilized by disulfide bonds when cooled (Verbeek & van den Berg, 2010). It is worth noting that the higher unit densities observed in formulations 3, 6 and 10 (Table 2.2) can be attributed to higher contents of both protein and fiber in lentil protein concentrate.

In terms of ingredient combinations, it was evident that the interaction of factors x1 and x3, and factors x2 and x3 (Table 2.3) exerted a strong influence on unit density. These interactions between x1 and x3, as well as x2 and x3, were characterized by negative regression coefficients (Table 2.3). This indicates that factor x1 and factor x2 acted antagonistically to factor x3 when combined, resulting in a decrease in unit density (Figure 2.3 and Table 2.2, particularly in formulations 6 and 8). This observation aligns with the findings of Ek et al., (2021), who noted that high starch ingredients tend to reduce unit density. Starch at high proportion restricts the aggregation of protein chains during extrusion, limiting crosslinking and the formation of stable disulfide bonds and resulting in a decrease in density (Verbeek & van den Berg, 2010). Hence, it can be understood that the decline in unit density of factor x3 when interacting with higher-starch ingredients such as x1 or x2 can be attributed to this effect. Furthermore, we observed that a three-way interaction involving all three lentil puff formulation factors x1, x2 and x3 at equal proportions of 33% did not influence the unit density (Table 2.3).

Expansion Ratio The expansion ratio serves as a crucial indicator of the extent to which a sample undergoes puffing as it emerges from the extruder (Asare et al., 2004). The expansion ratio is an important characteristic of snack extrusion (Asare et al., 2004) given that higher expansion ratios are sought-after attributes in extruded snacks (O'Shea et al., 2014). Within our lentil puff formulations, the expansion ratio exhibited a range from 0.53 ± 0.06 to 4.03 ± 0.21 . Notably, factor x3 exhibited the lowest average expansion ratio (Figure 2.2) (Tables 2.2 and 2.3), while factors x1 and x2 demonstrated higher average expansion ratios individually (Tables 2.2 and 2.3). In accordance with the findings of Ek et al., (2021), it is observed that the expansion ratio of extruded snacks increases with higher starch and is inversely proportional to unit density and its influencing factors.

As demonstrated in Figure 2.3, lentil puff formulations characterized by higher ratios of x3 (specifically, formulations 3, 6, and 10), featuring lower starch content and higher protein levels (as outlined in Table 2.1), displayed lower expansion ratios. Conversely, formulations with elevated ratios of x1 (such as 1 and 7) and x2 (including 2, 5, and 9), which had higher starch quantities (as indicated in Table 2.1), exhibited higher expansion ratios. From Table 2.3, our evidence indicates that the expansion ratio of lentil puff formulations was dependent on factor x1 and factor x2 which have higher amounts of starch. Factor x3, as well as the two-way and three-way interactions of factors x1, x2 and x3 were found not to influence the expansion ratio (Table 3).

Overall, our findings suggest that incorporating higher levels of lentil flour and starch concentrate can be advantageous in producing snack products with a more substantial expansion. Additionally, the inclusion of lentil starch concentrate is particularly suitable for ingredient

mixes that include protein sources, facilitating the attainment of the desired expansion.

Interestingly, a three-way interaction between all three lentil puff formulation factors x_1 , x_2 , and x_3 at equal proportions of 33% was observed to not influence the expansion ratio (Table 2.3).

These insights provide valuable guidance for optimizing lentil-based snack formulations.

In Table 2.2, when we compare lentil puff formulations to the reference corn puffs, we observe that formulations 8, 10, 11, and 12 show no significant differences in unit density when compared to corn puffs. On the other hand, formulations 1, 2, 4, 5, 7, and 9 exhibit lower unit densities than corn puffs. It's worth noting that corn is a pivotal ingredient in snack extrusion processes, as highlighted in previous studies (Dogan et al., 2013; Jatav & Bhatt, 2020; Kaisangsri et al., 2016) and for this reason, the extrudate properties serve as an adequate standard for assessing the extruded lentil puff formulations. Notably, when compared to this reference, lentil puff formulations 3 and 6 exhibit a higher mean unit density than corn puffs (Table 2.2).

Regarding the expansion ratio, lentil puff formulations with ratios of factor x_3 of 50% and above (formulations 3, 6, 8, and 10) displayed a lower mean expansion ratio than corn puffs (Table 2.2). Conversely, formulations 4, 11 and 12 had no noticeable differences in expansion ratio with corn puffs whilst formulations 1, 2, 5, 7, and 9 presented higher expansion ratios than corn puffs. As emphasized by Kaisangsri et al., (2016), a lower density and a higher expansion ratio are essential qualities of an extruded snack. Remarkably, nine formulations (1, 2, 4, 5, 7, 9, 10, 11 and 12) showing comparable or superior expansion ratios than corn puffs suggest a promising market potential for extruded lentil puffs.

Instrumental Texture

The desirability and consumer acceptance of extruded snack products are significantly influenced by the texture they possess. The hardness of the lentil puff formulations ranged from $71.91 \pm 15.91\text{N}$ to $290.16 \pm 21.87\text{N}$ with notably similar trends with unit density (Table 2.2). Figure 2.4 shows that akin to unit density, the hardness of lentil puff formulations rose in correlation with an increase in factor x3, while it declined with increasing levels of factor x1 and x2. Notably, lentil puff formulations with higher ratios of factor x3 (Table 2.2: formulations 3,6 and 10) were found to be harder than formulations with higher ratios of factor x1 and x2. The increasing effect of factor x3 on the hardness of lentil puff formulations is attributed to its higher protein and fiber content which act as diluents to the expansion of starch (Allen et al., 2007; Kaisangsri et al., 2016; Moraru & Kokini, 2003).

Despite the superior effect of factor x3 on the hardness of lentil puff formulations, the interaction between factor x2 and x3 presented a negative estimate of the regression coefficient. This suggests that factor x2 has an adverse interaction with factor x3, leading to a reduction in the hardness of lentil puff formulations. Once more, we observe that the influence of factor x3 on extrudate characteristics is attenuated by factor x2, which is consistent with findings reported by (Allen et al., 2007; Kaisangsri et al., 2016) that lowered starch behavior is only observed at greater concentrations of fiber or protein. Interactions between factors x1 and x2, factors x1 and x3 as well as the three-way interaction between all three lentil puff formulation factors were found to have no influence on the hardness texture (Table 2.3).

The crunchiness of lentil puff formulations ranged from 587.38 ± 65.09 to 884.36 ± 80.19 Ns. Figure 2.4 shows the crunchiness of lentil puff formulations was influenced by all three formulation factors. However, we observe that formulations 3 and 10 with higher ratios of factor

x3 (greater than 50%) possess noticeably higher crunchiness levels (Table 2.2) evidenced by x3's higher estimate in Table 2.3. The crunchiness of extruded snacks has been associated with a denser cell structure and less expansion (Lazou et al., 2010) which can be found in higher ratios of factor x3. From Table 2.3, we see that crunchiness was influenced by the three lentil puff formulation factors x1, x2, and x3. O'Shea et al., (2014), asserting that extruded snacks have a cell-like structure typically filled with air which influences the crispy/crunchy quality. Starch, protein, and fiber influence the air cell structure of extruded snacks (Moraru & Kokini, 2003) therefore, it becomes evident that the three formulation factors, each with varying levels of starch, protein, and fiber, are crucial in explaining the changes in crunchiness.

The crispiness of lentil puff formulations varied between 120.20 ± 11.61 to 223.40 ± 35.10 . In contrast to crunchiness, figure 2.4 shows that the crispiness of lentil puff formulations was mostly dependent on factors x1 and x2 with some contribution by factor x3 at ratios below 60%. Lentil puff formulations 1, 2, 5, 7, and 9 with higher amounts of x1 and x2 presented higher levels of crispiness (Table 2.2). Additionally, formulations with less than a 60% ratio of x3 such as formulations 4, 8, 11, and 12 had higher levels of crispiness. Table 2.3, showed all three formulation factors showing a positive influence on crispiness texture with factor x1 and factor x2 estimated to have a stronger influence on crispiness compared to factor x3. Richlea lentils recognized for their high protein solubility (Jarpa-Parra, 2018) which may contribute to crispiness however higher levels of starch found in lentil flour and lentil starch concentrate greatly influence crispiness (Kaisangsri et al., 2016).

In comparison to the reference, lentil puff formulations 3, 6, and 10 had a higher mean hardness than corn puffs (Table 2.2). Proteins affect water distribution during extrusion and

through their macro-molecular structure, increasing covalent and non-bonding interactions that reduce air cell size and create a rigid structure (Moraru & Kokini, 2003). The action of protein is observed largely in formulations with ratios of factor x3 above 60% (formulations 3 and 10) as well as interactions between factors x1 and x3, which reduce the expansion of lentil puff formulations. Formulations with ratios of x2 above 30% were observed to have a lower hardness texture than corn puffs, which can be attributed to the diminishing effect of starch on protein (Allen et al., 2007).

The mean crunchiness of corn puffs was found to be higher than all 12 lentil puff formulations whilst the crispiness of corn puffs was lower only than formulation 12 and not different to formulation 1,2,4, 5, 7, 8, and 9. A comparable occurrence was documented by Kaisangsri et al., (2016) in a study to include apple pomace as a value-added ingredient in an extruded corn puff. The extruded snacks that contained 100% corn created a crunchier snack compared to snacks containing higher amounts of apple pomace which created a crispier snack (Kaisangsri et al., 2016). The higher crispiness of lentil puff formulations can be attributed to its high protein solubility (Jarpa-Parra, 2018) which like pectin in apple pomace (Kaisangsri et al., 2016) causes a lubricating effect to increase crispiness.

Sensory Evaluations of Lentil Puff Formulations

Overall Liking Research has shown that incorporating flavoring into extruded snacks can improve their sensory acceptance (Prinyawiwatkul et al., 1993). The overall liking scores of lentil puff formulations presented with no flavoring therefore ranged from 3.83 ± 2.23 to 5.94 ± 2.16 for lentil puff formulation 3 (100% lentil protein concentrate) and lentil puff formulation 2 (100% lentil starch concentrate) respectively. According to the regression coefficients presented

in Table 2.4, the collective influence of all three factors played a crucial role in determining the overall liking. Interestingly, we observe comparable contour plots of crispiness (Figure 2.4), and sensory acceptance (Figure 2.5), with the highest values located at the centroid for both plots. This implies a connection between the instrumental crispiness texture and sensory acceptance scores, highlighting the significance of all three component factors in improving sensory acceptance.

It was however evident from formulation 3 and 10 that extreme values of factor x3 were linked to reduced sensory acceptance scores (Table 2.5). The declining influence of factor x3 has also been observed by Choi et al., (2007), who noted that incorporating 50% additional protein in extruded snacks has been associated with a decrease in sensory acceptance scores (Choi et al., 2007). The reduction in sensory acceptance scores associated with increased protein content can be explained by its characteristics, including reduced expansion (Allen et al., 2007) and a heightened perception of burnt flavor (Lazou et al., 2010). Moreover, Lazou et al., (2010) discovered that incorporating 50% lentil flour in the manufacturing of expanded snacks led to an overall acceptability score of 5, further supporting this observation.

Congruent to literature, lentil puff formulations 2, 4, 9, 11, and 12 presented 5.94 ± 2.16 , 5.92 ± 1.79 , 5.90 ± 1.76 , 5.85 ± 2.00 , and 5.86 ± 1.97 acceptance scores respectively (Table 2.2). Nonetheless, the five lentil puff formulations achieved higher scores than the corn puff reference (5.23 ± 2.26) when produced under identical extrusion conditions of water rate and screw speed with adjustments to the feed rate (w.b). Corn has been recognized as the preferred ingredient for snack extrusion within the food industry (Kaisangsri et al., 2016). Studies attempting to enhance the nutritional value of corn by adding protein or fiber have only managed to achieve acceptance

scores similar to corn puffs when incorporating 30% or less of these additives (Lazou et al., 2010) or less (Kaisangsri et al., 2016). The fact that five formulations made entirely from Richlea lentils scored higher than corn puffs highlight the market potential for extruded lentil puffs.

Texture Liking Texture is one of the most essential characteristics influencing the sensory acceptance of extruded snack products (Paula & Conti-Silva, 2014; Suknark et al., 2006). The texture liking scores for lentil puff ranged from 3.92 ± 2.38 to 6.51 ± 1.67 (Table 2.5). Lentil formulation 3 showed the lowest texture liking score, which is similar to its overall acceptance, underscoring the impact of higher protein content on extruded snacks (Allen et al., 2007; Choi et al., 2007). Smaller ratios of factor x3 were however found to be necessary to enhance the texture liking score with Table 2.4 showing dependence on the interaction of all 3 factors and formulations 4, 9, 11 and 12 possessing a minimum x3 ratio of 17%.

Higher texture liking scores were obtained by lentil puff formulations 2, 4, 9, 11, and 12 (Table 2.4), which is linked to their higher instrumental crispiness texture (Table 2.2). Crispiness is a significant feature of extruded snacks, known to enhance participant satisfaction with these snacks (Devi et al., 2013). Texture liking scores of 6.0 were located approximately at the centroid of the contour plot indicating contributions by factor x3 (Figure 2.5). This implies that a 33% maximum ratio of factor 3 can be included in ingredient mixes without compromising desirable attributes.

We however observe that the interaction between factor x1 and x3 and the extreme points of factor x3 were associated with lower texture liking scores. The increasing effect of factor x3 on the unit density and instrumental hardness of lentil puff formulations seen in formulations 3,6 and 10 (Table 2.2) can be associated with the lower texture scores of formulations 3 and 10. The

diminishing effect of higher protein on texture liking scores can be linked to its characteristics of increased unit density and lower expansion and crispiness observed in Figures 2.3 and 2.4.

Just About Right (JAR) The just about right penalties represent the weighted difference between the mean of liking for JAR - the mean of liking for the two other levels of “too little” or “too much” taken together (XLSTAT 2020.1, Addinsoft Inc, New York, NY, USA). The mean drops represent the difference between the liking mean for the JAR levels minus the "too much" or "too little" levels. Table 2.5 therefore shows how many points of liking were lost by the lentil puff formulations for not being as expected by the participants whilst Table 2.6 shows how many points of liking were lost by the lentil puff formulations for being “too little” or “too much”. Regarding hardness, none of the 12 lentil puff formulations were deemed “just right.” (Table 5) and from Table 2.6 we see that more formulations (2, 3, 4, 7, 9, 12) lost points for being less hard than too hard. Formulations 3 and 10 with ratios of x3 above 50% were identified as being too hard (Table 2.6) which aligns with the instrumental texture results (Figure 2.4).

Excluding formulation 9, participants perceived all other lentil puff formulations as lacking the desired level of crunchiness and crispiness (Table 2.5). Notably, formulations 3 and 10 were tagged as being too crunchy, and formulations 1 and 7 with ratios of x1 above 50% were identified as being too crispy. Formulation 9 with a 66% ratio of x2 and 17% of x2 and x3 was regarded as having the ideal level of crunchiness and crispiness but needed a bit more hardness. In turn, formulation 11 was penalized for not having the right amount of hardness, crunchiness, and crispiness (Table 2.5) but the textural attributes were not noticeably “too little” or “too much”. Interestingly our findings show that formulations 4, 9, and 11 with higher sensory acceptance and texture liking scores (Table 2.5) were not regarded as being a little crispy or too

crispy (Table 2.6). The corn puffs reference was penalized for being a little crispy and crunchy (Table 2.6) which was reflected in its sensory acceptance and texture liking scores (Table 2.5).

Check All That Apply (CATA) The findings from the check all that apply (CATA) test (Figure 2.6) revealed that sensory attributes of empty, chewy, thick, hard, gritty, crunchy, and crispy were selected by participants out of 10 presented attributes to describe the texture of lentil puff formulations. From Figure 2.6, we see that lentil puff formulations 4, 7, and 9 with higher sensory scores were associated with the crispy texture attribute which was congruent with the instrumental crispiness texture (Table 2.2). Lentil puff formulation 12 as well as formulation 1 and 11, also with higher sensory scores were found in between attributes of crispy and empty, and crispy and crunchy respectively. Lentil puff formulation 2 was associated with the texture attribute of empty but it still received higher sensory scores. Other attributes of thick, hard and gritty were associated with lentil puff formulations 3 and 10 respectively. These formulations, in turn, received lower overall liking and texture liking scores.

The texture of extruded snacks has been reported to be the determinant of sensory acceptance (Paula & Conti-Silva, 2014) with crispiness being the ruling attribute (Devi et al., 2013; Kaisangsri et al., 2016; Paula & Conti-Silva, 2014). Figure 2.7 shows that sensory attributes including crunchy and crispy were essential for the acceptance of lentil puff formulations. Most participants however preferred to have crispier lentil puffs than crunchy lentil puffs, so crispier formulations received higher sensory acceptance scores (Table 2.2 and 2.5). This finding agrees with a study by Devi et al., (2013) where participants reported satisfaction with a protein-rich sorghum snack because it was tasty, appealing, light, crispy, and low fat. Crunchiness was considered important but preferred at a lower level (Figure 2.7) so crunchier

lentil puff formulations 3 and 10 (Table 2.2) received lower sensory acceptance scores. Attributes of hardness, thickness, chewy, gritty, and empty were identified by participants as present (Figure 2.7) but did not increase the sensory acceptance and texture liking scores.

The reference corn puffs were crunchier than all the lentil puff formulations (Table 2.2). However, the participants did not lower the sensory acceptance scores for corn puffs (Table 2.4). Corn puffs were identified by participants as chewy (Figure 2.6) but not crunchy which contradicted measurements of instrumental crunchiness texture (Table 2.2). Chewiness is measured instrumentally by the total positive area under the test curve (Texture Technologies, 2020) whilst crunchiness is measured as the linear distance of the test curve (Li et al., 2022). Such closeness in testing parameters may explain corn puffs having higher crunchiness than the lentil puff formulations and gives reason to conducting consumer sensory evaluations to confirm instrumental texture data (Paula & Conti-Silva, 2014).

Relationship between Instrumental Texture and Sensory Evaluation

Crispiness texture was identified as an essential contributor to the overall liking of lentil puff formulations from our CATA analysis (Figure 2.6). To understand the effect of the instrumental texture on sensory evaluation scores, we fit linear models with overall acceptance scores, texture liking, CATA_hardness, CATA_crunchiness and CATA_crispiness as responses and the instrumental textures of hardness, crunchiness, and crispiness as predictors.

Our findings (Table 2.7) revealed a weak interaction between hardness and crunchiness on the overall liking with a negative estimate. This indicates that the hardness and crunchiness may act antagonistically to decrease the overall acceptance scores of lentil puff formulations. The effect of the interaction between hardness and crunchiness acts contrary to our hypothesis of

hardness and crunchiness increasing the overall liking at a “just about right” level. We also observe in Table 2.7 that the instrumental crunchiness did not have a noticeable relationship with the CATA_hardness measured by participants, unlike the instrumental hardness and crispiness.

The interaction between hardness and crispiness however provided a noticeable positive effect on both the overall liking and texture liking scores (Table 2.7). The positive interaction indicates that the instrumental hardness and crispiness textures act synergistically to improve the overall acceptance and texture liking scores of lentil puff formulations. This confirms our hypothesis of increasing crispiness texture increasing the sensory acceptance of lentil puff formulations and explains the penalties given by participants to all lentil puff formulations for not having the right amount of hardness (Table 2.5). The instrumental hardness and crunchiness also had a positive relationship with the CATA texture descriptors indicating that participants were able to identify hard and crispy formulations accurately.

Weak evidence of an interaction between crunchiness and crispiness was observed for both the overall acceptance and texture liking scores (Table 2.7) with negative regression coefficients indicating a probable antagonistic relationship between crunchiness and crispiness. This corroborates our findings in Figure 2.6 which showed a preference for lower crunchiness in the lentil puff formulations. The crunchiness texture of lentil puff formulations acted contrary to our hypothesis including interactions with both hardness and crispiness. We attribute the deviation in our hypothesis to the attributes of hardness, brittleness, firmness and crispness mostly used to describe snacks (Paula & Conti-Silva, 2014) and the participants being unfamiliar with crunchiness as a descriptor of snack texture. The three-way interaction between hardness,

crunchiness, and crispiness was observed to influence neither the overall liking nor the texture liking scores.

Optimization of the Lentil Formulation

The mixture model fit for both overall consumer acceptance and for the texture liking was used to predict the optimum lentil puff formulation. Since consumer acceptance has been used as a measure of market potential (International Food Information Council, 2020) and texture greatly influences the sensory acceptance of snack products (Paula & Conti-Silva, 2014) the predicted maximum score was used to determine the optimum formulation. The maximum overall acceptance score was predicted to be 6.07 and the maximum texture liking score was predicted to be 6.73 located at $x_1 = 0.25$, $x_2 = 0.5$, and $x_3 = 0.25$. The optimum lentil puff formulation was therefore predicted to be a 50% ratio of lentil starch concentrate (x_2) to 25% ratios of both lentil flour (x_1) and lentil protein concentrate (x_3) produced at an adjusted feed rate of 10.1 kg/h and screw speed of 350 rpm. The predicted formulation located at $x_1 = 0.25$, $x_2 = 0.5$ and $x_3 = 0.25$ was also found to provide a crispiness of 205.24 Ns found to be crucial for consumer sensory acceptance (Table 2.7)

Conclusions

The lentil puff formulations showed noticeable differences in their extrudate properties and instrumental texture which translated into their perceived sensory characteristics. The responses of expansion ratio, unit density, and instrumental hardness, crunchiness, and crispiness textures were dependent on the component factors of lentil flour, lentil starch concentrate, and lentil protein concentrate studied under a mixture design. The inclusion of protein concentrate in the

mixture design generally decreased the expansion ratio and instrumental crispiness of the lentil puff formulations at ratios above 33%. The sensory attribute of crispiness was found to be essential for enhancing sensory acceptance and was found to be dependent on all 3 formulation factors providing a balance of desirable sensory properties and increased protein and fiber. The optimum lentil puff formulation was therefore predicted from a maximum overall acceptance score of 6.03 and texture score of 6.70, at a 50% ratio of lentil starch concentrate (x2) to 25% ratios of both lentil flour (x1) and lentil protein concentrate (x3). Evidently, the successful development of a desirably expanded and crispy puffed snack from the incorporation of lentil starch and lentil protein concentrate in ingredient mixes with lentil flour highlights their feasibility as ingredients in snack extrusion. Moreover, lentil puff formulations receiving higher sensory scores than the commercially preferred corn puffs, shows the potential of extruded lentil puffs created from a single ingredient. The created lentil puffs provide a ready-to-eat puffed snack with increased protein and fiber, and a pleasant crispy texture, promising to encourage the convenient consumption of pulses in the US. The product quality of the developed lentil puffs would benefit from further research on the effect of varied extrusion feed rates and extrusion screw speed on the physical and sensory properties. Additionally, sensory studies on the addition of flavoring to lentil puff formulations would also need to be conducted to correctly gauge the market potential.

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Tables

Table 2.1: Ingredient ratios, corresponding feed rates, moisture content, and nutrient content of lentil puff formulations in the mixed model design.

Formulation	Weight ratios of the factors (d.b.)			Feed rate (kg/h, w.b.)			Moisture content (%)	Nutrient content of feed (kg/10 kg batch, d.b.)			
	Lentil flour (x1)	Lentil starch concentrate (x2)	Lentil protein concentrate (x3)	Lentil flour	Lentil starch concentrate	Lentil protein concentrate		Starch	Protein	Fiber	Ash
1-100/0/0	1.00	0.00	0.00	10.00	0.00	0.00	8.21	5.70	2.17	0.55	0.27
2-0/100/0	0.00	1.00	0.00	0.00	10.10	0.00	8.70	7.10	1.30	0.40	0.20
3-0/0/100	0.00	0.00	1.00	0.00	0.00	9.90	7.55	2.20	4.70	1.20	0.60
4-33/33/33	0.33	0.33	0.33	3.30	3.30	3.30	8.15	5.00	2.72	0.72	0.36
5-50/50/0	0.50	0.50	0.00	5.00	5.10	0.00	8.46	6.40	1.74	0.48	0.24
6-50/0/50	0.50	0.00	0.50	5.00	0.00	5.00	7.88	3.95	3.44	0.88	0.44
7-66/17/17	0.66	0.17	0.17	6.60	1.70	1.70	8.18	5.35	2.45	0.63	0.31
8-0/50/50/	0.00	0.50	0.50	0.00	5.00	5.00	8.13	4.65	3.00	0.80	0.40
9-17/66/17	0.17	0.66	0.17	1.70	6.70	1.70	8.43	6.05	2.01	0.56	0.28
10-17/17/66	0.17	0.17	0.66	1.70	1.70	6.60	7.85	3.60	3.71	0.96	0.48
11-33/33/33	0.33	0.33	0.33	3.30	3.30	3.30	8.15	5.00	2.72	0.72	0.36
12-33/33/33	0.33	0.33	0.33	3.30	3.30	3.30	8.15	5.00	2.72	0.72	0.36

a. d.b. and w.b. denote dry base and wet base, respectively.

b. The feed rate of each ingredient in the formulation was adjusted based on the total moisture content of the mixture across the 12 formulations.

Table 2.2: Extrudate properties and instrumental texture of lentil puff formulations compared to the reference product.

Formulation	Extrudate properties		Instrumental Texture		
	Unit density (g/cm ³)	Expansion ratio	Hardness (N)	Crunchiness (Ns)	Crispiness
1-100/0/0	0.12 ± 0.02 ^{efgh}	3.49 ± 0.34 ^b	80.98 ± 7.49 ^{fg}	684.05 ± 165.15 ^c	183.80 ± 31.67 ^{bc}
2-0/100/0	0.09 ± 0.01 ^h	3.45 ± 0.13 ^b	102.14 ± 6.56 ^{de}	611.17 ± 70.43 ^c	191.60 ± 20.17 ^{bc}
3-0/0/100	0.51 ± 0.04 ^a	0.53 ± 0.06 ^g	290.16 ± 21.87 ^a	884.36 ± 80.19 ^b	120.20 ± 11.61 ^e
4-33/33/33	0.12 ± 0.02 ^{efgh}	2.97 ± 0.34 ^c	85.35 ± 6.46 ^{fg}	613.87 ± 87.81 ^c	202.40 ± 27.79 ^{ab}
5-50/50/0	0.10 ± 0.02 ^{gh}	4.03 ± 0.21 ^a	82.61 ± 6.48 ^{fg}	589.94 ± 93.53 ^c	193.40 ± 32.76 ^{bc}
6-50/0/50	0.22 ± 0.03 ^b	1.86 ± 0.17 ^e	188.38 ± 20.09 ^b	869.32 ± 56.02 ^b	146.20 ± 13.05 ^{de}
7-66/17/17	0.11 ± 0.03 ^{fgh}	3.89 ± 0.30 ^a	62.84 ± 8.51 ^h	588.25 ± 85.65 ^c	210.60 ± 24.09 ^{ab}
8-0/50/50	0.14 ± 0.01 ^{defg}	2.66 ± 0.18 ^d	110.32 ± 7.83 ^d	683.33 ± 65.13 ^c	193.80 ± 12.56 ^b
9-17/66/17	0.10 ± 0.01 ^{gh}	3.49 ± 0.14 ^b	79.13 ± 4.07 ^{fg}	695.65 ± 117.96 ^c	206.60 ± 28.06 ^{ab}
10-17/17/66	0.20 ± 0.01 ^{bc}	1.57 ± 0.12 ^f	202.56 ± 9.88 ^b	871.61 ± 69.93 ^b	142.00 ± 9.30 ^{de}
11-33/33/33	0.14 ± 0.004 ^{def}	2.92 ± 0.20 ^c	91.04 ± 7.95 ^{ef}	612.57 ± 109.38 ^c	164.40 ± 14.81 ^{cd}
12-33/33/33	0.15 ± 0.01 ^{de}	2.96 ± 0.26 ^c	71.91 ± 15.91 ^{gh}	587.38 ± 65.09 ^c	223.40 ± 35.10 ^a
Corn puffs	0.16 ± 0.02 ^{cd}	2.95 ± 0.22 ^c	152.05 ± 1018 ^c	1837.38 ± 125.05 ^a	199.00 ± 13.66 ^a

For the same column, values (means ± SD) followed by the same letter are not significantly different based on Fisher's least significant difference test ($\alpha = 0.05$).

Table 2.3: Regression coefficients for the determinations of the extrudate properties and instrumental texture of lentil puff formulations.

Parameter	Extrudate properties		Instrumental texture		
	Unit density (g/cm ³)	Expansion ratio	Hardness (N)	Crunchiness (Ns)	Crispiness
x1	0.12**	3.65***	72.83*	656.38***	190.29***
x2	0.10*	3.43***	103.60**	636.32***	191.97***
x3	0.50***	0.45	298.57***	907.26***	113.73**
x1:x2	-0.01	2.52	-49.19	-235.71	36.53
x1:x3	-0.41*	-0.45	11.75	330.93	-23.15
x2:x3	-0.66**	2.48	-323.55*	-161.57	139.39
x1:x2:x3	0.18	-0.09	-911.05	-2711.36	406.80

- a. x1 = coded level for the weight ratio of lentil flour, x2 = coded level for the weight ratio of lentil starch concentrate; x3 = coded level for the weight ratio of lentil protein concentrate ; x1: x2 = interaction of factors 1 and 2; x1: x3 = interaction of factors 1 and 3; x2: x3 = interaction of factors 2 and 3 and x1:x2:x3 = interaction of all 3 factors
- b. Values superscripted with “***”, “**”, “*” and “.” indicated that the regression provided noticeable evidence at $p < 0.001$, $p < 0.01$, $p < 0.05$ and $p < 0.1$ respectively.

Table 2.4: Regression coefficients for the determinations of the consumer acceptance score, and texture liking score of selected lentil puff formulations.

Parameter	Overall liking score	Texture liking score
x1	5.48 ***	5.81***
x2	5.94 ***	6.35***
x3	3.83 ***	3.92***
x1:x2	-2.69*	-2.23 .
x1:x3	-2.55*	-6.99**
x2:x3	1.98 .	0.56 .
x1:x2:x3	31.40**	49.64 **

- a. x1 = coded level for the weight ratio of lentil flour, x2 = coded level for the weight ratio of lentil starch concentrate; x3 = coded level for the weight ratio of lentil protein concentrate ; x1: x2 = interaction of factors 1 and 2; x1: x3 = interaction of factors 1 and 3; x2: x3 = interaction of factors 2 and 3 and x1:x2:x3 = interaction of all 3 factors
- b. Values superscripted with “***”, “**”, “*” and “.” indicated that the regression provided noticeable evidence at $p < 0.001$, $p < 0.01$, $p < 0.05$ and $p < 0.1$ respectively.

Table 2.5: Overall liking score, texture liking score and Just About Right Penalties for texture attributes of selected lentil puff formulations compared to the reference product.

Formulation	Overall liking score	Texture liking score	Just About Right Penalties		
			Hardness	Crunchiness	Crispiness
1	5.48 ± 1.75 ^{ab}	5.81 ± 1.96 ^b	1.37*	1.27*	1.56*
2	5.94 ± 2.16 ^a	6.35 ± 2.19 ^a	1.62*	2.11*	1.96*
3	3.83 ± 2.23 ^d	3.92 ± 2.38 ^e	1.99*	2.35*	1.33*
4	5.92 ± 1.79 ^a	6.35 ± 1.78 ^a	1.22*	1.37*	0.93*
7	5.30 ± 1.94 ^{bc}	5.50 ± 2.13 ^{bc}	1.40*	1.04*	0.94*
9	5.90 ± 1.76 ^a	6.51 ± 1.67 ^a	0.94*	0.30	0.36
10	4.87 ± 2.07 ^c	4.92 ± 2.26 ^d	1.71*	1.62*	1.42*
11	5.85 ± 2.00 ^a	6.41 ± 1.69 ^a	1.65*	0.90*	1.04*
12	5.86 ± 1.97 ^a	6.42 ± 1.73 ^a	1.71*	0.96*	0.91*
Corn puffs	5.23 ± 2.26 ^{bc}	5.02 ± 2.48 ^{cd}	1.94*	2.15*	2.34*

For the same column, values (means ± SD) followed by the same letter are not significantly different based on Fisher's least significant difference test ($\alpha = 0.05$). For the same row, values with * indicate a significant difference between the means within the same category based on a two-sample t-test ($\alpha = 0.05$).

Table 2.6: Just About Right mean drops for texture attributes of selected lentil puff formulations compared to the reference product.

Formulation	Hardness		Crunchiness		Crispiness	
	Too little	Too much	Too little	Too much	Too little	Too much
1	1.75	1.04*	1.51	1.17*	1.93	1.38*
2	1.55*	2.99	2.05*	2.92	2.09*	1.62
3	1.70*	2.16*	2.27*	2.57*	1.29*	1.54
4	1.24*	1.13	1.44*	0.94	1.08	0.54
7	1.33*	1.95	0.83	1.33	0.56	1.16*
9	0.79*	1.46	0.16	0.44	0.32	0.41
10	1.75	1.70*	1.06	1.85*	1.47*	1.37*
11	1.31	2.16	1.13	0.67	0.99	1.11
12	1.82*	-0.08	1.04*	0.71	1.05*	0.64
Corn puffs	2.60*	1.54*	2.21*	2.03	2.28*	2.50

For the same row, values with * indicate a significant difference between the means within the same category based on a two-sample t-test ($\alpha = 0.05$).

Table 2.7: Regression coefficients for the interactions between instrumental texture and sensory evaluation scores.

Instrumental texture	Overall liking score	Texture liking score	CATA hardness	CATA crunchiness	CATA crispiness
intercept	2.49×10^0	4.84×10^0	-0.07	3.21×10^{-1}	0.13
Hardness	$6.53 \times 10^{-4} ***$	$-1.44 \times 10^{-2} ***$	0.002***		
Crunchiness	7.86×10^{-3}	$5.91 \times 10^{-3} *$		-8.65×10^{-6}	
Crispiness	1.22×10^{-2}	4.07×10^{-3}			0.003***
Hardness: crunchiness	$-3.43 \times 10^{-5} .$	-2.25×10^{-5}			
Hardness: crispiness	$4.38 \times 10^{-5} **$	$1.23 \times 10^{-4} **$			
Crunchiness: crispiness	$-3.41 \times 10^{-5} .$	$-2.71 \times 10^{-5} .$			
Hardness: crunchiness: crispiness	1.16×10^{-7}	5.67×10^{-8}			

- a. x_1 = coded level for the weight ratio of lentil flour, x_2 = coded level for the weight ratio of lentil starch concentrate; x_3 = coded level for the weight ratio of lentil protein concentrate ; $x_1: x_2$ = interaction of factors 1 and 2; $x_1: x_3$ = interaction of factors 1 and 3; $x_2: x_3$ = interaction of factors 2 and 3 and $x_1:x_2:x_3$ = interaction of all 3 factors
- b. Values superscripted with “***”, “**”, “*” and “.” indicated that the regression provided noticeable evidence at $p < 0.001$, $p < 0.01$, $p < 0.05$ and $p < 0.1$ respectively.

Figures

Figure 2.1: Lentil puffs made from formulations (left to right) in the mixed model design.



Figure 2.2: Cross section of lentil puffs made from formulations 1 to 13 (left to right) in the mixed model design.

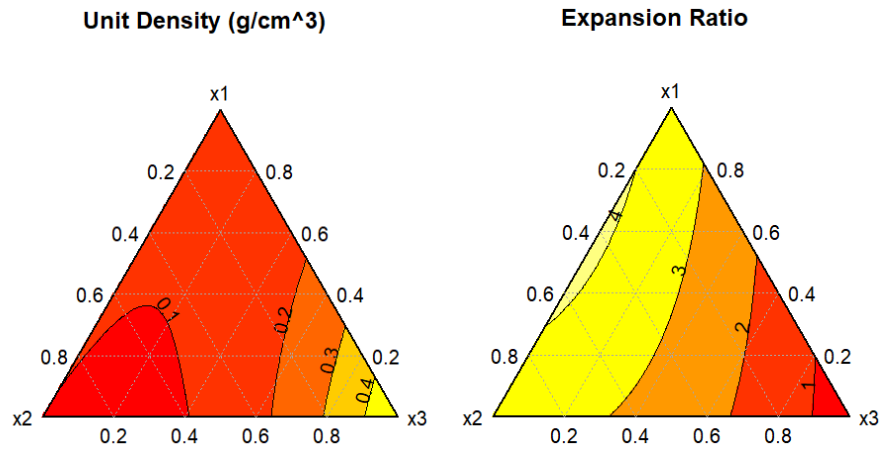


Figure 2.3: Contour plots of unit density and expansion ratios of lentil puff formulations.

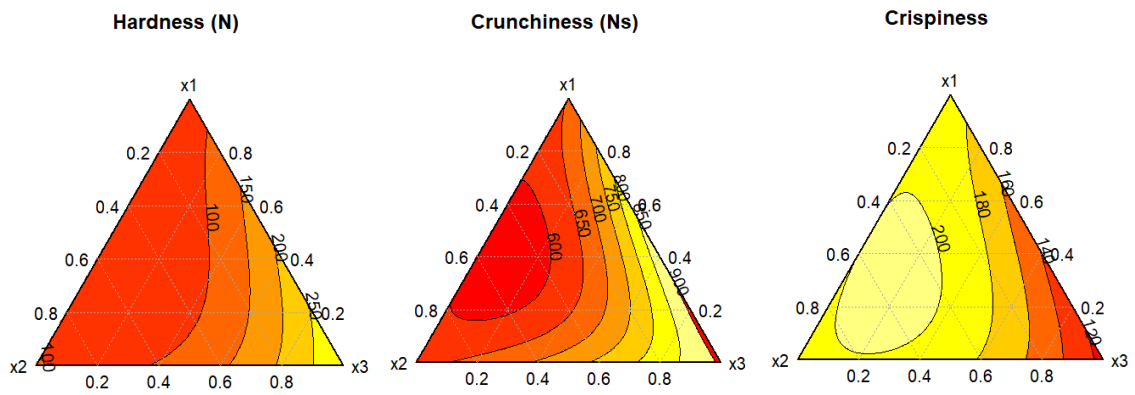


Figure 2.4: Contour plots of instrumental hardness, crunchiness, and crispiness texture of lentil puff formulations.

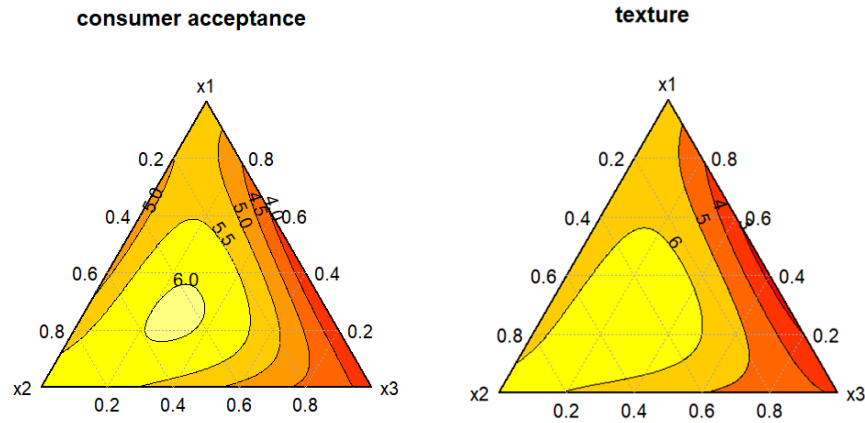


Figure 2.5: Contour plot of consumer acceptance and texture liking score of lentil puff formulations.

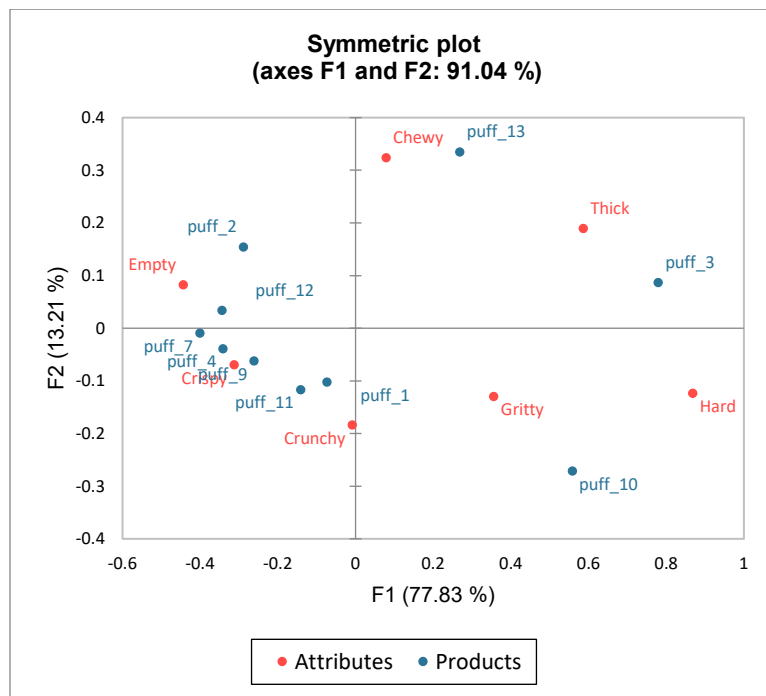


Figure 2.6: Scree plot showing the corresponding CATA attributes of lentil puff formulations.

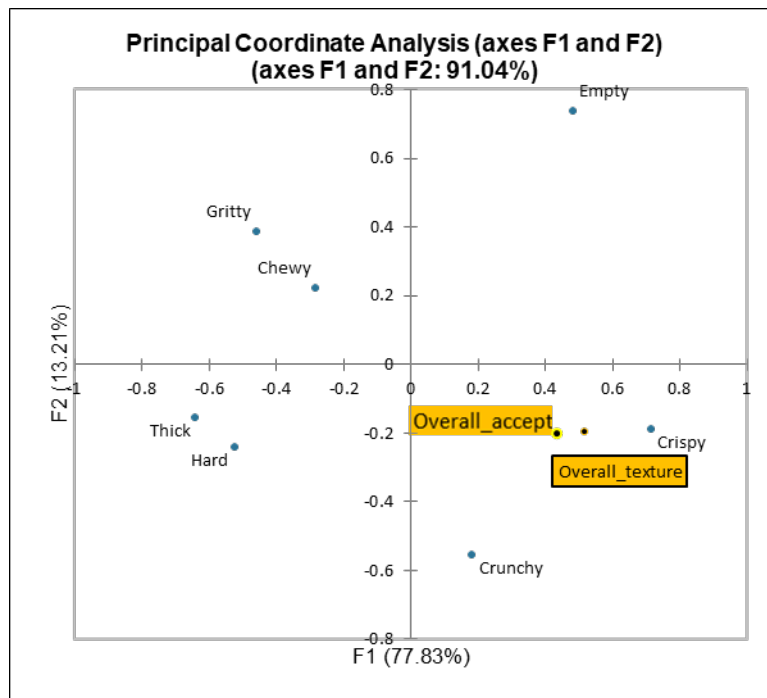


Figure 2.7: Bi-plot of the relationship between CATA attributes and overall liking and texture liking score.

CHAPTER THREE

EXPLORING THE USE OF LENTIL FLOUR AND LENTIL PROTEIN CONCENTRATE IN
DEVELOPING COUSCOUS WITH A PILOT-SCALE TWIN-SCREW EXTRUDER.Contribution of Authors and Co-Authors

Manuscript in Chapter [3]

Author: Edwin Allan

Contribution: Edwin Allan developed the recipes and process parameters for all couscous formulations, performed the analysis, interpreted results and wrote the manuscript.

Co-Author: Dr. Wan-Yuan Kuo

Contribution: Dr. Wan-Yuan Kuo provided expertise in recipe development and analysis and edited the manuscript, figures and tables.

Co-Author: Dr. Dilpreet Bajwa

Contribution: Dr. Dilpreet Bajwa provided expertise in process development and edited the manuscript, figures, and tables.

Co-Author: Girish Ganjyal

Contribution: Girish Ganjyal provided expertise in process development and edited the manuscript, figures, and tables.

Co-Author: Chidimma Ifeh

Contribution: Provided expertise in conducting sensory consumer acceptance tests of lentil couscous formulations and edited the manuscript, figures, and tables.

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Abstract

Lentil couscous was developed from the ratio of lentil protein concentrate to lentil flour and different levels of extruder screw speeds ranging from 200-300 rpm using a twin-screw pilot scale extruder at a dry feed rate of 10 kg/h and a water feed rate of 3 kg/h. An analysis of the bulk density, the water absorption index, instrumental texture (hardness, springiness, cohesiveness, gumminess, chewiness, and resilience) was conducted for all twelve formulations. Five lentil couscous formulations were then presented to 120 participants to evaluate sensory properties (overall liking in terms of acceptance, appearance, flavor and texture, as well as check all that apply) and purchasing indicators (purchase intent and willingness to pay). An increase in ratio of lentil protein concentrate to lentil flour increased the bulk density of lentil couscous formulations whilst decreasing the water absorption index, chewiness and resilience texture. An increase in extruder screw speed decreased the hardness and gumminess texture. Higher sensory acceptance, appearance, flavor and texture scores for couscous samples presented to participants were observed for formulations with lentil protein concentrate levels below 30% and a screw speed of 250 rpm. Check all that apply tests revealed nutty and a nutty aftertaste positively impact overall flavor and acceptance scores whilst texture attributes of smooth and soft/tender positively impacted the overall texture and acceptance scores. Clusters obtained from grouping the sensory scores of participants into low and high the high scoring cluster were willing to buy 10 oz of lentil couscous for \$3.37.

Introduction

Couscous is a popular food in Northern Africa, some Sub-Saharan African countries, and recently North America (Igrejas et al., 2020). Couscous is a granular product of 1-2 mm in diameter (Chemache et al., 2021; Moussa et al., 2022a), typically made from durum wheat but has also been made with sorghum, millet, or corn in West Africa (Aboubacar & Hamaker, 1999). Couscous is considered a healthy food, ideal for vegetarians (Coskun, 2013) and provides complex carbohydrates, B vitamins, and minerals (Çelik et al., 2004). Once mostly in the healthy food sections of grocery stores in North America (Coskun, 2013), the increased consumption of couscous has widened its use in other recipes like salads (Nur et al., 2018) and breakfast cereals (Aboubacar & Hamaker, 1999), and potentially gluten-free products. Approximately one-third of adult Americans were found wanting to reduce or avoid gluten consumption ((NPD group, 2015), reflecting the \$4.6 billion sale of gluten-free products in 2017 (Marketsandmarkets.com, 2018). With the United States ranking number four in the importation of couscous (Nur et al., 2018), attempts are being made to develop gluten-free couscous (Aboubacar & Hamaker, 1999; Demir & Demir, 2016).

Lentil flour is gluten-free and contains higher amounts of protein, fiber, and phytochemicals than traditional cereal flour such as wheat, rice, and corn (Bresciani et al., 2021; Romano et al., 2021), making it a viable candidate in making nutritionally enhanced couscous. Traditional couscous is prepared by mixing and aggregating flour with water into small granules, steaming, and then drying (Moussa et al., 2022b). The gluten in wheat couscous forms a strong viscoelastic network, creating optimal dough properties which, after hydration, presents favorable culinary qualities such as stability, higher weight per volume, lower hydration times

and lower loss of solids (da Silva et al., 2016). Creating more nutritious gluten-free couscous has been a challenge due to the lowered structural integrity (Benayad et al., 2021). One study showed a decrease textural stability in cooked couscous when replacing more than 25% durum wheat flour with lentil flour (Benayad et al., 2021; Demir & Demir, 2016a) . Gluten-free pasta made from lentil flour can present desirable culinary properties (Bresciani et al., 2021), if stronger texture is created from new mechanized methods such as extrusion (Romano et al., 2021).

Extrusion processing generates elevated temperature and pressure which is valuable for processing gluten-free ingredients (Pasqualone et al., 2020). Extrusion is performed with a single or twin-screw extruder and with the aid of a rotating screw (s), food ingredients are partially or fully cooked in a chamber called the barrel, and then shaped and pushed out of a hole called a die (Berrios et al., 2022; Pasqualone et al., 2020). The rotation of the extruder screw against the lining of the extrusion chamber generates temperature and pressure with increasing screw speed resulting in higher temperature and pressure (Berrios et al., 2022). Extrusion processing uses the elevated temperature and pressure to pregelatinize and gelatinize starch and crosslink proteins to create a strong dough matrix (Ek et al., 2021). The gluten-free products created from extrusion therefore have a uniform and cohesive texture to withstand additional cooking processes (Bresciani et al., 2021). Gluten-free pearl millet couscous developed from an extrusion study was observed to have comparable sensory acceptance to traditional durum wheat couscous (Moussa et al., 2022b).

Extrusion also presents the opportunity to increase nutrition content of couscous by including high fiber or protein found to be lacking in some gluten-free products (Woomer & Adedeji, 2021). The recent production of protein concentrates from lentil flour using mechanical

separation methods (González & Pérez, 2002) presents the potential of creating a 100% lentil couscous with increased protein content using extrusion. Consequently, the development of a lentil couscous with desired structural integrity addresses the consumer appreciation for whole lentil products, and the desire for a “clean label” in the US (Milner et al., 2020). Therefore, the objective of this study is to examine the use of a twin-screw extruder in developing a 100% lentil couscous and the effect of increasing protein and screw speed on the culinary quality, texture and sensory properties of the lentil couscous.

Hypothesis 1: We hypothesize that decreasing factor x_1 decreases water absorption index, instrumental cohesiveness, and consumer sensory acceptance score of hydrated lentil couscous whilst the bulk density, instrumental hardness and gumminess increase with increasing factor x_1 .

Hypothesis 2: Increasing factor x_2 increases water absorption index, instrumental cohesiveness, and consumer sensory acceptance score of hydrated lentil couscous whilst decreasing bulk density, instrumental hardness, and gumminess texture.

Materials and Methods

Materials

Organic Richlea lentil flour and Richlea lentil protein concentrate were obtained from Montana Pure Protein (Billings, MT). Durum wheat semolina couscous from Bob's Red Mill Natural Foods Inc. (Milwaukie, OR) was used as a reference for physical, culinary, and sensory tests.

Proximate Analysis Organic Richlea lentil flour,, and Richlea lentil protein concentrate samples were dispatched to the NP Analytical Laboratories /(St. Louis, MO, USA) for a proximate analysis of ash, , dietary fiber, crude protein, moisture, and carbohydrate (by calculation) contents, and the fatty acid profile (AOAC International, 2005) Methods 942.05, 922.06, 2011.25, 990.03, 930.15, and 996.06, respectively.

Methods

Central Composite Design for Lentil Couscous Formulations Preliminary trials were conducted to choose suitable ranges of formulations for making the lentil couscous. Based on preliminary trials the ratio of lentil flour to lentil protein concentrate and the extruder screw speed were critical determinants of the product cooking quality, flavor, and texture. A central composite design was conducted with RStudio Version 1.2.1335 (RStudio Inc., Boston, MA, USA) to evaluate the effects of two formulation factors on the cooking properties, texture properties, and consumer acceptance of the lentil couscous. Factor I represents the weight ratio (d.b.) of lentil flour to lentil protein concentrate and factor II represents the extruder screw speed (Table 3.1). The corresponding values for the two factors with three levels of variation (2^3 , $\alpha = 2$) and their combinations for each formulation were determined using a central composite design (Table 3.1). The response variables were analyzed and modelled using the equation:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j + \varepsilon$$

Where Y is the predicted response; β_0 is a constant; β_i is the linear coefficient; β_{ii} is the squared coefficient; β_{ij} is the cross-product coefficient, and ε is the random error associated with the response. 3-dimensional response surfaces for each response variable were made using SAS 9.4 according to the predicted model equation.

Extrusion Cooking Extrusion cooking was performed with a pilot scale twin-screw extruder (BCTM-30, Buhler Inc., St. Paul, MN) with a screw diameter of 30 mm and a 20L:D process length. A feed rate of 10 kg/h, a 20-hole circular extruder die with a diameter of 2.00 mm and a feed moisture level of 30% was used. A variable speed die face cutter with three blades was used to cut extrudates. Extrudates were collected when the system was stabilized with constant torque and backpressure at the die. The extrudates were then dried in a proofing oven (Metro C5 3 Series Insulated Holding/Proofing Cabinet, InterMetro Industries Corp, USA) at 40°C for 18 hr. The dried extrudates were stored in air-tight plastic bags at room temperature until further analysis. All extrusion trials were run in triplicate.

Physical Properties Bulk density was evaluated according to (Kaur et al., 2013). The samples were gently filled in 10 ml graduated cylinder, previously tared. The bottom of the cylinder was gently tapped on a laboratory bench several times until there was no further diminution of the sample level after filling to the 10 ml mark. Then, bulk density was calculated according to the equation below.

$$\text{Bulk density} = \left(\frac{a}{b} \right)$$

Where a= mass of sample and b=volume of sample

The water absorption index was determined according to (Anderson et al., 1970) with modifications. A 1 g sample was dispersed in 10 g of distilled water, using a glass rod to break any lumps. After stirring for 30 min, the dispersions were rinsed into tared centrifuge tubes, made up to 32.5 g and centrifuged at 3000×g (1500 rpm) () for 10 min. Then, the sediment was

weighed for its solid content and was used to calculate the water absorption index according to the equation below:

$$WAI = \left(\frac{a}{b} \right)$$

Where WAI = water absorption index, a = weight of sediment, and b =weight of dry solid.

Texture Analysis Texture profile analysis was performed with the TA.XT Plus C texture analyzer and software (Texture Technologies, La Crescenta, CA). The texture analyzer was equipped with a TA-35c 4-inch diameter cylindrical probe and a 50 kg load cell. Couscous was prepared by rehydrating extruded and dried couscous with hot water at a 1;1.5 ratio (w/w) and left to soak for 30 minutes with intermittent stirring. The couscous was placed in a fitting cylinder and compressed at 1.0 mm/sec at room temperature until reaching 50% strain, after which the probe returned to starting position and repeated another compression using the same speed and strain. Six attributes of hardness (peak force after the first compression, N), cohesiveness (area of work, Ns during the second compression divided by the area of work, Ns during the first compression) springiness (height during the second compression divided by the original compression distance) and gumminess (hardness*cohesiveness), chewiness (gumminess * springiness which is distance2/distance1) and resilience (dividing the upstroke energy of the first compression by the downstroke energy of the first compression, area 4/area 3) of the samples is calculated based on the force of compression and the height of compressed samples (Texture Technologies, 2020).

Sensory Evaluations The sensory evaluation was performed according to (Demir & Demir, 2016a). Couscous samples will be prepared by adding hot water to samples for 15 min to cook them. 120 participants were recruited for the study and instructed to taste the sample and rate the attributes on a 9-point hedonic scale for the overall liking from “Dislike extremely” (1), “Neither like nor dislike” (5) to “Like extremely” (9). The participants were asked to score the couscous in terms of overall acceptance, appearance, flavor and texture. Participants were also asked to check all the terms that apply from a list of terms they consider to be appropriate to describe the appearance, flavor, taste, and texture of the samples. The samples were coded with 3 digits and the order of sample presentation completely randomized for serving to the participants to guard against any bias. Participants had access to water to help cleanse their palates prior to proceeding to the next sample. Sensory evaluation was performed in a room with appropriate temperature in open sitting. Five-point scales were also used to determine purchase intent (PI) and willingness-to-pay (WTP). The participants were presented with the local market price of couscous (\$4.71) before answering the PI and WTP questions. For PI, they were asked if lentil couscous becomes available in Montana, how likely are they to buy it if it is \$ _4.71 _ per 10 oz (dried weight) at a grocery store? The 5-point scale was anchored from “Definitely will not buy” (1), “Might or might not buy” (3), and “Definitely will buy (5)” (Delgado et al., 2013b). For WTP, they were asked to indicate how much they are willing to pay for 10 oz (dried weight) of this lentil couscous, on the scale anchored between \$1.71 and \$5.71, with incremental intervals of \$1. Demographic information of participants was also taken.

Statistical Analyses The data in this study were processed using R Version 1.2.1335 (R Core Team., Boston, MA). A multilinear regression model was fit to determine the effects of

formulation factors x_1 and x_2 on the physical properties and instrumental texture of lentil couscous formulations. We applied a one-way analysis of variance (ANOVA) to determine the differences in means among different samples, and to the reference wheat couscous. A Fisher's least significant difference test ($\alpha=0.05$) was used to compare means. Mean, standard deviation and Pearson's correlation coefficients for the relationships between all properties were calculated using XLSTAT (XLSTAT 2020.1, Addinsoft Inc, New York, NY, USA).

Results and Discussion

Physical Properties

Bulk Density The bulk density of lentil couscous formulations presented a range from 0.82 ± 0.14 to 1.12 ± 0.13 (Table 3.2). Weak evidence of differences was observed between lentil couscous formulations with $p\text{-value} = 0.09$ and consequently, no significant differences in bulk density were observed between formulations 3 – 11 (Table 3.2). Comparing the lentil couscous formulations 1 vs. 3 and 2 vs. 5, at the same screw speed, formulations 1 and 2 with a higher level of lentil protein concentrate (30%) had had significantly lower bulk densities than their counterpart formulations 3 and 4 (lentil protein concentrate 10%) (Table 2).

Contrary to our hypothesis, factors x_1 and x_2 did not influence bulk density (Figure 3.3, Table 3.3). The unexpected results may be attributed to the high protein solubility of lentil protein ranging from 78% to 91% (Can Karaca et al., 2011; Joshi et al., 2011), especially influential during couscous extrusion which utilizes a higher extrusion water rate (20% d.b.). The normal restriction of starch gelatinization and swelling by protein as reported by Allen et al., (2007) is therefore limited by the solubility of lentil protein. Weak evidence of differences observed between lentil couscous formulations in Table 3.2 can be attributed to the high protein solubility of lentil protein concentrate.

Furthermore, all couscous formulations were extruded to a minimum 105°C temperature, more than sufficient for starch gelatinization and swelling (Ek et al., 2021), which explains the absence of a relationship between bulk density and factor x_2 .

In Table 3.2, we further compare the bulk density of the lentil couscous formulations to wheat semolina couscous. Remarkably, the bulk density of wheat semolina was not significantly different from the lentil couscous formulations, except formulation 2 (which had a noticeably lower bulk density (Table 3.2). The above comparison suggests that the handling, transport, and storage of the lentil couscous formulations 1, 3, and 4 - 11 will be similar to commercial wheat couscous.

Water Absorption Index (WAI) The WAI provides information on the amount of water a sample can absorb (Giovanelli et al., 2023) and is a notable property of couscous (Ren et al., 2021). From Table 3.2, our finding show that the WAI of lentil couscous formulations ranged from 4.29 ± 0.87 to 5.58 ± 0.53 (Table 3.2). No differences in water absorption index were found between all lentil couscous formulations excluding formulation 8. Formulation 8 with 40% lentil protein concentrate, was found to have a lower water absorption index than formulations 3,4,5, 7.

Protein content above 15% in couscous formulations was reported by (Debbouz, 1992) to be detrimental to the water absorption index. Consistent with this literature, x_1 was negatively correlated with the WAI (Table 3.3) which supports our hypothesis of the effect of increasing x_1 . Starch polymers are hydrophilic in nature and therefore in extrusion processing, starch granules swell after absorbing moisture with their free hydroxyl groups (Xiaoya Shang et al., 2008). The high moisture and heat process during the extrusion thus created pre-gelatinized starch in the extruded and dried couscous which can readily absorb water when rehydrated. The increase in

lentil protein concentrate levels (x_1) lowered starch contents in the couscous formulations (Table 3.1), thus reduced the concentration of free hydroxyl groups for moisture absorption when rehydrated.

In comparison to the reference, the water absorption index of lentil puff formulations 1 to 11, excluding formulation 8, were found not to be noticeably different (Table 3.2). Our findings align with reports by (Benayad et al., 2021) on lentil flour having higher water absorption properties compared to wheat semolina flour. Water absorption also serves as an indicator of the swelling index of couscous with high swelling couscous considered a desirable characteristic (Benayad et al., 2021; Hammami et al., 2022). Formulation 8 had the highest proportion of lentil protein concentrate of 40% (Table 3.1) which explains its lower water absorption (Table 3.2).

Texture Analysis

Couscous of good quality should possess a texture that is not too hard/firm and should be easy to chew after hydration (Hammami et al., 2022). Gumminess is derived from the product of hardness and cohesiveness measurements (Texture Technologies, 2020) and therefore trends in gumminess observed were similar to hardness trends in lentil couscous formulations (Table 3.4). From Table 3.4, we observe that lentil couscous formulations 1 and 2 with an x_1 level of 30%, hardness and gumminess were found to increase with an increase in factor x_2 . Meanwhile, in formulations 3 and 4 with an x_1 level of 10%, hardness and gumminess were found to decrease with an increase in factor x_2 . Formulations 5, 6, 9, 10 and 11 with an x_1 level of 20% showed a decrease in hardness and gumminess in moving from screw speeds of 200 to 300 rpm. No differences in hardness and gumminess were observed in moving from screw speeds of 200-250

rpm and from 250-300 rpm. For formulations 7 and 8 with equal x_2 levels, hardness and gumminess were found to decrease with an increase in factor x_1 .

Despite the trends observed in the measured hardness and gumminess (Table 3.4), both hardness and gumminess were found not to be influenced by factors x_1 and x_2 (Table 3.5). Here again, the unexpected results may be attributed to the high protein solubility of lentil protein (Can Karaca et al., 2011; Joshi et al., 2011), which limits the restriction of starch gelatinization and swelling by protein as reported by Allen et al., (2007). Higher ratios of factor x_1 (beyond 40% lentil protein concentrate) and x_2 (beyond 300 rpm) may be needed to understand the effect of the selected formulation factors on hardness and gumminess.

Springiness and cohesiveness measurements are both associated with the resistance of a product to a 2nd deformation (Texture Technologies, 2020). Formulations 1 and 8 with x_1 levels of 30% and higher were found to have lower springiness and cohesiveness (Table 3.4). The springiness was observed to decrease with an exponential increase in x_1 (Table 3.5) and cohesiveness was observed to decrease with an increase in x_1 (Table 3.5, Figure 3.4). Higher levels of x_1 results in lower starch levels in couscous which reduce the concentration of free hydroxyl groups for moisture absorption when rehydrated (Xiaoya Shang et al., 2008). The effect of increasing protein on the springiness and cohesiveness texture can be improved by an increase in extruder screw speed which disrupts starch granules and increases water absorption and swelling (Ek et al., 2021). Formulations 2, 5, 6, 8, and 11 with lower x_1 and higher x_2 (Table 3.4) notably presented higher springiness and cohesiveness which demonstrates good quality couscous (Giovanelli et al., 2023; Hammami et al., 2022). However, higher levels of x_2 , may be needed to observe a noticeable increase in cohesiveness and springiness (Table 3.5, Figure 3.4).

Formulations 1,6,7,8,9 and 10 were observed to have a lower chewiness whilst formulations 1, 8, 10, and 11 had a lower resilience compared to other lentil couscous formulations (Table 3.4). Chewiness is a product of gumminess and springiness measurements, and resilience denotes the resurgence of a product to its original height after compression (Texture Technologies, 2020). Generally, chewiness and resilience were both observed to decrease with an increase in x_1 (Figure 3.4) meaning an increase in the ratio of lentil protein concentrate decreases the recovery of lentil couscous to its original height after compression. Chewiness and resilience are textural parameters derived from springiness (Texture Technologies, 2020), therefore the decrease in springiness observed with an exponential increase in x_1 is reflected in the decrease in chewiness and resilience. In addition to cohesiveness, smoothness, and springiness, chewiness is recognized as an important characteristic of couscous (Giovanelli et al., 2023; Hammami et al., 2022).

In comparison to the reference wheat semolina couscous, lentil couscous formulations 7 and 10 were found to not be different in hardness, formulations 1, 5, 10, and 11 were not different in gumminess and formulations 10 and 11 were not different in chewiness texture. Remarkably, lentil couscous formulations 2-7 and 9-11 were found not to be different in springiness texture from wheat semolina couscous. Springiness is an important attribute in couscous and in wheat semolina is reported to originate from the action of the protein gluten (Benayad et al., 2021). Lentil flour and lentil protein concentrate are gluten-free ingredients (Romano et al., 2021), and therefore the successful development of “springy” lentil couscous highlights its potential as a noteworthy gluten-free alternative to wheat semolina couscous. Lentil couscous formulations

were however found to have lower cohesiveness and resilience texture compared to wheat semolina couscous.

Sensory Evaluations

The sensory scores of 5 selected lentil couscous formulations against wheat semolina are shown in Table 3.6. The overall acceptance scores of the selected samples ranged from 5.72 ± 1.62 to 6.37 ± 1.69 (Table 3.6). Lentil couscous formulation 6 presented the highest overall acceptance scores of 6.37 ± 1.69 but was found not to be different from formulations 7, 8 and 9 (Table 3.6). Remarkably, formulations 6 and 7 were found to have noticeably higher scores than wheat semolina which emphasizes the concluding remarks of Benayad et al., (2021) on developing a 100% legume-based couscous. Conversely, differences in overall acceptance scores were not found between lentil formulations 8,9, 10, and wheat semolina. This was in accordance with findings from Demir & Demir, (2016) who observed that the overall acceptance scores of a wheat semolina control did not differ from formulations with lentil flour inclusions.

The overall appearance scores of the selected formulations ranged from 5.74 ± 1.73 to 6.84 ± 1.43 (Table 3.6). Lentil couscous formulation 6 exhibited the highest overall appearance score of 6.84 ± 1.43 but was found not to be different from formulations 7, 9, and wheat semolina. This matched with findings from Demir & Demir, (2016) who reported that the inclusion of lentil flour to wheat semolina in the production of couscous did not reduce overall appearance scores. Lower appearance scores were observed for formulation 8 with higher x_1 levels and formulation 10 with lower x_2 levels (Table 3.6). Formulation 10 was however found not to be different from formulations 7, 9, and wheat semolina however formulation 6 was noticeably lower in appearance scores than all other selected formulations (Table 3.6).

Formulation 6 with 40% of lentil protein concentrate was observed to have a comma shape (Figure 3.1 and 3.2) different from the typical spherical shape of couscous seen in formulations 6,7, 9, and wheat semolina (Figure 3.1 and 3.2). The contrasting shape of formulation 8 can be attributed to the reduced starch content which causes a reduction in gelatinization and slight expansion needed to provide the ideal spherical shape of couscous (Giovanelli et al., 2023).

The overall flavor scores of selected samples exhibited a range from 5.83 ± 2.24 to 6.32 ± 1.75 with formulation 6 presenting the highest score and wheat semolina, the lowest overall flavor score. The higher scoring of formulation 6 compared to wheat semolina can be attributed to the presence of slight beany flavors of pulse-based nutrition bars after high-temperature processing which was observed by Szczygiel et al., (2017) to result in higher acceptance scores. Therefore, the overall flavor scores of lentil couscous formulations 7-10 were found not to be different from formulation 6.

The overall texture scores of the selected couscous formulations ranged from 5.80 ± 1.82 to 6.56 ± 1.63 with formulation 6 presenting the highest overall texture scores of 6.56 ± 1.63 which was found to be different than formulations 8, 10, and wheat semolina. The structure and texture of gluten-free couscous is reported to be contingent on starch gelatinization and retrogradation (Marti & Pagani, 2013). Formulations 8 and 10 have higher levels of protein concentrate and lower screw speeds respectively which limit starch gelatinization and retrogradation. Gluten in wheat semolina has also been reported by Chemache et al., (2021) to produce strong cohesive forces that can impact the final texture of couscous when hydration is used instead of traditional steaming.

Consequently, the overall acceptance scores of the presented couscous formulations were found to be strongly positively correlated with the overall flavor and texture scores (Table 3.7) . Texture especially is reported to have a significant impact on the culinary quality of couscous (Benayad et al., 2021; Giovanelli et al., 2023; Hammami et al., 2022). Formulations 6 and 7 with higher flavor and textural scores were therefore found to have higher overall acceptance scores (Table 3.6). In addition, formulations 6 and 7 observably obtained higher scores in all sensory categories, which can be attributed to a sensory halo effect.

A halo effect according to Thorndike, (1920) refers to a perceptual bias in which one notable attribute determines the overall impression of a person or object. In sensory studies, the halo effect is experienced when the assessment of one specific quality of a product attribute strongly influences the perception of other characteristics of the same product (Apaolaza et al., 2017). Participants in the sensory test were asked to evaluate the overall appearance, flavor, and texture scores immediately after evaluating the overall acceptance of each sample provided. Formulations 6 and 7 receiving higher scores for appearance, flavor, and texture following the high overall acceptance scores could be perceived as a probable halo effect. However, wheat semolina received higher overall appearance scores and formulation 8 received higher overall flavor scores despite initial low overall acceptance scores which contradict the workings of a halo effect.

Check All That Apply (CATA) The results from the check all that apply (CATA) test (Figure 3.5, Table 3.8) shows the sensory attributes selected by participants out of 18 presented attributes to describe the flavor of lentil couscous formulations. From Figure 3.5, we see that lentil puff formulations 6,7 and 9 with higher overall flavor scores were in the region featuring

the attributes of nutty, roasted pea/beany, umami aftertaste, nutty aftertaste, pea/beany aftertaste. Formulations 8 and 10 were found in an adjacent region featuring smoky, earthy, earthy aftertaste and smoky aftertaste attributes. Wheat semolina however was located in a distant region and featured bland and sweet aftertaste attributes.

Lentil couscous formulations 6,7 and 9 and formulations 8 and 10 were found not to differ significantly in flavor attributes. Wheat semolina however was mainly separated from lentil couscous formulations by the reduced presence of roasted pea/beany, nutty, nutty aftertaste, earthy, earthy aftertaste attributes and the increased presence of bland, and sweet aftertaste attributes (Table 3.8). Figure 3.6 further shows that high overall flavor scores were featured in a region close to umami aftertaste, nutty, nutty aftertaste, roasted pea/beany, and pea/beany aftertaste. Nutty and nutty aftertaste attributes were found to noticeably increase the overall flavor scores whilst the bland flavor attribute was found to decrease overall flavor scores (Table 3.9).

The texture attributes of cohesive, sticky, smooth, soft/tender, al dente, gummy, springy, chewy, chewy, mushy, sticky aftertaste, grainy aftertaste, and creamy aftertaste were selected by participants to describe the selected couscous formulations (Figure 3.5, Table 3.8). Unlike the flavor attributes, the textural attributes were evenly dispersed around couscous formulations (Figure 3.5). Therefore, clear differences in textural attributes were not found between lentil couscous formulations and wheat semolina. Moreover, none of the samples presented were however found close to the region featured by the mushy texture attribute. Figure 3.6 shows that high overall texture scores were located near the smooth and soft/tender texture attributes and Table 3.9 revealed that the smooth and soft/tender texture attributes indeed increase the overall

texture scores of formulations. Gummy and grainy aftertaste attributes were however found to decrease the overall texture scores of couscous formulations (Table 3.9). Quality couscous is considered a soft, non-sticky granular product which explains participants' appreciation for smooth, soft/tender couscous and a decrease in sensory scores for gummy attributes which denote stickiness.

Overall acceptance scores were strongly correlated with overall flavor and texture scores and this is reflected in associated CATA attributes. Figure 3.7 therefore shows that the marker for high overall acceptance scores were situated near the attributes of smooth, nutty, and nutty aftertaste attributes. Table 3.9 therefore shows the attributes of nutty, nutty aftertaste, smooth, and soft/tender increased the overall acceptance score whilst attributes of bland, gummy, and grainy aftertaste reduced the overall acceptance scores of presented couscous formulations.

Relationship between Instrumental Texture and Frequency of CATA Texture Attributes

The texture and structure of couscous plays an important role in the culinary quality of couscous (Abecassis et al., 2012; Hammami et al., 2022). The instrumental texture was compared to the CATA texture attributes of lentil couscous formulations to understand the relationship between instrumental and subjective tests of couscous and understand its effect on sensory acceptance scores. Instrumental hardness, gumminess, and chewiness texture were found to be positively correlated with the CATA attribute of springy whilst cohesiveness and resilience were negatively correlated with a grainy aftertaste (Table 3.10). The correlation coefficients between these variables are 0.855, 0.925, and 0.971 between hardness and springy, gumminess and springy, and chewiness and springy respectively (Table 3.10). Instrumental cohesiveness and grainy aftertaste

and resilience and grainy aftertaste presented correlation coefficients of -0.862 and -0.939 (Table 3.10).

Chewiness according to Texture Technologies, (2020) is derived from hardness, cohesiveness, and springiness measurements. The observed higher positive correlation between chewiness and the springy CATA attribute demonstrates that participants were able to detect outstanding qualities of lentil couscous formulations. Remarkably, instrumental cohesiveness and resilience, derived from springiness (Texture Technologies, 2020) are reported to be desirable characteristics of couscous (Hammami et al., 2022) and were negatively correlated with the grainy aftertaste CATA attribute (Table 3.10). The grainy aftertaste shown in Table 3.8 negatively impacts the overall acceptance scores of lentil couscous formulations.

Cluster Analysis of the Consumers in relation to Purchase Indicators of Formulation 6

Demographic information was collected from participants who signed up for the sensory test to help explain purchase indicators of lentil couscous formulation 6. From the demographic survey results, participants were labeled by gender, age (18–34, 35–44, and above 44), ethnicity, and how often they eat lentils and couscous. Agglomerative hierarchical clustering identified two consumer clusters (Table 3.11), which appeared to be differentiated based on the consumer scores for formulation 6. The consumers in Cluster 1 issued lower overall acceptance, appearance, flavor, and texture scores compared to Cluster 2 (Table 3.11). Cluster 2 had a significantly higher proportion of women participants as well as participants who eat lentils once a week compared to the total pool (Table 11).

The median purchase intent of participants in cluster 1 was 3 (Table 3.12) which indicates that participants may or may not buy formulation 6 if it was available in the market.

Participants in cluster 1 also had a mean willingness to pay score of $\$3.00 \pm 0.76$ (Table 3.12) for 10 oz of lentil couscous which is below the market price of wheat couscous on the market.

Participant in cluster 2 validated their higher overall sensory scores with a median purchase intent of 4 (Table 3.12) which indicates that participants are willing to buy lentil couscous formulation 6. However, the willingness to pay scores of $\$3.36 \pm 0.78$ for 10 oz of lentil couscous in cluster 2 (Table 3.12) was still lower than the market price for wheat semolina.

Purchase intent was found to be positively correlated with overall acceptance, flavor, and texture scores whilst WTP was positively correlated with overall flavor and texture scores (Table 3.13).

Conclusions

Lentil couscous formulations presented sufficient differences in their bulk density, water absorption and instrumental texture properties, which translated into small differences in sensory scores and CATA attributes perceived by participants. The water absorption index, cohesiveness, chewiness, and resilience texture of lentil couscous formulations decreased with an increase in the ratio of lentil protein concentrate in line with our hypothesis. Bulk density, hardness and gumminess texture were found to be independent of both factors of weight ratio of lentil protein concentrate to lentil flour and extruder screw speed. Higher sensory acceptance, appearance, flavor and texture scores for couscous samples presented to participants were observed for formulations with lentil protein concentrate levels at 20% and a screw speed of 250 rpm. Sensory flavor attributes of nutty and a nutty aftertaste were found to positively impact overall flavor and acceptance scores whilst texture attributes of smooth and soft/tender positively impacted the overall texture and acceptance scores.

Clusters obtained from grouping the sensory scores of participants into low and high revealed that participants who issued higher sensory score had more women compared to the total pool of participants. The high scoring cluster were also willing to buy the presented sensory samples and were willing to spend \$3.37 for 10 oz of lentil couscous. The successful development of nutty, smooth, and soft couscous from a 100% lentil base highlights the feasibility of lentils as a viable replacement for wheat in couscous and other pasta-based products. Moreover, one lentil couscous formulation receiving higher sensory scores than wheat semolina shows the potential of lentil couscous as a novel food product. The created lentil couscous provides a gluten-free pasta-based product with increased protein and fiber, a pleasant nutty flavor, smooth and soft texture, promising to increase US appreciation for whole lentil products. The novelty of a 100% lentil couscous would benefit from research on the effect of higher ratios of lentil protein concentrate and higher levels of screw speed on the physical and sensory attributes. Additionally, studies on the glycemic index, phytochemical properties, and protein digestibility of lentil couscous formulations would help to correctly assess its nutrition potential.

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Tables

Table 3.1: Coded levels and corresponding values for the lentil couscous formulations generated from a central composite design.

Formulation number	Block	Factor I		Factor II		Protein content per 100 g serving size (g)
		Coded level (x ₁)	Weight ratio of lentil protein concentrate to lentil flour (d.b.)	Coded level (x ₂)	Extruder screw speed/rpm	
1-30/70/225	1	1	30:70	-1	225	29.29
2-30/70/275	1	1	30:70	1	275	29.29
3-10/90/225	1	-1	10:90	-1	225	24.23
4-10/90/275	1	-1	10:90	1	275	24.23
5-20/80/250	1	0	20:80	0	250	26.76
6-20/80/250	1	0	20:80	0	250	26.76
7-0/100/250	2	-2	0:100	0	250	21.7
8-40/60/250	2	2	40:60	0	250	31.82
9-20/80/300	2	0	20:80	2	300	26.76
10-20/80/200	2	0	20:80	-2	200	26.76
11-20/80/250	2	0	20:80	0	250	26.76

a. d.b. and w.b. denote dry base and wet base, respectively.

b. The feed rate of each ingredient in the formulation was adjusted based on the total moisture content of the mixture across the 12 formulations.

Table 3.2: Bulk density and hydration properties of lentil couscous formulations compared to the reference product.

Formulation	Bulk density (g/cm ³)	Water absorption index (WAI)
1-30/70/225	0.91 ± 0.09 ^{bc}	5.09 ± 0.20 ^{ab}
2-30/70/275	0.82 ± 0.14 ^c	4.68 ± 0.66 ^{ab}
3-10/90/225	1.12 ± 0.13 ^a	5.37 ± 0.69 ^a
4-10/90/275	1.07 ± 0.00 ^{ab}	5.24 ± 0.14 ^a
5-20/80/250	1.11 ± 0.07 ^a	5.37 ± 0.12 ^a
6-20/80/250	1.00 ± 0.06 ^{abc}	5.10 ± 0.40 ^{ab}
7-0/100/250	1.00 ± 0.06 ^{abc}	5.58 ± 0.53 ^a
8-40/60/250	0.96 ± 0.21 ^{abc}	4.29 ± 0.87 ^b
9-20/80/300	0.97 ± 0.00 ^{abc}	5.02 ± 1.05 ^{ab}
10-20/80/200	0.95 ± 0.13 ^{abc}	4.80 ± 0.33 ^{ab}
11-20/80/250	0.95 ± 0.13 ^{abc}	5.36 ± 0.06 ^a
Wheat semolina	1.04 ± 0.06 ^{ab}	5.36 ± 0.06 ^a

For the same column, values (means ± SD) followed by the same letter are not significantly different based on Fisher's least significant difference test ($\alpha = 0.05$).

Table 3.3: Regression coefficients for the determinations of the physical properties of lentil puff formulations.

Parameters	Bulk density (g/cm ³)	Water absorption index
Intercept	1.016***	5.275***
X ₁	-0.044	-0.284**
X ₂	-0.009	-0.008
X ₁ :X ₂	-0.010	-0.069
X ₁ ²	-0.009	-0.085
X ₂ ²	-0.015	-0.092

- X₁ = coded level for the weight ratio of lentil protein concentrate to lentil flour; X₂ coded level for extruder screw speed; X₁:X₂ = interaction of factors 1 and 2; X₁² = second order of factor 1; X₂² = second order of factor 2.
- Values superscripted with “***”, “**”, “*” and “.” indicated that the regression provided noticeable evidence at $p < 0.001$, $p < 0.01$, $p < 0.05$ and $p < 0.1$ respectively.

Table 3.4: Instrumental texture of lentil couscous formulations compared the reference product.

Formulation	Hardness (N)	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
1-30/70/225	3840.99 ± 147.21 ^{de}	0.09 ± 0.01 ^c	0.10 ± 0.00 ^{fg}	365.95 ± 3.76 ^d	33.84 ± 1.89 ^{gh}	0.03 ± 0.00 ^{ef}
2-30/70/275	5891.20 ± 680.23 ^b	0.11 ± 0.01 ^{ab}	0.10 ± 0.00 ^{ef}	599.51 ± 77.81 ^b	63.82 ± 8.49 ^b	0.03 ± 0.00 ^{cde}
3-10/90/225	6739.65 ± 473.79 ^a	0.10 ± 0.00 ^{bc}	0.11 ± 0.01 ^{cde}	708.39 ± 11.33 ^a	71.89 ± 1.99 ^a	0.04 ± 0.00 ^b
4-10/90/275	5157.82 ± 83.14 ^c	0.10 ± 0.00 ^{bc}	0.11 ± 0.00 ^{bcd}	565.31 ± 16.13 ^b	57.26 ± 0.34 ^c	0.04 ± 0.00 ^{bc}
5-20/80/250	3686.65 ± 185.45 ^{de}	0.12 ± 0.01 ^a	0.11 ± 0.00 ^{bc}	411.86 ± 18.04 ^{cd}	48.15 ± 3.10 ^d	0.03 ± 0.00 ^{cde}
6-20/80/250	1907.22 ± 206.37 ^h	0.12 ± 0.00 ^a	0.11 ± 0.00 ^{bcd}	211.09 ± 18.78 ^f	24.38 ± 1.97 ^{ij}	0.03 ± 0.00 ^{cde}
7-0/100/250	2914.34 ± 27.70 ^g	0.10 ± 0.01 ^{bc}	0.11 ± 0.00 ^{bcd}	316.73 ± 8.96 ^e	32.25 ± 1.54 ^{gh}	0.04 ± 0.00 ^{bcd}
8-40/60/250	2214.42 ± 211.45 ^h	0.09 ± 0.00 ^c	0.09 ± 0.00 ^g	207.78 ± 21.57 ^f	19.62 ± 2.63 ^j	0.03 ± 0.00 ^g
9-20/80/300	2256.38 ± 224.00 ^h	0.11 ± 0.01 ^a	0.11 ± 0.00 ^b	252.43 ± 23.43 ^f	28.86 ± 3.12 ^{hi}	0.03 ± 0.00 ^{cde}
10-20/80/200	3473.13 ± 92.74 ^{ef}	0.10 ± 0.00 ^{bc}	0.11 ± 0.00 ^{de}	365.56 ± 9.24 ^d	37.41 ± 1.95 ^{fg}	0.03 ± 0.00 ^f
11-20/80/250	4000.81 ± 163.49 ^d	0.11 ± 0.01 ^{ab}	0.11 ± 0.01 ^{de}	418.61 ± 6.95 ^c	46.18 ± 2.77 ^{de}	0.03 ± 0.00 ^{def}
Wheat semolina	3105.04 ± 225.20 ^{fg}	0.11 ± 0.01 ^{ab}	0.12 ± 0.01 ^a	383.93 ± 39.73 ^{cd}	42.41 ± 3.42 ^{ef}	0.04 ± 0.00 ^a

For the same column, values (means ± SD) followed by the same letter are not significantly different based on Fisher's least significant difference test ($\alpha = 0.05$).

Table 3.5: Regression coefficients for the determinations of the instrumental texture of lentil puff formulations.

Parameters	Hardness (N)	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
Intercept	4230.24**	0.111***	0.10735***	449.663**	5.27***	0.0347***
X ₁	-297.09	-0.002	-0.00400*	-43.85	-0.28**	-0.0021**
X ₂	-163.76	0.003	0.00206	-11.31	-0.01	0.0004
X ₁ :X ₂	908.01	0.004	0.00050	94.16	-0.07	-0.0008
X ₁ ²	-222.96	-0.004*	-0.00186	-27.64	-0.09	-0.0008
X ₂ ²	-147.87	-0.001	-0.00002	-15.95	-0.09	0.0003

- X₁ = coded level for the weight ratio of lentil protein concentrate to lentil flour; X₂ coded level for extruder screw speed; X₁:X₂ = interaction of factors 1 and 2; X₁² = second order of factor 1; X₂² = second order of factor 2.
- Values superscripted with “***”, “**”, “*” and “.” indicated that the regression provided noticeable evidence at $p < 0.001$, $p < 0.01$, $p < 0.05$ and $p < 0.1$ respectively.

Table 3.6: Sensory scores of selected lentil couscous formulations compared to the reference product.

Formulation number	Overall acceptance	Overall appearance	Overall flavor	Overall texture
6-20/80/250	6.37 ± 1.69 ^a	6.84 ± 1.43 ^a	6.32 ± 1.75 ^a	6.56 ± 1.63 ^a
7-0/100/250	6.21 ± 1.50 ^{ab}	6.55 ± 1.46 ^{ab}	6.20 ± 1.47 ^{ab}	6.33 ± 1.50 ^{ab}
8-40/60/250	5.95 ± 1.68 ^{abc}	5.74 ± 1.73 ^c	5.89 ± 1.81 ^{ab}	5.98 ± 1.74 ^{bc}
9-20/80/300	6.10 ± 1.59 ^{abc}	6.50 ± 1.33 ^{ab}	6.15 ± 1.63 ^{ab}	6.10 ± 1.79 ^{abc}
10-20/80/200	5.72 ± 1.62 ^c	6.25 ± 1.50 ^b	5.85 ± 1.76 ^{ab}	5.80 ± 1.82 ^c
Wheat semolina	5.87 ± 2.01 ^{bc}	6.42 ± 2.09 ^{ab}	5.83 ± 2.24 ^b	5.89 ± 2.26 ^{bc}

For the same column, values (means ± SD) followed by the same letter are not significantly different based on Fisher's least significant difference test ($\alpha = 0.05$).

Table 3.7: Relationship between overall sensory scores.

Variables	Overall Opinion	Overall appearance	Overall flavor	Overall texture
Overall Opinion	1	0.636	0.952	0.980
Overall appearance	0.636	1	0.732	0.672
Overall flavor	0.952	0.732	1	0.948
Overall texture	0.980	0.672	0.948	1

Bold values indicate a significant correlation based on Fisher's least significant difference test ($\alpha = 0.05$).

Table 3.8: Multiple pairwise comparisons of CATA attributes between selected lentil couscous formulations including the wheat semolina.

Attributes	6-20/80/250	7-0/100/250	8-40/60/250	9-20/80/300	10-20/80/200	Wheat Semolina
Pea/beany	0.598 (a)	0.523 (a)	0.598 (a)	0.570 (a)	0.579 (a)	0.159 (b)
Nutty	0.561 (a)	0.561 (a)	0.495 (ab)	0.533 (a)	0.514 (a)	0.318 (b)
Earthy	0.505 (a)	0.430 (a)	0.542 (a)	0.430 (a)	0.458 (a)	0.215 (b)
Smoky	0.187 (a)	0.150 (ab)	0.224 (a)	0.112 (ab)	0.187 (a)	0.047 (b)
Herbal	0.196 (a)	0.159 (a)	0.178 (a)	0.112 (a)	0.196 (a)	0.150 (a)
Bitter	0.056 (ab)	0.065 (ab)	0.159 (a)	0.093 (ab)	0.121 (ab)	0.019 (b)
Floral	0.093 (a)	0.103 (a)	0.084 (a)	0.093 (a)	0.084 (a)	0.131 (a)
Bland	0.206 (b)	0.308 (b)	0.168 (b)	0.252 (b)	0.252 (b)	0.636 (a)
Cohesive	0.224 (a)	0.243 (a)	0.150 (a)	0.234 (a)	0.168 (a)	0.168 (a)
Sticky	0.271 (a)	0.178 (a)	0.234 (a)	0.224 (a)	0.150 (a)	0.196 (a)

Table 3.8 continued

Attributes	6- 20/80/250	7- 0/100/250	8- 40/60/250	9- 20/80/300	10- 20/80/200	Wheat Semolina
Smooth	0.374 (a)	0.280 (ab)	0.196 (b)	0.280 (ab)	0.178 (b)	0.299 (ab)
Soft/Tender	0.430 (a)	0.280 (ab)	0.430 (a)	0.346 (ab)	0.252 (b)	0.346 (ab)
Al dente	0.224 (b)	0.364 (ab)	0.215 (b)	0.318 (ab)	0.393 (a)	0.215 (b)
Gummy	0.327 (a)	0.243 (a)	0.243 (a)	0.290 (a)	0.308 (a)	0.402 (a)
Springy	0.290 (b)	0.280 (b)	0.299 (ab)	0.318 (ab)	0.383 (ab)	0.467 (a)
Chewy	0.290 (b)	0.449 (ab)	0.449 (ab)	0.449 (ab)	0.579 (a)	0.523 (a)
Mushy	0.121 (a)	0.019 (b)	0.131 (a)	0.093 (ab)	0.019 (b)	0.047 (ab)
Aftertaste_						
Pea/beany	0.467 (a)	0.383 (ab)	0.411 (a)	0.505 (a)	0.495 (a)	0.224 (b)
Aftertaste_						
Nutty	0.430 (a)	0.402 (a)	0.430 (a)	0.458 (a)	0.411 (a)	0.178 (b)
Aftertaste_						
Earthy	0.374 (a)	0.374 (a)	0.449 (a)	0.374 (a)	0.402 (a)	0.159 (b)
Aftertaste_						
Smoky	0.131 (ab)	0.121 (ab)	0.150 (a)	0.093 (ab)	0.159 (a)	0.019 (b)
Aftertaste_						
Floral	0.056 (a)	0.019 (a)	0.056 (a)	0.037 (a)	0.047 (a)	0.093 (a)
Aftertaste_						
Sweet	0.093 (b)	0.112 (ab)	0.084 (b)	0.075 (b)	0.075 (b)	0.224 (a)
Aftertaste_						
Salty	0.234 (a)	0.206 (a)	0.206 (a)	0.178 (a)	0.150 (a)	0.262 (a)
Aftertaste_						
Sour	0.037 (a)	0.047 (a)	0.047 (a)	0.019 (a)	0.056 (a)	0.009 (a)
Aftertaste_						
Umami	0.178 (a)	0.159 (a)	0.215 (a)	0.178 (a)	0.103 (a)	0.093 (a)
Aftertaste_						
Bitter	0.056 (a)	0.047 (a)	0.103 (a)	0.047 (a)	0.084 (a)	0.009 (a)
Aftertaste_						
Sticky	0.168 (a)	0.140 (a)	0.168 (a)	0.178 (a)	0.121 (a)	0.150 (a)
Aftertaste_						
Grainy	0.224 (ab)	0.234 (ab)	0.327 (a)	0.234 (ab)	0.299 (a)	0.112 (b)
Aftertaste_						
Creamy	0.206 (a)	0.112 (a)	0.131 (a)	0.140 (a)	0.112 (a)	0.178 (a)

Table 3.9: Mean impact of CATA attributes of selected lentil couscous formulations including wheat semolina.

Variable	Sum (Overall sensory acceptance)	Mean (Overall sensory acceptance)	Mean impact	Standardized difference	p-value	Significant
Roasted pea/beany	1904.000	5.987				
	1972.000	6.086	0.099	0.739	0.460	No
Nutty	1846.000	5.715				
	2030.000	6.364	0.648	4.929	<0.0001	Yes
Earthy	2171.000	5.932				
	1705.000	6.178	0.246	1.821	0.069	No
Smoky	3277.000	6.013				
	599.000	6.175	0.162	NA	NA	NA
Bitter	3605.000	6.141				
	271.000	4.927	-1.214	NA	NA	NA
Bland	2804.000	6.273				
	1072.000	5.497	-0.775	-5.444	<0.0001	Yes
Smooth	2759.000	5.870				
	1117.000	6.494	0.624	4.180	<0.0001	Yes
Soft/Tender	2396.000	5.718				
	1480.000	6.637	0.918	6.754	<0.0001	Yes
Al dente	2728.000	5.969				
	1148.000	6.205	0.236	1.598	0.110	No
Gummy	2756.000	6.152				
	1120.000	5.773	-0.379	-2.608	0.009	Yes
Springy	2551.000	6.017				
	1325.000	6.078	0.061	0.434	0.664	No
Chewy	2147.000	6.152				
	1729.000	5.901	-0.251	-1.870	0.062	No
Mushy	3647.000	6.119				
	229.000	4.978	-1.141	NA	NA	NA
Aftertaste_Pea/beany	2232.000	5.936				
	1644.000	6.180	0.244	1.800	0.072	No
Aftertaste_Nutty	2339.000	5.922				
	1537.000	6.223	0.301	2.195	0.029	Yes
Aftertaste_Earthy	2476.000	5.981				
	1400.000	6.140	0.160	1.141	0.254	No
Aftertaste_Smoky	3437.000	6.030				
	439.000	6.097	0.067	NA	NA	NA
Aftertaste_Sweet	3420.000	5.989				
	456.000	6.423	0.433	NA	NA	NA

Table 3.9 continued

Variable	Sum (Overall sensory acceptance)	Mean (Overall sensory acceptance)	Mean impact	Standardized difference	p-value	Significant
Aftertaste_Umami	3262.000	6.007				
	614.000	6.202	0.195	NA	NA	NA
Aftertaste_Bitter	3659.000	6.048				
	217.000	5.865	-0.183	NA	NA	NA
Aftertaste_Grainy	3006.000	6.147				
	870.000	5.686	-0.461	-2.951	0.003	Yes

Table 3.10: Relationship between instrumental texture and frequency of selected CATA texture attributes.

Variables	Cohesive	Sticky	Smooth	Soft /Tender	Al dente	Gummy	Springy	Chewy	Mushy	Aftertaste		
										Sticky	Grainy	Creamy
Hardness	-											
Springiness	0.619	-0.631	-0.364	-0.434	0.001	0.541	0.885	0.777	-0.578	-0.645	-0.366	-0.132
Cohesiveness	0.367	0.475	0.656	0.276	0.231	0.612	0.211	-0.362	0.281	0.500	-0.545	0.688
Gumminess	0.394	-0.092	0.603	-0.299	0.077	0.783	0.544	0.027	-0.432	-0.166	-0.862	0.470
Chewiness	-											
Resilience	0.491	-0.611	-0.223	-0.472	0.027	0.651	0.925	0.730	-0.630	-0.637	-0.505	-0.038
	0.436	-0.495	-0.088	-0.384	0.060	0.771	0.971	0.650	-0.548	-0.510	-0.625	0.110
	0.165	-0.203	0.487	-0.301	0.063	0.802	0.711	0.189	-0.513	-0.277	-0.939	0.401

Bold values indicate a significant correlation based on Fisher's least significant difference test ($\alpha = 0.05$).

Table 3.11: Demographic breakdown of the clusters based on sensory scores of formulation 6.

Demographic group		Total Pool	Cluster 1	Cluster 2
Gender	Man	38 (35.51%)	21 (45.65%)	17 (27.87%)
	Woman	67 (62.61%)	24 (52.17%)*	43 (70.49%)*
	Prefer not to answer	2 (0.02%)	1 (2.17%)	1 (1.64%)
Age	18-34	78 (72.90%)	33 (71.73%)	45 (77.77%)
	35-44	13 (12.15%)	6 (13.04%)	7 (11.48%)
	Above 44	16 (14.95%)	7 (15.22%)	9 (14.76%)
Ethnicity	White	87 (81.31%)	38 (82.61%)	49 (80.33%)
	Non-white	19 (17.76%)	8 (17.38%)	11 (18.04%)
	Prefer not to answer	1 (0.01%)	0 (0%)	1 (1.64%)
Eat lentils	Once a week	24 (22.43%)	7 (15.22%)	17 (27.87%)
	2-3 times a week	10 (9.35%)	2 (4.35%)	8 (13.12%)
	4-6 times a week	5 (4.67%)	4 (8.70%)	1 (1.64%)
	Never	5 (4.67%)	3 (6.52%)	2 (3.28%)
	Occasionally	63 (58.88%)	30 (65.21%)	33 (54.10%)
Eat couscous	Once a week	6 (5.61%)	4 (8.70%)	2 (3.28%)
	2-3 times a week	2 (1.87%)	0 (0%)	2 (3.28%)
	4-6 times a week	4 (3.74%)	2 (4.34%)	2 (3.27%)
	Never	27 (25.23%)	14 (30.44%)	13 (21.31%)
	Occasionally	68 (63.55%)	26 (56.52%)	42 (68.85%)

The results are displayed as the number of individuals and the corresponding percentage relative to the total in the cluster. The percentage followed by an * indicates a significant difference from the pooled percentage based on Fisher's exact test ($\alpha = 0.05$).

Table 3.12: Average consumer scores by clusters for sensory scores, and willingness-to-pay (WTP) for formulation 6 as well as median purchase intent (PI).

Cluster	Overall sensory acceptance	Overall appearance	Overall flavor	Overall texture	PI ^b	WTP (\$)
1	4.91 ± 1.19 ^b	5.83 ± 1.04 ^b	4.89 ± 1.25 ^b	5.30 ± 1.40 ^b	3 ^b	3.00 ± 0.76 ^b
2	7.48 ± 1.04 ^a	7.61 ± 1.19 ^a	7.39 ± 1.23 ^a	7.51 ± 1.06 ^a	4 ^a	3.36 ± 0.78 ^a

a. For the same column, values (means ± SD) followed by the same letter are not significantly different based on Fisher's least significant difference test ($\alpha = 0.05$).

b. For the same column, values (median) followed by the same letter are not significantly different based on Fisher's least significant difference test ($\alpha = 0.05$).

Table 3.13: Relationship between sensory scores and purchase indicators of formulation 6.

Variables	PI	WTP	Overall acceptance	Overall appearance	Overall flavor	Overall texture
PI	1	0.529	0.298	0.125	0.307	0.269
WTP	0.529	1	0.169	0.097	0.226	0.218
Overall Opinion	0.298	0.169	1	0.519	0.826	0.685
Overall appearance	0.125	0.097	0.519	1	0.337	0.439
Overall flavor	0.307	0.226	0.826	0.337	1	0.630
Overall texture	0.269	0.218	0.685	0.439	0.630	1

Bold values indicate a significant correlation based on Fisher's least significant difference test ($\alpha = 0.05$).

Figures

Figure 3.1: Lentil couscous formulations showing formulation 1 on the first row (blue tag) to the last row. The last cell shows the reference wheat semolina



Figure 3.2: Hydrated lentil couscous from selected formulations for the sensory consumer acceptance test. Formulation 6 on the first row (blue tag) to Formulation 10 on the last row. The last cell shows the reference wheat semolina.

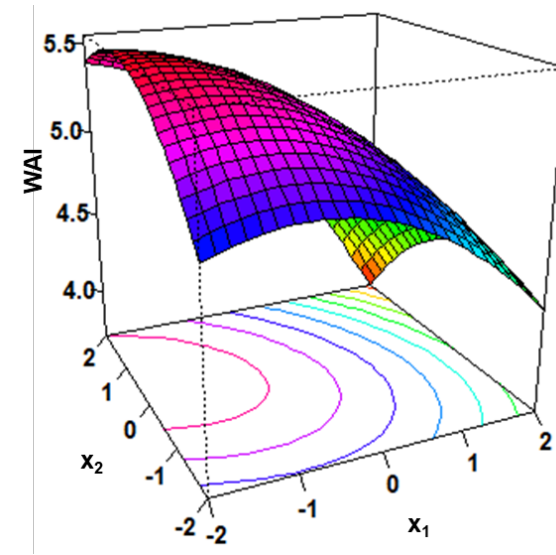
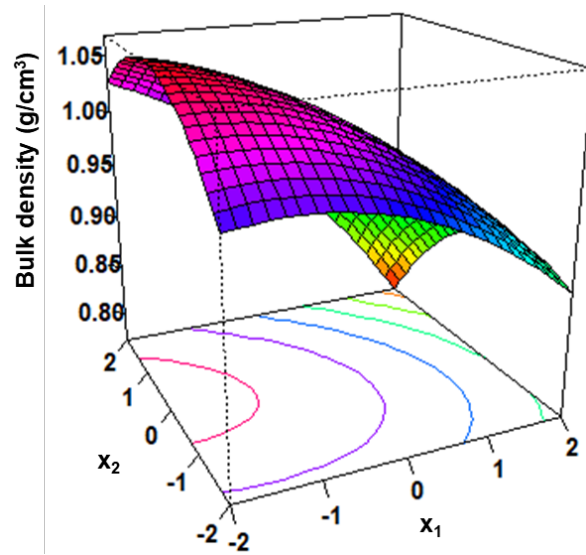


Figure 3.3: Response surfaces showing the bulk density and water absorption index, WAI of lentil couscous formulations. X1 and X2 are the RSM-coded levels for the weight ratio of lentil protein concentrate to lentil flour and extruder screw speed, respectively (Table 1).

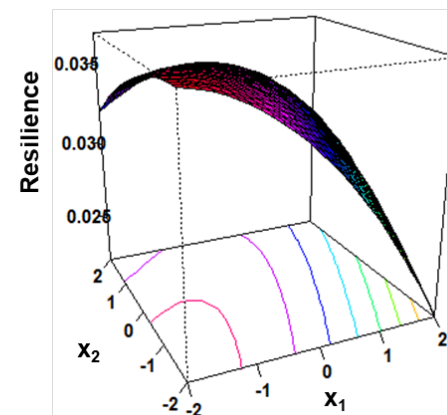
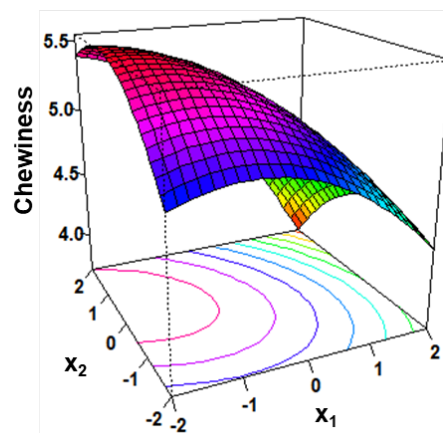
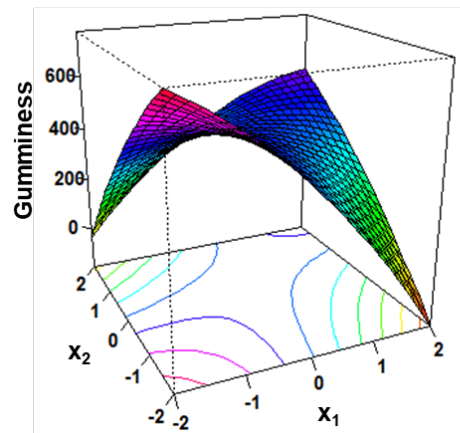
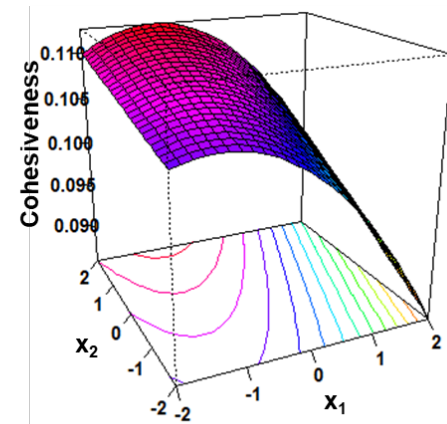
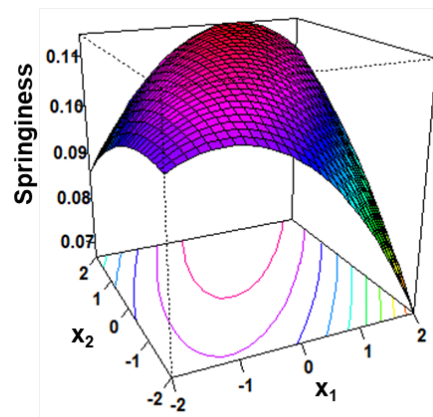
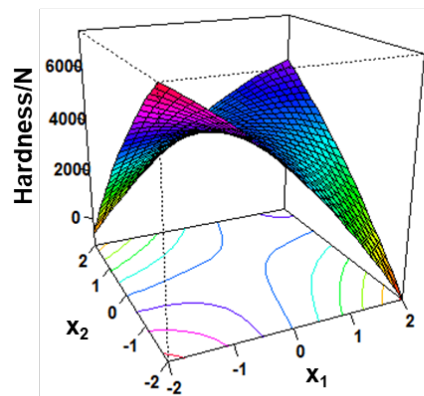
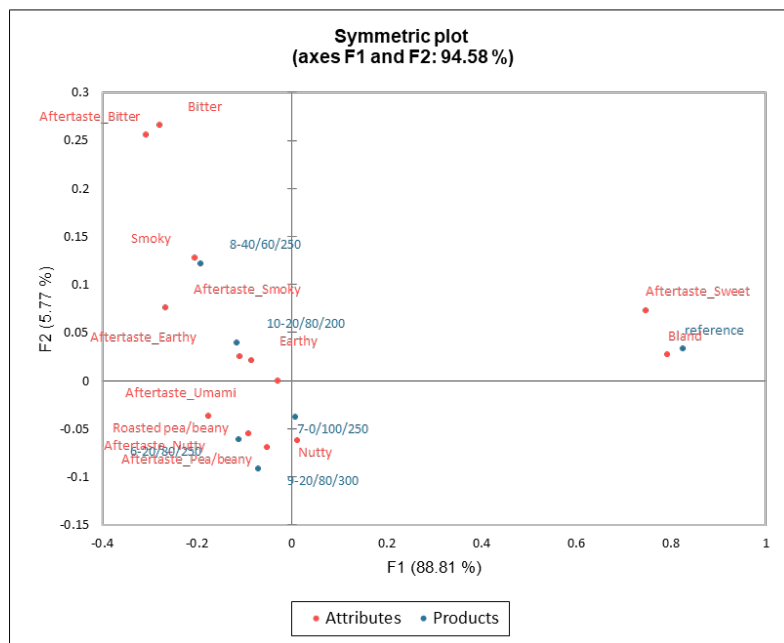


Figure 3.4: Response surfaces showing the instrumental texture, hardness, springiness, cohesiveness, gumminess, chewiness, and resilience of lentil couscous formulations. X1 and X2 are the RSM-coded levels for the weight ratio of lentil protein concentrate to lentil flour and extruder screw speed, respectively (Table 1).

(a)



(b)

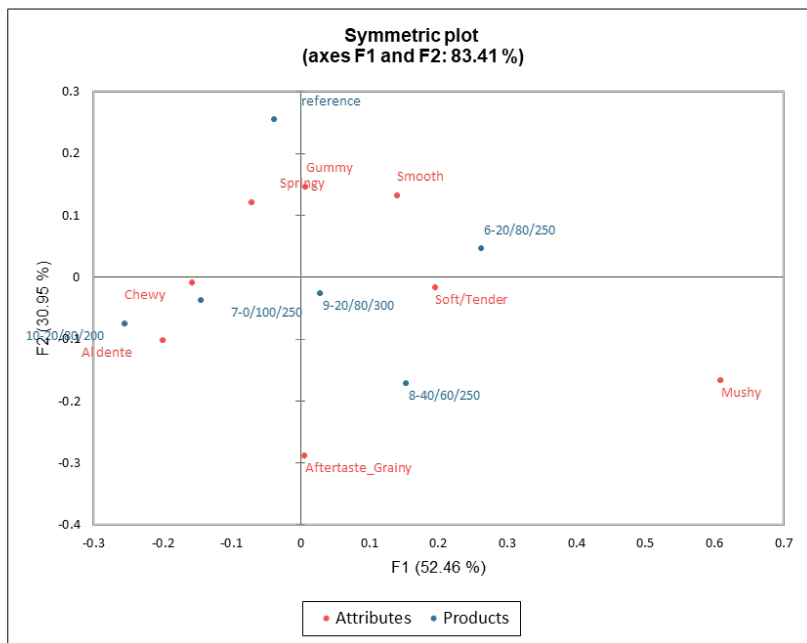
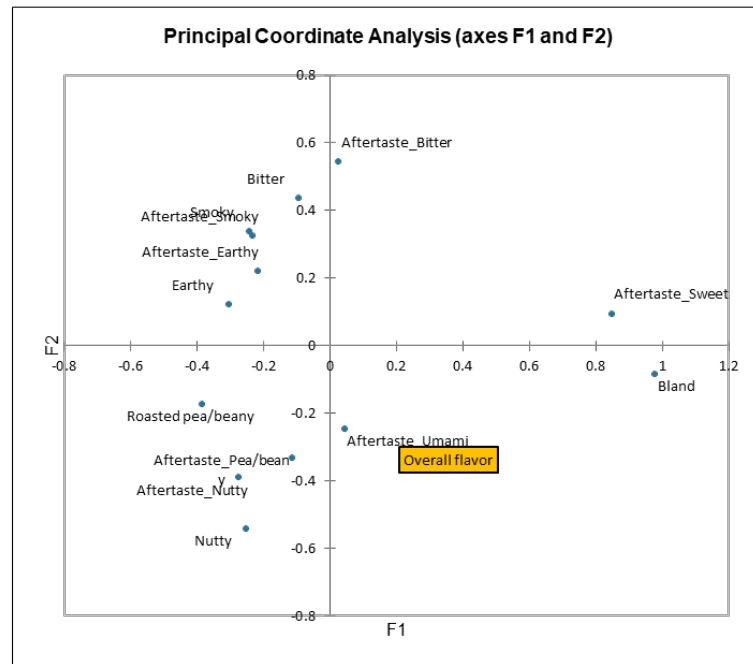


Figure 3.5: Bi-plot showing the corresponding CATA flavor (a), and texture (b) attributes of selected lentil couscous formulations.

(a)



(b)

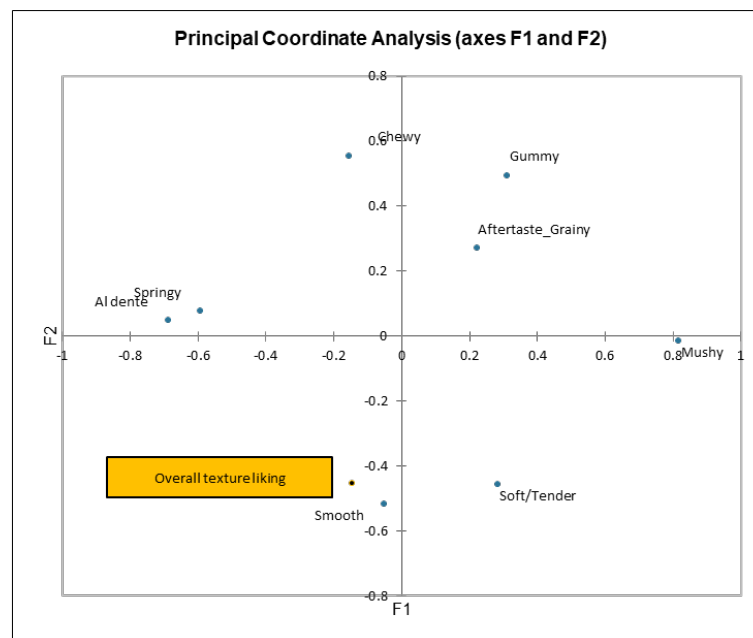


Figure 3.6: Bi-plot of the relationship between CATA flavor and texture attributes and overall sensory scores of selected lentil couscous formulations.

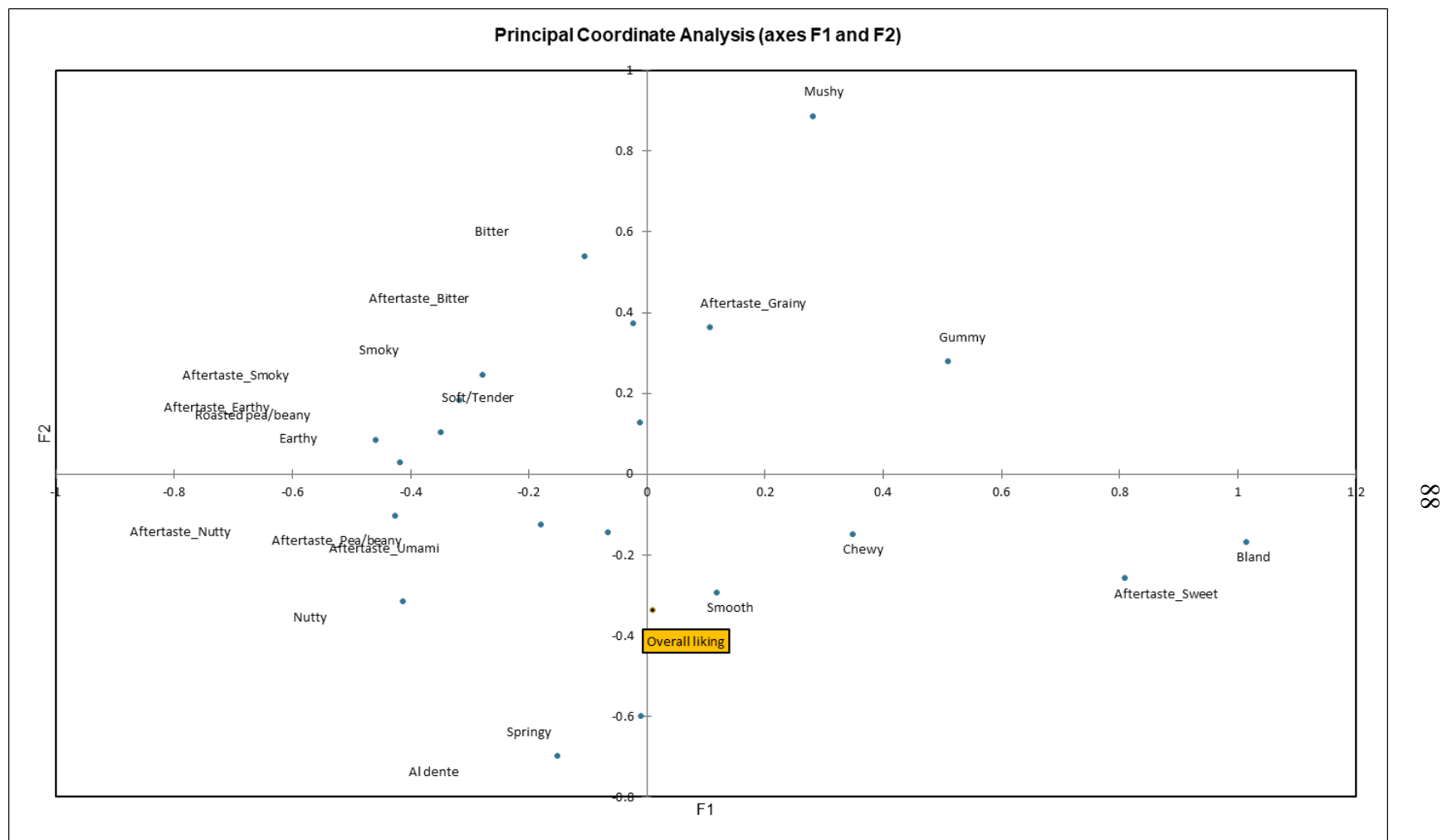


Figure 3.7: Bi-plot of the relationship between CATA attributes and overall sensory acceptance of selected lentil couscous formulations.

CHAPTER FOUR

EXPLORING THE USE OF LENTIL PROTEIN CONCENTRATE IN VEGETABLE PROTEIN
TEXTURIZATION USING A TWIN-SCREW PILOT-SCALE EXTRUDER.Contribution of Authors and Co-Authors

Manuscript in Chapter [4]

Author: Edwin Allan

Contribution: Edwin Allan developed the recipes and process parameters for all TVP formulations, performed the analysis, interpreted results and wrote the manuscript.

Co-Author: Dr. Wan-Yuan Kuo

Contribution: Dr. Wan-Yuan Kuo provided expertise in recipe development and analysis and edited the manuscript, figures and tables.

Co-Author: Dr. Dilpreet Bajwa

Contribution: Dr. Dilpreet Bajwa provided expertise in process development and edited the manuscript, figures, and tables.

Co-Author: Dr. Girish Ganjyal

Contribution: Dr. Girish Ganjyal provided expertise in process development and edited the manuscript, figures, and tables.

Co-Author: Dr. Jane Boles

Contribution: Dr. Jane Boles provided expertise in recipe development and edited the manuscript, figures, and tables

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Introduction

Extrusion processing has created dry and high moisture texturized vegetable proteins (TVPs) which are currently marketed as ingredients for meat alternatives and as meat analogs respectively (Ubbink & Muhialdin, 2022). Texturized vegetable protein developed from soy, pulse and cereal proteins as meat substitutes provide a solution to reduce the dependence on cattle and other animals for meat (Kumar et al., 2017). The inefficient use of land, feed and resources in the production of animal protein as well as the health and environmental issues involved have made consumers clamor for more sustainable food production. Texturized vegetable protein presents a more sustainable approach to providing protein in meals, however, the increased use of soy and wheat TVP substantiates the demand for commercial monocropping operations that negatively impact ecological diversity (Bouatrous et al., 2023; Kastens et al., 2017). Wheat and soy are also associated with metabolic disorders and challenges (Amoura et al., 2020) and allergen concerns (Riaz, 2011). The negative impact on the environment by intensive animal farming or monocropping, as well as consumers' demand for healthier and environmentally sound, plant protein options have set the stage for more sustainably produced pulses like lentils (Berrios et al., 2022).

Lentils, even after cooking, were found to generate 87 times lower carbon emissions than beef produced in Canada (Chaudhary & Tremorin, 2020). Lentils are not only drought-enduring crops but present a lower glycemic response compared to wheat, and are rich in fiber, antioxidants, and phytochemicals associated with maintaining healthy body weight (Joshi et al., 2017). The global consumption and environmental benefits of lentils have drawn the attention of farmers leading to an increase in lentil production in the USA from 240,000 tons in 2014 to

376,000 tons in 2020 (USDA, 2021). Montana leads the US in lentil production, growing 72% of the crop in 2020 (USDA, 2021). However, little is consumed domestically and almost 90% of the harvested lentils are exported (USA Pulses, 2016). Lentil research and processing in Montana would add value to lentils, increasing the income of small farmers and reducing the need for an external customer to make a profit.

Currently, lentils are emerging as an important ingredient in the food processing industry with the creation of lentil pasta and puffed snacks. However, lentils are seldom incorporated in the commercial production of texturized vegetable protein due to their presumed lowered structural integrity and firmness (Kim et al., 2021). Lentils contain an average of 26% of crude protein (Nosworthy et al., 2018) which is lower than the 47% protein content of soy (Frutos et al., 2008; Lopes-da-Silva & Monteiro, 2019), are limited in sulfur-containing methionine and cysteine, and have no gluten which is not ideal for texturization (Nosworthy et al., 2018). New technology in concentrating and isolating protein has however improved texturization outcomes for pulses other than soy. Pulses like peas and mung beans have therefore been successfully used in making commercial texturized protein from isolates (Maningat et al., 2022) with ingredients like peas being incorporated into mainstream plant-based meat analogs (Hu et al., 2019).

In research, lentils have been used in developing texturized vegetable protein from lentil protein concentrate with the addition of pea isolate to the ingredient mix to increase the protein content prior to extrusion (Kim, et al., 2021). However, extreme processing conditions within the extruder barrel as well as new technology in the production of texturizable protein isolates and the use of reducing and cross-linking agents, to increase molecular weight, gelation, and texturizing ability (Ganjyal, 2020) presents an opportunity to develop lentil TVP with increased

structural integrity, and firmness. Creating a commercially viable lentil TVP from Montana-grown lentils would provide an allergen-free and gluten-free alternative to soy and wheat TVPs with a lower environmental impact and also open markets for small-scale Montana lentil farmers with the overall goal of increasing diversity in crop production and consumption. The objective of this study is therefore to explore the use of lentil protein concentrate in developing a texturized vegetable protein with combinations of pea protein isolate and reviewed functional ingredients and extrusion feed rates and screw speeds (2) determine the physical and instrumental texture properties of developed lentil TVP.

Materials and Methods

Materials

Organic Red Crimson lentil protein concentrate (Muehlbauer and McPhee, 2004), and Richlea lentil protein concentrate with protein contents of 54% and 47% (d.b.) respectively were obtained from Montana Pure Protein (Billings, MT). Pea protein isolate (70001066CE Vitessence Pulse 1803C) with a protein content of 84.5% (d.b.) was obtained from Ingredion (South Sioux City, NE, USA), and pea protein isolate (Pea Protein 85-C) with a protein content of 86.8% (d.b.) was obtained from AGT Foods (Minot, ND, USA). Soy protein concentrate (Arcon F soy protein concentrate) with a protein content of 76.0% (d.b) was obtained from ADM (Decatur, IL, USA) to produce a reference TVP. In this research, varying amounts of pea protein isolate were added to lentil protein concentrate to increase protein content with a maximum of 66% (d.b.) protein content achieved.

Methods

Extrusion Processing Extrusion processing was done with a twin-screw co-rotating food extruder (BCTM-30, Buhler Inc., St. Paul, MN) with a screw diameter of 30 mm and a 20L:D process length. A feed rate of 15 kg/h and a feed moisture level of 2-4kg/h was used for each formulation. A circular extruder die with a diameter of 3.75 mm and a variable speed die face cutter with three blades was used to shape and cut extrudates. Extrudates was collected when the system is stabilized with specific mechanical energy 181-199 Wh/kg and die pressure ranging from 28.4 to 29 Bar. Subsequently, the extrudates underwent a 2-hour drying process at room temperature immediately after extrusion. These dried extrudates were then stored in airtight plastic bags at room temperature for subsequent analysis.

Physical Properties Water binding capacity and water solubility index of texturized proteins were determined according to Singh & Singh, (2003). A 4 g of ground samples were added to 36 mL of distilled water and placed in a shaking water bath for 30 minutes at room temperature and were taken out every 10 minutes and vortexed for 15 seconds. After hydration, samples were centrifuged for 10 minutes at 3000 g. The supernatant of the centrifuged samples was carefully poured out into moisture dishes and dried in a convection oven (Blodgett Convection Oven, Essex Junction, Vermont) at 110°C for 30 minutes. All measurements were done in triplicate.

$$\text{WBC (g/kg)} = \frac{\text{weight of pellet}}{\text{weight of dry solids}} \times 1000$$

$$\text{WSI (g/kg)} = \frac{\text{weight of dissolved solid in supernatant}}{\text{weight of dry solids}} \times 1000$$

Instrumental Texture: Firmness and Toughness Preliminary texture analysis was conducted on all extruded lentil TVP samples with the TA.XT Plus C texture analyzer and software (Texture Technologies, La Crescenta, CA) following the procedure outlined by Texture Technologies (2020). Samples were prepared by hydration for 30 minutes with boiling water at a 1:5 ratio (w/w) and 20 g of each hydrated sample placed on the TA-40-PP-AG platform. The texture analyzer was configured with a TA-40-PP-AG fixture and a 50 kg load cell. The hydrated TVP samples were compressed at 1.0 mm/sec at room temperature until reaching 75% strain. For each batch, six texture measurements were obtained. These measurements encompassed firmness (kg), denoting the peak force following the first compression, and toughness (kg.sec), (area under the curve).

Instrumental Texture: Texture Profile Analysis Texture profile analysis was then performed with the TA.XT Plus C texture analyzer and software (Texture Technologies, La Crescenta, CA) following the method described by Lee et al. (2022) with modifications. The samples included five lentil TVP samples, selected for their structural stability, and soy TVP. Samples were prepared by boiling for 30 minutes in water at a 1:5 ratio (w/w) and 20 g of each hydrated sample placed on the TA-40-PP-AG platform. The texture analyzer was equipped with a TA-40-PP-AG fixture and a 50 kg load cell. The hydrated TVP samples were compressed at 5.0 mm/sec at room temperature until reaching 50% strain, after which the probe is returned to starting position and repeated another compression using the same speed and strain. Six attributes of the samples are calculated based on the force of compression and the height of compressed samples (Texture Technologies, 2020). These six attributes included hardness (peak force after the first compression, g), cohesiveness (area of work, during the second compression

divided by the area of work, N_s during the first compression), springiness (Distance of the second compression divided by the distance of the first compression) and gumminess (hardness*cohesiveness), chewiness (gumminess *springiness), and resilience (dividing the upstroke energy of the first compression by the downstroke energy of the first compression).

Statistical Analysis The data was statistically analyzed using a two-way analysis of variance (ANOVA) and Fisher's least significant difference test by R Version 1.2.1335 (R Core Team., Boston, MA). Mean standard deviation and Pearson's correlation coefficients for the relationships between all properties were calculated using XLSTAT (XLSTAT 2020.1, Addinsoft Inc, New York, NY, USA).. The data was analyzed by RStudio Version 1.2.1335 (RStudio Inc., Boston, MA). Three to five replicates were used for each sample depending on the precision of each test depending on the precision of each method.

Results and Discussion

Physical properties

Water Binding Capacity The water binding capacity of lentil TVPs (Table 4.2, trial 1-9) ranged from 2623.33 ± 18.43 g/kg to 3176.67 ± 89.24 g/kg (Table 4.3). Compared to the raw ingredients of Richlea lentil protein concentrate (1781.67 ± 88.82) and Red crimson protein concentrate (1803.33 ± 44.74), all lentil TVPs had a higher water binding capacity. Water binding capacity or water absorption capacity as explained by Farooq & Boye, (2011) are used interchangeably to showcase the hydration properties of food and have been used as an indicator of TVP quality and functionality. The increase in the water binding capacity of lentil TVP samples in trial 1 to 9 (Table 4.3) provide evidence to the denaturation and texturization of lentil proteins.

We observed the effect of increasing protein on water binding capacity in Trial 4 and 6 (Table 4.3) where increasing pea protein isolate ratios resulted in an increase in water binding capacity. For the same ratio of pea protein isolate (10%), we observed that a decrease in pH from the addition of citric acid (trial 8) did not result in a significant decrease in water binding capacity. Conversely, an increase in pH from the addition of calcium hydroxide to a pea protein isolate ratio of 15% (trial 9) was observed to increase the water binding capacity to a level similar to trial 4 with 30% pea protein isolate. The pH of protein ingredients is known to play a role in its functionality with lower pH levels close to the isoelectric point, causing proteins to become hydrophobic and precipitate out of solution (Kim, 2018) leading to a decrease in water absorption after texturization. Conversely, an increase in pH increases the solubility of protein ingredients which results in higher water absorption and binding after texturization (Kim, 2018) as seen in trial 9.

Contrary to the effect of increasing protein on water binding capacity lentil TVP samples from trial 1 and 2 were found to produce a higher binding capacity compared to trial 5, 6, 7 and 8 despite possessing lower protein contents (Table 4.3). Trial 1 and 2 however utilized gums known for their water binding properties (Sánchez et al., 1995), with a study by Ya et al., (2020) confirming that the water binding effect of gums is maintained during extrusion. The lower water binding capacity of trial 5 especially compared to trial 4 and 6 with lower ratios of pea protein isolate can be attributed to a different brand of pea protein isolate used in production. Trial 5 utilized Ingredion's Pea protein isolate compared to trial 3, 4, 6, 7, 8 and 9 which utilized AGT's pea protein isolate. Differences in the water binding capacity between both brands can be attributed to differences in the protein isolation process (Riaz, 2011).

The absorption and binding of water to TVP is also dependent on the raw material used in production (Riaz, 2011). Notably, differences in water binding capacity were observed for lentil TVPs produced with different ingredients but under similar conditions of feed rate and screw speed. We see in trial 1 and 2 that red crimson protein TVP presents a higher water binding capacity compared to Richlea protein TVP produced under similar conditions (Table 4.3). The difference in water binding between the lentil varieties can be attributed to differences in the ratios of gum as well as differences in protein content. Red crimson lentils have a higher protein content than Richlea lentils (Ek et al., 2021b), and as proteins denature during the texturization process, the water absorption has been reported by Wagner & Anon, (1990) to increase resulting in Red crimson lentils with higher protein presenting a higher water binding capacity.

Compared to the reference soy protein concentrate (4063.33 ± 64.44 g/kg), lentil TVP trials (1 to 9) had a lower water binding capacity. As previously discussed, higher protein content in ingredients leads to higher water binding capacity, and despite the addition of pea protein isolate to Red crimson protein concentrate, the protein content of soy protein concentrate remained higher (Table 4.2). Soy is also a complete protein and contains all 9 essential amino acids in adequate proportions (Yeu et al., 2007) compared to lentils which are limited in sulfur-containing cysteine and methionine (Jarpa-Parra, 2018). Texturization of proteins, in addition to protein content is dependent on the content of sulfur containing amino acids which are responsible for cross-linking after protein denaturation. Cross-linking results in strongly aggregated structures with a lower solubility leading to a higher water binding capacity observed in trial 10 with soy protein concentrate (Zhao et al., 2020).

Water Solubility Index The water solubility index of lentil TVPs (Table 4.2, trial 1-9) ranged from 175.00 ± 66.14 g/kg to 283.33 ± 52.04 g/kg (Table 4.3). Texturization involves a structural change from globular to fibrous changes on the application of heat and pressure. The fibrous shapes produced are stabilized by hydrophobic interactions which reduce water solubility (Wagner & Anon, 1990). Compared to the raw ingredients of Richlea lentil protein concentrate (1066.67 ± 128.29 g/kg) and Red crimson protein concentrate (1058.33 ± 28.87 g/kg), all lentil TVPs therefore had a significantly lower water solubility index.

In contrast to the WBC, differences in water solubility were not observed with changes in the protein content of formulations (Table 4.3). Water solubility was found not to noticeably differ between trials 3 and 4 with 30% pea protein isolate and trial 6, 7, and 8 with 10% pea protein isolate. We however see a higher water solubility of trial 1 (no pea protein isolate) and trial 8 (10% citric acid) compared to trial 4 (30% pea protein isolate). Texturization is characterized by a reduced solubility of the end product and the higher solubility of trial 1 can be attributed to a partial texturization (Asif, Rooney, Ali, & Riaz, 2013; Riaz, 2011) due to its lower protein, lower feed rate and lower screw speed (Table 4.2). The higher solubility of trial 8 compared to trial 4 can be attributed to the lower protein content of trial 8 as well as the inclusion of citric acid reported by (xxx) to hydrolyze starch while crosslinking takes place.

The reference soy TVP with a higher degree of texturization was lower in water solubility than trials 1, 2, 3, and 5-9. Lentil protein is recognized as having a high solubility compared to other pulse proteins (Jarpa-Parra, 2018) which explains the higher water solubility index of lentil TVP samples compared to soy TVP. The WSI of trial 4 was not noticeably different from the reference trial 10. This can be attributed to a higher degree of texturization of

trial 4 with higher protein content (Table 2) during the extrusion process compared to other lentil TVP trials.

Instrumental Texture: Firmness and Toughness

The firmness of lentil TVP trials ranged from 2.22 ± 0.2 kg to 6.91 ± 0.97 kg (Table 4.3) and the toughness ranged from 1.94 ± 0.22 kg.sec to 6.78 ± 0.76 kg.sec. Trials 3,4, and 5 with higher protein content were observed to present a higher firmness and toughness (Table 4.2) seen in their denser structures after hydration (Figure 4.2). Generally, higher protein ingredients are more easily texturized and produce tougher and firmer textures after hydration (Riaz, 2004). Trials 1, 2, 6, and 7 with lower ratios of pea protein isolate and lower overall protein contents consequently presented lower firmness texture and toughness (Table 4.3) which could be observed in their flaky texture after hydration (Figure 4.2).

Additionally, changes in the pH of trials from the addition of citric acid or calcium hydroxide were observed to have an effect on the firmness texture and toughness. Trial 8 (10% pea protein isolate), which contained citric acid that decreases the pH, had firmness and toughness comparable to trial 4 (30% pea protein isolate). This phenomenon can be attributed to the increased thermal denaturation at a lower pH which causes proteins to aggregate and form denser networks from stronger protein-protein interactions (Dahl & Villota, 1991). Conversely, trial 9 (15% pea protein isolate), which contained calcium hydroxide that increases the pH, had significantly lower firmness and toughness than trial 6 (10% pea protein isolate). Pulse proteins have been reported to solubilize when exposed to alkaline conditions (Riaz, 2011; Boye et al., 2010) and, according to Dahl & Villota (1991), leading to weaker structural development.

Additives including calcium chloride, transglutaminase, cysteine, and methionine used in trials 3 and 7 were not observed to improve the firmness texture or toughness compared to trials 4 and 6.

The reference soy TVP (trial 10) presented significantly higher firmness and toughness compared to the lentil TVP samples. Soy protein concentrate has been used extensively in producing texturized vegetable food proteins and is characterized by a spongy-meaty structure that provides a chewy texture (Riaz, 2011). Soy possesses excellent gelation properties and contains all 9 essential amino acids, including methionine and cysteine, which are important for cross-linking (Zhang et al., 2021). Texturized soy proteins are therefore reported to present denser and tougher fibrous anisotropic structures compared to other pulse proteins (T. Kim, Riaz, et al., 2021; Riaz, 2011; Zhang et al., 2021).

Relationship between Water Binding Capacity, Water Solubility Index and Instrumental Texture

The functionality of proteins is generally determined by measurements of water binding capacity and water solubility index which can be correlated with properties like hydration and texture properties. From Table 4.4, we see that both the water binding capacity and the water solubility index have a positive relationship with the firmness texture and toughness ($p\text{-value} < 0.001$). This implies that increasing water binding capacity or water solubility index of TVP samples leads to an increase in firmness texture and toughness. The interaction between water binding capacity and water solubility index however produces a negative impact on the firmness and toughness of TVP samples ($p\text{-value} < 0.001$). It can be inferred from the negative impact that a TVP sample with both high-water binding and water solubility would present lower firmness texture and toughness.

Instrumental Texture: Texture Profile Analysis

In contrast to the firmness texture and toughness, 50% strain was used for texture profile analysis of TVP samples as recommended by (Texture Technologies, 2020). The hardness and gumminess of lentil TVP samples generally decreased with a decrease in the ratio of pea protein isolate. Trial 5 with higher ratios of pea protein isolate (40%) presented higher hardness and gumminess values compared to trial 4, 6, 8 and 9 (Table 4.5). Protein content is especially important in texturization and trials with higher protein would most likely possess firmer and denser structures (T. Kim, Riaz, et al., 2021; Riaz, 2011) leading to increased hardness and gumminess texture.

Compared to the developed lentil TVP samples, the reference soy TVP produced in trial 10, had significantly higher hardness and gumminess texture (Table 4.5). As discussed in previous sections, soy protein concentrate used in trial 10 possesses a higher protein content as well as ample amounts of sulfur containing amino acids which produce firmer and denser structures after texturization. The lower hardness and gumminess texture of lentil TVP samples compared to soy has been reported in a study by (Kim et al., 2021) where developed lentil TVP samples possessed approximately half the hardness and gumminess of soy TVP at equal levels of protein. According to Lin et al., (2002) however, the most texturized products with harder and gummier texture seen in soy protein meat analogs may not always be appreciated by consumers.

Slightly contrasting to hardness and gumminess, the springiness and cohesiveness of lentil TVP samples was found to be higher in trial 3 (30% pea protein isolate) compared to trial 6 (10% pea protein isolate), 8 (10% pea protein isolate) and 9 (15% pea protein isolate) with lower ratios of pea protein isolate (Table 4.5). The springiness and cohesiveness of TVP samples from trial 4 however did not differ from samples from trial 5 (40% pea protein isolate), and trial 5 did

not differ from trials 6, and 8, indicating that an increase in springiness and cohesive was not solely dependent on protein content. Trial 5 also did not differ from trial 9 in springiness but had a noticeably higher cohesiveness (Table 4.5)

Compared to lentil TVP samples, soy TVP was lower in springiness and found not to be different in cohesiveness from samples produced in trial 6, 8 and 9. Springiness is a measure of the amount of sample deformed by the first compression (J. S. Lee et al., 2022) whilst cohesiveness texture evaluates the maintenance of the structure of the sample and the force that prevents initial decomposition upon biting (Shahbazi et al., 2021). The lower springiness and cohesiveness of soy TVP can be attributed to the lower dry feed rate used in texturization associated implying a limitation on protein-protein interactions from higher moisture conditions (T. Kim, 2018).

The chewiness of lentil TVP samples generally increased with an increase in protein content with trial 5 with 40% pea protein isolate possessing the highest chewiness. Notably, TVP samples produced from trial 9 with 15% pea protein isolate and 1% calcium hydroxide (Table 4.2) had a lower chewiness compared to trials 6 and 8 with 10% pea protein isolate. The lower chewiness of trial 8 lentil TVP can be attributed to the alkaline conditions created by calcium hydroxide which as discussed in earlier sections limits protein-protein interactions during texturization .

Compared to trial 4 and 5 lentil TVP samples with higher pea protein isolate, soy TVP was found to have a lower chewiness texture (Table 4.5). The lower chewiness of soy protein concentrate can be attributed to extrusion at lower dry feed rates of 71% compared to lentil TVP samples extruded at 88% feed rate (Table 4.2). According to (Lin et al., 2002), lower moisture

content (higher feed rates) in extrusion increases die pressure and temperature resulting in a more layered and fibrous structure with a chewier and more cohesive texture. The higher chewiness of lentil TVP samples from trial 4 and 5 attributed to the higher extrusion dry feed rates (Table 4.2), is made possible by the characteristic lower bulk density (Funke et al., 2022) and higher protein solubility (Jarpa-Parra, 2018) of lentil protein compared to soy protein concentrate (Deak et al., 2008). Notably, higher dry feed rates were explored for soy protein concentrate but did not result in texturization (Table 4.1) due to the bulkiness of soy protein concentrate which causes the extruder to over-torque at high feed rates. Conversely lower feed rates explored in the development of lentil TVP resulted in partial texturization (Table 4.1).

The resilience of TVP samples was seemingly dependent on protein content with samples produced in trials 4, 5 and 10 with higher protein contents having higher resilience compared to samples produced in trials 6, 8 and 9. Resilience denotes the resurgence of a product to its original height after compression (Texture Technologies, 2020) and higher protein content generally results in higher texturization which creates firm and dense structures (Riaz, 2011) which present higher resilience.

Conclusion

The trials employed in the development of lentil texturized vegetable protein (TVP) in this study highlight strategies explored in recent studies to develop and improve the quality of lentil and pulse TVP. The significant higher water binding capacity and lower water solubility index of lentil TVP samples compared to lentil protein concentrates indicate the successful protein texturization. Generally, physical properties of lentil TVPs were dependent on increasing protein content and extrusion conditions of increasing dry feed rate and increasing screw speed. A

change in pH was also observed to influence physical properties of lentil TVP samples, despite exploring the addition of gums, salts, transglutaminase and cross-linking agents. Higher firmness texture and toughness were observed with the addition of citric acid and lower firmness texture and toughness were observed with the addition of calcium hydroxide.

The effect of increasing protein contents on texturization is also observed during texture profile analysis where trials with higher protein contents had higher chewiness compared to lower protein trials. The reference TVP produced from soy protein concentrate presented higher water binding capacity, firmness, and toughness at 75% strain, as well as hardness and gumminess at 50% strain compared to the developed lentil TVP samples. However, higher springiness, cohesiveness, and chewiness were seen in lentil TVP samples with higher contents of pea protein isolate (30-40% w/w, d.b. in dry feed) compared to the developed soy TVP. This suggests a market potential for lentil TVP not only as an allergen-free and gluten-free meat analog but providing a desirable springy, cohesive and chewy texture in vegetarian meal applications. The novelty of a springy, cohesive, and chewy lentil TVP would benefit from research on using lentil protein isolate to increase the protein content and create a 100% lentil TVP. Additionally, studies on the effect of pH on the textural properties of lentil TVP would help to improve firmness texture and toughness, found to be lacking in comparison to soy TVP.

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Tables

Table 4.14: Ingredient combinations and processing parameters explored in the development of lentil TVP.

Main ingredient	Other ingredients	Dry feed rate (%, d.b.)	Screw speed (rpm)	Texturized ?
Richlea protein concentrate	Xanthan gum (0.2%), calcium hydroxide (0.06%)	77	250-300	No
	xanthan gum (0.2%), L-cysteine (0.1%), calcium hydroxide (0.5%)	85	250	Some (1)
Red Crimson protein concentrate	NA	83	325-350	No
	calcium hydroxide (0.1%, 1%)	77	250	No
	calcium hydroxide (1%), sodium bisulfite (1%)	77-87	250-300	No
	calcium hydroxide (0.1%), sodium bisulfite (1%), locus bean gum (0.2%)	77-83	250-350	No
	guar gum (5%), L-cysteine (0.18%)	77	400	No
	pea protein isolate (3.75%, 1.5%, 5%, 10%, 5%), guar gum (1.875%, 7%, 5%)	80	250-400	No
	guar gum (5%, 5%), d-methionine (0.18%, 0.18%), L-cysteine (0.18%, 0.18%), transglutaminase (0.1%)	77, 83	250-300, 400	No
	pea protein isolate (5%, 47.5%), guar gum (5%, 5%), calcium hydroxide (0.12%), sodium bisulfite (0.10%, 0.11%)	81	250-375, 150-400	No
	rice protein concentrate (10%, 22%), pea protein isolate (20%), guar gum (2%)	71	300	Some
	soy protein concentrate (10%, 30%)	77-83	400	Some
	pea protein isolate (10-40%), transglutaminase (0.2%), L-cysteine (0.18%), D-methionine (0.18%), calcium chloride (1%), citric acid (1%), calcium hydroxide (0.1%, 1%), sodium bisulfite (0.1%)	88	400	Successful (2-9)
Soy protein concentrate	None	77, 63-69	250, 200-300	No
	None	71	350	Successful (10)

The numbers following the terms “Some” or “Successful denotes the trial numbers in Table 4.2.

Table 4.15: Successful ingredient combinations and processing parameters explored in the development of Lentil TVP.

Trial	Main ingredient (kg, d.b.)	Pea Protein Isolate (kg, d.b.)	Additives (kg, w.b.)	Protein content (% d.b.)	Dry feed rate (% w.b.)	Screw speed (rpm)	Objective	Results
1	Richlea protein concentrate (9.74 kg)	0.0	xanthan gum (0.2 kg), L-cysteine (0.01 kg), calcium hydroxide (0.05kg)	47	77	300	Determine the effect of the addition of a gum and a cross-linking agent on lentil TVP development and quality at a basic pH	Some texturization but sample produced is easily squished between the fingers when hydrated.
2	Red Crimson protein concentrate (9.48 kg)	0.25 (Ingredion)	guar gum (0.25 kg), sodium meta bisulfite (0.01 kg), calcium hydroxide (0.012 kg)	55	77	300	Determine the effect of increased protein content, and the addition of a gum and a cross-linking agent on lentil TVP development and quality at a basic pH	Some texturization but sample produced is easily squished between the fingers when hydrated.
3	Red Crimson protein concentrate (6.94 kg)	3 (AGT)	transglutaminase (0.02 kg), L-cysteine (0.018 kg), D-methionine (0.018 kg)	63	88	400	Determine the effect of an increased protein content the addition of cross-linking agents and increasing dry feed rate and screw speed on lentil TVP development and quality	Successful texturization. TVP produced was firm and tough after hydration.
4	Red Crimson protein concentrate (7 kg)	3 (AGT)	None	64	88	400	Determine the texturization quality of a lentil protein concentrate-pea protein isolate mix in the absence of enzymes and cross-linking agents	Successful texturization. No difference in the firmness and toughness with the absence of protein enzymes and cross-linking agents

Table 4.2 Continued

Trial	Main ingredient (kg, d.b.)	Pea Protein Isolate (kg, d.b.)	Additives (kg, w.b.)	Protein content (% d.b.)	Dry feed rate (% w.b.)	Screw speed (rpm)	Objective	Results
5	Red Crimson protein concentrate (6 kg)	4 (Ingredion)	None	66	88	400	Determine the texturization quality of a lentil protein concentrate with an increased pea protein isolate content	Successful texturization. TVP produced had a higher firmness and toughness.
6	Red Crimson protein concentrate (9 kg)	1 (AGT)	None	57	88	400	Determine the texturization quality of a lentil protein concentrate with a lower pea protein isolate content	Successful texturization. TVP produced had a lower firmness and toughness.
7	Red Crimson protein concentrate (8.9 kg)	1 (AGT)	calcium chloride (0.1 kg)	57	88	400	Determine the effect of calcium chloride salt on the physical and textural properties of lentil TVP	No detectable difference in physical and textural properties of lentil TVP with the addition of calcium chloride.
8	Red Crimson protein concentrate (8.9 kg)	1 (AGT)	citric acid (0.1 kg)	57	88	400	Determine the texturization quality of a lentil protein concentrate with a lower pea protein isolate content at a lower pH	Successful texturization. TVP produced at lower pH had a higher firmness and toughness than TVP with similar pea protein isolate.
9	Red Crimson protein concentrate (8.4 kg)	1.5 (AGT)	calcium hydroxide (0.1 kg)	58	88	400	Determine the texturization quality of a lentil protein concentrate with a lower pea protein isolate content at a basic pH	Successful texturization. TVP produced at a higher pH had a lower firmness and toughness than TVP with similar pea protein isolate.

Table 4.2 Continued

Trail	Main ingredient (kg, d.b.)	Pea Protein Isolate (kg, d.b.)	Additives (kg, w.b.)	Protein content (% d.b.)	Dry feed rate (% w.b.)	Screw speed (rpm)	Objective	Results
10	Soy protein concentrate			76	71	350	Produce a texturized vegetable protein as a reference for the development of a lentil TVP	Reference produced had the highest firmness and toughness of all samples produced

Table 4.16: Water binding capacity, water solubility index and texture properties of successful TVP trials.

Trial	Water binding capacity (g/kg)	Water solubility index (g/kg)	Texture	
			Firmness (kg)	Toughness (kg.sec)
1-RP-XG-CaOH-cy	2885.83 ± 39.71 ^d	283.33 ± 52.04 ^b	2.22 ± 0.2 ^f	2.24 ± 0.16 ^f
2-RCP-PI-GG-CaOH-Na ₂ S ₂ O ₅	3015.83 ± 45.02 ^c	225.00 ± 25.00 ^{bc}	3.23 ± 0.34 ^{de}	2.53 ± 0.36 ^{ef}
3-RCP-30%PI-TG-cy-me	3137.50 ± 40.93 ^b	216.67 ± 14.43 ^{bc}	3.84 ± 0.66 ^{cd}	3.82 ± 0.66 ^c
4-RCP-30%PI	3135.83 ± 14.43 ^b	175.00 ± 66.14 ^{cd}	4.24 ± 0.29 ^c	4.06 ± 0.36 ^c
5-RCP-40%PI_Inng	2623.33 ± 18.43 ^f	258.33 ± 57.74 ^{bc}	6.91 ± 0.97 ^b	6.78 ± 0.76 ^b
6-RCP-10%PI	2708.33 ± 28.43 ^e	241.67 ± 14.43 ^{bc}	2.93 ± 0.29 ^e	2.49 ± 0.31 ^{ef}
7-RCP-10%PI-CaCl ₂	2670.00 ± 36.83 ^{ef}	258.33 ± 14.43 ^{bc}	3.12 ± 0.28 ^e	3.00 ± 0.27 ^{de}
8-RCP-10%PI-CA	2640.00 ± 21.79 ^{ef}	266.67 ± 28.87 ^b	3.97 ± 0.25 ^c	3.44 ± 0.28 ^{cd}
9-RCP-15%PI-CaOH	3176.67 ± 89.24 ^b	258.33 ± 14.43 ^{bc}	2.61 ± 0.21 ^{ef}	1.94 ± 0.22 ^f
10-SPC	4063.33 ± 64.44 ^a	108.33 ± 28.87 ^d	13.55 ± 1.30 ^a	11.66 ± 1.24 ^a
Red crimson protein concentrate	1803.33 ± 44.74 ^g	1058.33 ± 28.87 ^a		
Richlea protein concentrate	1781.67 ± 88.82 ^g	1066.67 ± 128.29 ^a		

- a. For the same column, values (means ± SD) followed by the same letter are not significantly different based on Fisher's least significant difference test ($\alpha = 0.05$).
- b. Full names and recipes of trial codes used in Table 4.3 found in Table 4.2

Table 4.17: Regression coefficients for the relationship between Water binding capacity, water solubility index and texture properties of successful TVP trials.

	Firmness	Toughness
Intercept	-3.79x10 ¹ ***	-3.29x10 ¹ ***
WBC	1.462x10 ⁻² ***	1.27x10 ⁻² ***
WSI	1.78x10 ⁻¹ ***	1.64x10 ⁻¹ ***
WBC* WSI	-6.30x10 ⁻⁵ ***	-5.78x10 ⁻⁵ ***

Values superscripted with “***”, “**”, “*” and “.” indicated that the regression provided noticeable evidence at $p < 0.001$, $p < 0.01$, $p < 0.05$ and $p < 0.1$ respectively.

Table 4.18: Texture profile analysis of selected lentil TVP trials compared to soy TVP.

Trial	Hardness (g)	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
4-RCP-30%PI	1157.98 ± 266.41 ^c	0.42 ± 0.01 ^a	0.11 ± 0.01 ^a	131.01 ± 20.76 ^c	55.67 ± 9.32 ^b	0.036 ± 0.003 ^a
5-RCP-40%PI_Ing	2085.47 ± 165.49 ^b	0.38 ± 0.05 ^{abc}	0.11 ± 0.01 ^{ab}	224.36 ± 15.07 ^b	84.59 ± 12.97 ^a	0.034 ± 0.002 ^a
6-RCP-10%PI	825.14 ± 149.01 ^{cd}	0.40 ± 0.03 ^b	0.10 ± 0.01 ^{bc}	78.26 ± 12.29 ^{de}	31.22 ± 5.46 ^c	0.029 ± 0.003 ^b
8-RCP-10%PI-CA	939.71 ± 100.46 ^{cd}	0.34 ± 0.07 ^{bc}	0.10 ± 0.01 ^{bc}	89.25 ± 7.33 ^d	30.22 ± 4.99 ^c	0.027 ± 0.003 ^b
9-RCP-15%PI-CaOH	607.95 ± 101.84 ^d	0.33 ± 0.08 ^c	0.09 ± 0.01 ^c	51.29 ± 7.33 ^e	16.58 ± 3.64 ^d	0.028 ± 0.003 ^b
10-SPC	6105.77 ± 789.17 ^a	0.06 ± 0.003 ^d	0.09 ± 0.004 ^c	520.75 ± 46.52 ^a	29.08 ± 2.31 ^c	0.035 ± 0.002 ^a

a. For the same column, values (means ± SD) followed by the same letter are not significantly different based on Fisher's least significant difference test ($\alpha = 0.05$).

b. Full names of trial codes used in Table 4.5 found in Table 4.2

Figures



1-RP-XG



2-RCP-PI-GG



3-RCP-30%PI-TG-cy-me



4-RCP-30%PI



5-RCP-40%PI_Ing



6-RCP-10%PI



7-RCP-10%PI-CaCl₂



8-RCP-10%PI-CA



9-RCP-15%PI-CaOH



10-SPC

Figure 4.8: Lentil TVP samples produced from trials 1-9 from left to right and the reference soy TVP in the last cell.



1-RP-XG



2-RCP-PI-GG



3-RCP-30%PI-TG-cy-me



4-RCP-30%PI



5-RCP-40%PI_Ing



6-RCP-10%PI



7-RCP-10%PI-CaCl₂



8-RCP-10%PI-CA



9-RCP-15%PI-CaOH



10-SPC

Figure 4.9: Hydrated lentil TVP samples produced from trials 1-9 from left to right and the reference soy TVP in the last cell.

CHAPTER FIVE

CONCLUSION

This study demonstrates that lentil flour and its fractionates of lentil starch concentrate and lentil protein concentrate are viable ingredients for use in the development of value-added food products of lentil puffs, lentil couscous and lentil texturized vegetable protein (TVP) with a twin-screw pilot-scale extruder. Regarding the development of lentil puffs, the overall liking and texture liking scores were dependent on all three formulation factors, lentil flour, lentil starch concentrate, and lentil protein concentrate. An optimum lentil puff formulation was predicted based on sensory overall liking to contain 50% (w/w, d.b.) of lentil starch concentrate and 25% of both lentil flour and lentil protein concentrate, produced at an extrusion feed rate of 20 kg/h, a moisture rate of 10% (w/w) and a screw speed of 350 rpm.

For the development of lentil couscous, increasing the weight ratio of lentil protein concentrate to lentil flour decreased the water absorption index, instrumental texture in cohesiveness, chewiness, and resilience of lentil couscous formulations. Higher sensory overall liking, appearance, flavor, and texture liking scores for lentil couscous samples were observed for formulations with lentil protein concentrate levels at 20% and a screw speed of 250 rpm. Clustering analysis revealed that participants who issued higher sensory liking scores were willing to buy the presented lentil couscous and on average were willing to spend \$3.37 for 10 oz of lentil couscous with 20% lentil protein concentrate and extruded at a 250 rpm screw speed.

The development of a lentil TVP with lentil protein concentrate revealed that the technological properties of lentil TVPs were dependent on the total protein content, extrusion conditions and pH. Adding pea protein isolate to lentil protein concentrate in formulations

increased the protein content to levels ideal for texturization. Increasing the extrusion dry feed rate and screw speed also increased the degree of texturization of ingredient mixes. Despite exploring the addition of gums, salts, transglutaminase as well as cross-linking agents, a change in pH, with additives of citric acid and calcium hydroxide, was the only strategy observed to influence the techno functional properties of lentil TVP samples. In comparison to the reference soy TVP produced from soy protein concentrate, lentil TVP samples produced from trials with higher contents of pea protein isolate ($\geq 30\%$, w/w, d.b. in the dry feed) presented higher instrumental springiness, cohesiveness, and chewiness texture.

This study demonstrated development of three lentil value-added extruded food products, highlighting the versatility and potential of lentil flour and its fractionates not only as a nutritious, allergen-free and gluten-free alternatives but capable of presenting sensory attributes desired by consumers and comparable to commercial ingredients like corn, wheat, and soy. Further studies are needed to understand the relationship between extrusion process responses such as die temperature, die pressure, and specific mechanical energy, and extrudate characteristics for scale-up production. This will be a step forward to truly add value and commercialize lentils geared towards increasing the incomes of Montana lentil farmers and encouraging the exploration of other pulses and specialty crops cultivated locally.

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