Techno-Functional and Sensory Properties of Salad Dressing-type Emulsions Prepared with Pulse Flours and Pulse Fractions

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Suggested Short Title: Pulse Flour and Fractions in Salad Dressing Applications

This thesis is dedicated to my beloved parents, Xihan Ma and Qin Wei,

and my soulmate Cheng Lu

Your love and smile has been my biggest encouragement to make this thesis possible

Foreword

The thesis is submitted in the form of original papers prepared for journal publications. The first two sections comprise a general introduction and a literature review presenting the theory and previous knowledge on this topic. The next seven sections contain the body of the thesis; each chapter represents a complete manuscript. The last section is a summary of the major conclusions. This format has been approved by the Faculty of Graduate Studies and Research, McGill University, and follows the conditions outlined in the Guidelines for Thesis Preparation, Thesis Specification, section 3 entitled "Traditional and manuscript-based theses" which are as follows:

"Candidates have the option of including, as part of the thesis, the text of a paper(s) submitted or to be submitted for publication, or the clearly duplicated text of a published paper(s). These texts must be bound as an integral part of the thesis.

If this option is chosen, connecting texts that provide logical bridges between the different papers are mandatory. The thesis must be written in such a way that it is more than a mere collection of manuscripts; in other words, results of a series of papers must be integrated.

The thesis must still conform to all other requirements of the "Guidelines for Thesis Preparation". The thesis must include: A Table of Contents, an abstract in English and French, an introduction which c1early states the rationale and objectives of the study, a comprehensive review of the literature, a final conclusion and summary, and a thorough bibliography of reference list.

Additional material must be provided where appropriate (e.g., in appendices) and in sufficient detail to allow a clear and precise judgment to be made of the importance and originality of the research reported in the thesis.

In the case of manuscripts co-authored by the candidate and others, the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent. Supervisors must attest to the accuracy of such statements at the doctoral oral defense. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the responsibilities of all authors of the co-authored papers. Under no circumstances can a co-author of any component of such a thesis serve as an examiner for that thesis. "

Abstract

Pulses, the low-fat dried seeds of legumes, including peas, chickpeas, lentils, beans and lupins, have received increased attention due to their numerous health-promoting benefits. Significant opportunities exist for using whole pulses as well as their fractions in a variety of innovative value-added food products. Salad dressings, which are important oil-in-water emulsions, account for a large part of the semi-solid foods market and are widely consumed in North America and in many other countries. The development of salad dressings supplemented with pulse flours and pulse fractions represents a novel avenue of study with great potential for improving the techno-functional and nutritional quality of salad dressing products by enhancing protein, fibre, vitamin and mineral content and, in some cases, promoting amino acid complementation.

The preliminary study focused on the characterization of selected raw and thermally treated pulse flours, since pre-cooking may be a potential way to enhance the nutritional value of pulse flours. The results of the scanning electron microscope analyses confirmed the significant differences in the effects of the treatments on the functional properties, particularly wet treatment, such as the increases in fat and water absorption capacity, gelling and emulsifying activity. The significant decrease observed in the total peak area of the pulse flavour profile (obtained by GC/MC method) induced by cooking pointed to a loss of volatile and/or hydrophilic compounds. A variety of alkyl pyrazines were detected in cooked pulses. The insights derived from the characterization of pulses could help in selecting the right starting materials for product development, thereby helping to meet related flavour and techno-functional challenges. The findings demonstrated the potential for using raw and thermally treated pulse flours in various food applications.

Salad dressing emulsions supplemented with raw and thermally treated pulse flours were developed. The effect of pulse addition on the dressing formulations was studied by comparing the physical properties of dressings prepared with or without supplementation. The results showed that lentil flours have considerable potential for use as an ingredient in salad dressings. The rheological parameters (including consistency coefficient, apparent viscosity, plateau modulus, and recoverable strain) were significantly increased, pointing to a thickening effect associated with pulse flour supplementation. Pre-boiling of pulse flours further increased the thickening effect, giving the highest rheological

properties observed. The relationship between the physical properties and structural characteristics was explored by comparing the rheological behaviour and the scanning electron microscope observations. The results of the quantitative descriptive analysis (evaluation of firmness) were consistent with the rheological data. All the dressings maintained acceptable stability over 28 days of storage. With respect to dressing appearance, lentil supplementation significantly increased the yellowness hue and the total colour intensity of the dressing samples.

The effects of reducing the fat and cholesterol (egg yolk) contents of the supplemented dressings, as well as increasing the amount of pulse flour added, upon the color and rheological properties were investigated by using a central composite design and response surface methodology. The results showed that changes in the main ingredients had a large effect on the responses observed. The scanning electron microscope results indicated that the dressings with lower oil and egg yolk contents had a less densely packed network and looser aggregated droplets. This finding was in good agreement with the observed response surface plots of the rheological parameters. Consumer sensory tests on showed that the dressings supplemented with whole green lentil flour, low concentrations of yellow pea flour, and chickpea flour with high oil content hold promise for commercial applications. The results should prove useful in selecting the most suitable ingredients for the production of supplemented salad dressings. Additionally, pulse protein fractions derived from green lentil, yellow pea and Desi chickpea were used in the preparation of pulse-supplemented dressings. The response surface plots showed that an increase in oil and emulsifier (pulse protein or egg yolk) concentration led to a linear or non-linear increase in the rheological and textural properties. Response surface methodology was used to optimize the salad dressing formulations based on selected response (dependent) variables. The validation test confirmed the overall adequacy of this modelling approach for predicting the behaviour of the dressing systems under different factor combinations, with the ultimate goal of meeting market specifications.

Miscellaneous full factorial design and response surface methodology were also employed in this research to explore the possibility of using various types of gum and combinations of gums to stabilize the lentil flour-supplemented dressings. The results

showed that an increase in both gum and oil concentrations resulted in increased emulsion firmness and viscosity as a result of the more compact emulsion structure and increased network formation. Large droplets (D[3,2] and D[4,3]) formed in the presence of increased gum contents at lower oil content. Validation tests showed the overall adequacy of the modelling approach for predicting variations in dependent variables of the dressings under optimal conditions.

The research work underpinning this thesis provides information to support the development of whole pulse flours and pulse fraction supplemented dressings with nutritional and techno-functional potential for commercial and industrial applications.

Resumé

Les légumineuses (sous forme de graines sèches avec une faible teneur en gras) tel que les petits pois, les pois chiches, les lentilles, les haricots et les graines de lupins, ont reçu une attention accrue en raison de leurs nombreux avantages favorables à la santé. Il existe plusieurs façons d'utiliser les légumineuses entières ainsi que leurs fractions dans une variété de produits alimentaires innovants à haute valeur ajoutée. Les vinaigrettes, qui sont des émulsions huile-en-eau, représentent une grande partie du marché des aliments semi-solides et sont largement consommés en Amérique du Nord ainsi que dans de nombreux autres pays. Le développement de vinaigrettes supplémentées avec des farines de légumineuses ou des fractions de légumineuses représente une nouvelle avenue avec un grand potentiel pour améliorer la qualité technico-fonctionnelle et nutritionnelle des vinaigrettes en augmentant la teneur en protéines, en fibres, en vitamines et minéraux, et dans certains cas complémentant les acides aminés du produit.

L'étude préliminaire a porté sur la caractérisation de certaines farines de légumineuses traitées thermiquement ou non, car la pré-cuisson peut être potentiellement un moyen d'améliorer la valeur nutritionnelle des farines de légumineuses. Les résultats des analyses au microscope électronique à balayage ont révélé des différences significatives selon les traitements thermiques sur les propriétés fonctionnelles particulièrement pour les traitement thermiques impliquant de l'eau, comme sur l'augmentation de la capacité d'absorption de la matière grasse et de l'eau, ainsi que sur l'activité gélifiante et d'émulsifiante. Au chromatographe en phase gazeuse couplé à un spectrophotomètre de masse, une importante diminution a été observée pour le total de l'aire sous les pics lors du dosage des saveurs des légumineuse induites par la cuisson indiquant une perte de composés volatils et/ou hydrophile. Une variété d'alkyle pyrazines a été détectée dans les légumineuses cuites. Les connaissances issues de la caractérisation des légumineuses pourraient aider à choisir les matières premières adéquates à la fabrication de produits alimentaires, contribuant ainsi à relever les défis reliés à la saveur et aux techno-fonctionnalités. Les résultats ont démontré la possibilité d'utiliser des farines de légumineuses brutes et traitées thermiquement dans des applications alimentaires diverses.

Des émulsions de vinaigrettes supplémentées avec des farines de légumineuses brutes et traitées thermiquement ont été développées. L'effet de l'addition de légumineuses dans les formulations des vinaigrettes a été étudié en comparant les propriétés physiques des vinaigrettes préparées avec ou sans supplémentation. Les résultats ont montré que les farines de lentilles ont un potentiel considérable comme ingrédient dans les vinaigrettes. Les paramètres rhéologiques ont été considérablement augmentés, montrant un effet d'épaississement associé à la supplémentation de la farine de légumineuses. L'addition de farines de légumineuses pré- bouillies a en outre augmenté l'effet épaississant, conduisant à des propriétés rhéologiques observées plus élevées. La relation entre les propriétés physiques et les caractéristiques structurelles a été explorée en comparant le comportement rhéologique et les observations faites à l'aide d'un microscope électronique à balayage. Les résultats de l'analyse descriptive quantitative (évaluation de la fermeté) étaient compatibles avec les données rhéologiques. Toutes les vinaigrettes ont maintenu une stabilité acceptable de plus de 28 jours d'entreposage. En ce qui concerne l'apparence, la supplémentation avec de la farine de lentilles a augmenté de façon significative la teinte jaune et l'intensité totale des couleurs des échantillons de vinaigrettes.

Les effets de la réduction des matières grasses et du cholestérol (jaune d'oeuf) dans les vinaigrettes supplémentées et de l'augmentation de la quantité de farine de légumineuses ajoutée, sur la couleur et les propriétés rhéologiques ont été étudiés en utilisant un plan central composite et une méthode de surface de réponse. Les résultats ont montré que des changements des principaux ingrédients ont eu un effet important sur les réponses observées. Les résultats au microscope électronique à balayage ont indiqué que les vinaigrettes contenant moins d'huile et de jaune d'œuf disposaient d'un réseau moins dense et des gouttelettes agrégées plus souples. Cette constatation est en accord avec les courbes de surface de réponses observées pour les paramètres rhéologiques. Des tests d'analyses sensorielles ont montré que les vinaigrettes supplémentées avec de la farine de lentilles vertes entières, de faibles concentrations de farine de pois jaune ou de farine de pois chiche avec haute teneur en huile, sont prometteuses pour des applications commerciales. Les résultats devraient s'avérer utiles dans le choix d'ingrédients plus appropriés pour la production de vinaigrettes supplémentées. Des fractions protéiques de

légumineuses provenant de lentilles vertes, pois jaune et le pois chiche Desi ont, également, été utilisé dans la préparation de vinaigrettes supplémentées. Les courbes de surface de réponses ont montré que l'augmentation de la concentration de l'huile et de l'émulsifiant (protéine de légumineuse ou le jaune d'œuf) conduit à une augmentation linéaire ou non linéaire dans les propriétés rhéologiques et texturales. La méthode de surface de réponse a été utilisée pour optimiser la formulation des vinaigrettes et a été basée sur les variable réponses (dépendantes) choisies. Le test de validation a confirmé la pertinence globale de cette approche de modélisation pour prédire le comportement des systèmes vinaigrettes avec différentes combinaisons de facteurs, dont le but ultime est de répondre aux spécifications du marché.

Divers plans factoriels complets et méthodes de surface de réponses ont également été utilisés dans cette recherche pour explorer la possibilité d'utiliser différents types de gomme et combinaisons de gommes pour stabiliser les vinaigrettes supplémentées avec de la farine de lentilles entières. Les résultats ont montré que l'augmentation à la fois de la gomme et des concentrations d'huile a entraîné une augmentation de la fermeté et de la viscosité des émulsions dues à une structure de l'émulsion plus compacte et à la formation accrue d'un réseau. De grosses gouttelettes (*D [3,2]* et *D [4,3]*) ont été formées à cause de l'augmentation de la concentration de gomme et du faible contenu en huile des vinaigrettes. Des tests de validation ont montré la pertinence globale de l'approche par modélisation pour prédire les variations de variables dépendantes des vinaigrettes dans des conditions optimales.

Les travaux de recherche de cette thèse fournissent des informations importantes et pertinentes pour permettre le développement de vinaigrettes supplémentées avec des farines de légumineuses entières ou avec des fractions protéiques avec un potentiel nutritionnel et techno-fonctionnel intéressants pour des applications commerciales et industrielles.

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Contributions of Authors

This thesis is submitted in the form of original papers prepared for refereed journal publications. The contributions of the different authors to the prepared manuscripts are as follows:

The author of this thesis, Zhen Ma, is the Ph.D. candidate who designed and conducted the experiments and handled data gathering and analysis, as well as manuscript preparation, under the guidance of Dr. Joyce Boye, Dr. Shiv Prasher and Dr. Benjamin Simpson.

Dr. Shiv Prasher, a co-supervisor and director of the thesis, co-authored all the manuscripts, and helped plan and direct the research as well as co-edit and review the scientific papers. Dr. Benjamin Simpson, a thesis co-supervisor and director, provided valuable guidance and knowledge for the research work, as well as editorial support throughout the thesis and preparation of the associated scientific research papers. Dr. Joyce Boye is the Principal Investigator of the research under whose guidance the overall research thesis was planned and conducted. She helped the author of this thesis identify and solve problems and provided direct advice as the research work progressed. Dr. Boye co-authored all the manuscripts which have been published or submitted for publication and helped correct, edit and review the papers.

Diane Montpetit co-authored the third and the sixth chapters and assisted with the experiments of the scanning electron microscopy work. Dr. Linda Malcolmson, who co-authored the third and sixth chapters, provided the pulse flour samples. Dr. Kevin Swallow, co-author of the sixth chapter, helped the candidate conduct the consumer acceptability testing at the Consumer Product Testing Centre in Alberta. Jacinthe Fortin, who co-authored the fifth chapter, helped with the sensory evaluation work done at Agriculture and Agri-Food Canada's Food Research and Development Centre in Saint-Hyacinthe. Dr. Sorayya Azarnia, co-author of the fourth chapter, assisted with data collection in the GC/MS experiments.

The details of the papers that have been published or submitted are provided below:

List of publications and scientific presentations

A: This thesis has been published or prepared for submission as follows:

Ma, Z., Boye, J. I., Simpson, B. K., Prasher, S. O., Montpetit, D., & Malcolmson, L. (2011). Thermal processing effects on the functional properties and microstructure of lentil, chickpea, and pea flours. *Food Research International*, *44*(8), 2534-2544.

Boye, J. I., **Ma, Z.** (2012). Finger on the pulse. *Food Science & Technology*, *26*(2), 20-24.

Ma, Z., Boye, J. I., Advances in the design and production of reduced-fat and reduced-cholesterol salad dressing and mayonnaise: A review. *Food and Bioprocess Technology*, (accepted).

Ma, Z., Boye, J. I., Fortin J., Simpson, B. K., Prasher, S. O., Rheological, physical stability, microstructural and sensory properties of salad dressings supplemented with raw and thermally treated lentil flours. *Journal of Food Engineering*, (under revision).

Ma, Z., Boye, J. I., Swallow K., Montpetit, D., Malcolmson L., Simpson, B. K., Prasher, S. O., Techno-functional characterization of pulse flour supplemented salad dressing emulsions. *Journal of Food Engineering*, (submitted).

Ma, Z., Boye, J. I., B. K., Prasher, S. O., Preparation of salad dressing emulsions using lentil, chickpea and pea protein isolates: a response surface methodology study. *Journal of food Science*, (submitted).

Boye, J. I., **Ma, Z.** Advances in legume processing perspective from North American Food Industry. In: Food Processing: Principles and Applications, 2nd edition. (S. Clark, S. Jung, & B. Lamsal, eds.). Wiley-Blackwell (Expected to be published in May, 2013).

B: Part of this thesis has been or will be presented at scientific conferences as follows:

Ma, Z., Boye, J. I., Simpson, B. K., Prasher, S. O., Montpetit, D., & Malcolmson, L., "Influence of thermal processing on the functional properties and microstructure of lentil, chickpea, and pea flours." *The 2010 Canadian Institute of Food Science and Technology/Agriculture and Agri-Food Canada (CIFST/AAFC) Conference*, Winnipeg, Manitoba, Canada, May 30 to June 1, 2010 (Poster Presentation).

Ma, Z., Boye, J. I., Simpson, B. K., Prasher, S. O., Montpetit, D., "Microstructure, physical stability and rheological properties of salad dressing-type emulsions supplemented with pulse flours." *The 2010 PURENet Annual General Meeting & Conference*, North Calgary, Alberta, November 1, 2010 (Poster Presentation).

Ma, Z., Boye, J. I., Simpson, B. K., Prasher, S. O., Montpetit, D., "Microstructure,

physical stability, and rheological properties of salad dressing-type emulsions supplemented with pulse flours, *8th Canadian Pulse Research Workshop*, Calgary, Alberta, November 3 to 5, 2010 (Oral Presentation)

Ma, Z., Boye, J. I., Simpson, B. K., Prasher, S. O., Fortin J., "Influence of processing on the rheological, physical stability, microstructural and sensory properties of lentil flour-supplemented salad dressings." *12th Annual Meeting of the Institute of Food Technologists (IFT)*, Las Vegas, Nevada, USA, June 25 to 28, 2012 (Poster Presentation).

Ma, Z., Boye, J. I., B. K., Prasher, S. O., "Use of response surface methodology to optimize conditions for the production of pulse protein-supplemented salad dressing using protein isolates from lentil, chickpea and pea." *12th Annual Meeting of the Institute of Food Technologists (IFT)*, Las Vegas, Nevada, USA, June 25 to 28, 2012 (Poster Presentation).

Contributions to Knowledge

The present work contributes to the expansion of the scientific knowledge in the general area of salad dressing supplementation using pulse fractions as techno-functional ingredients and studying their influences on the colour, rheology, texture, particle size and sensory properties of the supplemented products. The specific contributions of this thesis to knowledge are described below:

1) The research carried out in this thesis on the development of salad dressings supplemented with pulse flour and pulse fractions represents a new avenue of research since no such studies have been undertaken to date.

2) The present work expands the knowledge of the impact of thermal treatments applied to pulse flours as apposed to pulse seeds on trypsin inhibitor activity, microstructure, and functional properties; and the first study to use scanning electron micrographs of dressings to confirm the increased gelling capacity associated with pre-boiling treatment, as well as to compare the disparities in the results obtained in other studies. Little scientific information is available on the volatile flavour profiles of different pulse varieties, especially those subjected to different thermal and drying treatments. The present work expands also knowledge of the volatile flavour profiles of raw and thermally processed pulse varieties.

3) This is the first study to demonstrate that pulse flour supplementation increased the viscoelasticity of salad dressing-type emulsions, with a significant increase in several rheological parameters, which point to the thickening effect of pulse flours. In addition, thermal processing of pulses, specifically pre-boiling-freeze/spray-drying, significantly increased the thickening effect of pulse flours in supplemented dressings compared with dressing prepared with raw samples, as evidenced by rheological testing, sensory testing and scanning electron microstructural observations. This finding has economic significance, since the information is useful for the development of commercial and industrial applications using pulses as techno-functional food ingredients.

4) Multiple regression models were generated to predict the physical behaviour of salad dressing systems supplemented with pulse flours and pulse protein isolates under a wide range of main component compositions, using a central composite design. Desirable formulations were developed using response surface methodology. The adequacy of these

models was validated under optimized conditions. The modelling used in this study provides a scientific approach for designing and improving the formulation of pulse fraction-supplemented salad dressings with desirable physical properties. This is also the first comprehensive analysis of the effects of main ingredient contents on the physical properties of salad dressings supplemented with pulse fractions. Several rheological parameters obtained in steady-state flow tests, dynamic oscillation tests as well as creep and recovery tests were selected as responses and used to compare supplemented salad dressings based on different formulations. The connection between the physical properties and structural characteristics was explored by comparing the rheological behaviour and scanning electron microscope observations for various formulations. Quantitative descriptive analysis and consumer evaluation testing were used to study the sensory attributes and to identify product acceptability.

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Nomenclature

a_w	Water activity
Gi	Gas entrapped in the foam (%)
FE	Foam expansion (%)
R5	Liquid retained in foam after 5 min (%)
G′	Storage modulus (Pa)
G″	Loss modulus (Pa)
G_N^0	Plateau modulus (Pa)
δ	Phase angle
n value	Flow behavior index (dimensionless)
m value	Consistency coefficient ($Pa.s^n$)
η_{ap}	Apparent viscosity (Pa.s)
η	Shear viscosity (Pa.s)
γ	Shear rate (s^{-1})
σ	Shear stress (Pa)
σ_0	Yield stress (Pa)
Q(t)%	Recoverable strain
J	Compliance (Pa^{-1})
C	Chroma
ΔE	Color difference
ϕ	Disperse phase volume fraction (%)
D*[3,2]*	Specific weighted mean diameter (μm)
D*[4,3]*	Volume weighted mean diameter (μm)
η^*	Complex viscosity (Pa.s)
*L**	Lightness
*a**	Red (+*a**) and green (-*a**) coordinate
*b**	Yellow (+*b**) and blue (-*b**) coordinate
d(*v*,0.1)	Particle size value (μm) below which 10% of the cumulative distribution lies.

List of Abbreviation

BS	Back scattering
C	Control
CCD	Central composite design
DCPI	Desi chickpea protein isolate
DDC	Dehulled Desi chickpea
DGL	Dehulled green lentil
DKC	Dehulled Kabuli chickpea
DRL	Dehulled red lentil
DYP	Dehulled yellow pea
EAI	Emulsifying activity index
ESI	Emulsifying stability index
FAC	Fat absorption capacity
GC/MS	Gas chromatography/mass spectrometry
GA	Gum Arabic
GG	Guar gum
GLPI	Green lentil protein isolate
GLWH	Green lentil with hull
HS-SPME	Head space-solid phase microextration
LDL	Low density lipoprotein
LVE	Linear viscoelastic region
O/W	Oil in water
PCFD	Pre-cooked-freeze-dried
PCSD	Pre-cooked-spray-dried
PCSE	Pre-cooked seed
PCSL	Pre-cooked slurry
PE	Pectin
PGA	Propylene glycol alginate
QDA	Quantitative descriptive analysis
R	Raw
RF	Roasted flour

RLWH	Red lentil with hull
RPA	Relative peak area
rpm	Revolutions per minute
RS	Roasted seed
RSM	Response surface methodology
SEM	Scanning electron microscopy
TAC	Total area counts
TIA	Trypsin inhibitor activity
WHC	Water holding capacity
W/O	Water in oil
XG	Xanthan gum
YPPI	Yellow pea protein isolates

Chapter 1. Introduction

1.1 General Introduction

Pulses, the edible seeds of legume plants, including dry beans, peas, chickpeas and lentils, are emerging ingredients in North America. Pulses are gaining recognition for their potential as ingredients that can boost the nutritional profile of foods and for their environmental benefits. They are a good or excellent and inexpensive source of protein, complex carbohydrates, fibre, and minerals. Consumption of pulses has been associated with many health benefits, including the reduction of the risks of type 2 diabetes and cardiovascular disease and prevention of the onset of various cancers (Lanza et al. 2006; Trinidad et al. 2010; Barbana et al. 2011; Meisel 2006). Canada is one of the largest pulse producers and exporters in the world. Unfortunately, the market for whole pulse seeds and pulse foods in North America is small. Around 75% of Canadian pulse production is exported to over 150 different markets and accounts for nearly 40% of the global pulse trade (AAFC 2012).

With the growing demand for adequate supplies of food to feed the ever-increasing world population, opportunities exist for food processors to develop novel foods fortified with pulse ingredients or based primarily on pulse ingredients, in order to exploit their health benefits and convenience as well as the techno-functional properties of pulse flours and fractions. These foods could also serve as hypoallergenic alternatives for individuals with food sensitivities. Supplementation of traditional foods with pulse flours and fractions can help to improve the nutritional quality of foods by enhancing the protein, fibre, vitamin, mineral content and, in some cases, promoting amino acid complementation. A significant amount of work has been done in the last two decades on the primary, secondary, and tertiary processing of pulses. This has led to the commercial use of pulse flours and fractions as well to the development of some novel pulse-supplemented foods such as breads, high fibre snacks, and spaghetti (Serdaroglu et al. 2005; Lazou & Krokida 2009; Rasmay et al. 2001; Petitot et al. 2010). None of the studies done so far have focused on salad dressing supplementation with pulse flours or pulse fractions. Salad dressings are important oil-in-water

emulsions that are widely consumed in North America and in many other countries. Salad dressings therefore represent a good avenue for using pulse ingredients in order to develop innovative value-added products. Significant opportunities exist for using pulses in the development of health promoting foods as consumers are looking for functional foods with disease prevention qualities. In addition to nutritional value, appearance, and texture attributes, sensory and rheological characteristics are important for high quality food products. This research project focusing on the development and optimization of new, value-added emulsion type food products using pulse ingredients, was undertaken with the aim of increasing our understanding of how different factors influence emulsion characteristics of pulse supplemented salad dressing. The expected outcome is increased consumption of pulses in the North American diet, as well as creating new market outlets abroad.

1.2 Objectives of Study

The principal objective of this study was to use pulse flours and fractions to supplement salad dressings and investigate the techno-functional and sensory properties of the final products.

The specific objectives of this study are listed below, along with the related chapters that describe each study component in detail.

(1) Investigate the effects of thermal processing (roasting and boiling) on the trypsin inhibitor activity, functional properties, and scanning electron microstructure of flours prepared from different varieties of lentil, chickpea, and pea; and correlate certain functional properties with changes in the microstructural characteristics (Chapter 3).

(2) Identify and semi-quantify the volatile flavour composition of navy bean, red kidney bean, green lentil, and yellow pea, and gain insights into the flavour changes caused by thermal processing, including cooking and drying (i.e., roasting of flour, roasting of seeds, pre-cooking seeds, pre-cooking slurry, pre-cooking-freeze-drying, and pre-cooking-spray-drying) by using the headspace-solid phase microextraction-gas chromatography-mass spectrometry method (Chapter 4).

(3) Examine the effect of processing techniques (raw, roasting of seeds, roasting of flour, pre-boiling-freeze-drying, and pre-boiling-spray-drying) applied to lentils on the colour, physical stability, and rheological, microstructural, and sensory properties of lentil-flour supplemented salad dressings; and explore the relationship between their rheological behaviour and their sensory attributes (Chapter 5).

(4) Evaluate the effect of three main components, namely the concentrations of pulse flour, egg yolk, and oil, on the rheological and colour characteristics of pulse flour-supplemented salad dressings by using a three-factor face-centered central composite design (CCD) and response surface methodology (RSM); and examine the sensory properties and consumer acceptability of selected dressings samples (Chapter 6).

(5) Study the physical properties (i.e., static and dynamic rheological behaviour, texture, color, water activity, and droplet size) of salad dressings prepared with proteins from lentil, pea, and chickpea, as affected by pulse protein, egg yolk, and oil content, using response surface methodology; and verify the adequacy of the generated multiple regression models (Chapter 7).

(6) Systematically examine the impacts of different types and concentrations of gums and gum blends (including xanthan gum, and mixtures of xanthan and gum arabic, xanthan gum and propylene glycol alginate, xanthan gum and pectin, as well as xanthan gum and guar gum) on the physical properties (rheological, textural, colour and particle-size characteristics) of lentil flour-supplemented salad dressings using response surface methodology; and study the effect of reducing the fat content of the dressing formulation on their physical properties; as well as optimize the composition of salad dressing emulsions using commercial products as a target (Chapter 8).

Chapter 2. Literature Review

2.1 The Salad Dressing and Mayonnaise Market

Salad, which can be served as an appetizer, a side dish or an entire meal, is one of the most popular and customizable foods. Salad dressings and mayonnaise are sauces used to enhance and modify the flavor of salads and other similar foods. Together, they account for a large part of the semi-solid foods market. The market for salad dressings and mayonnaise is estimated at about $25,925 million/yr, which makes it a very important food category. Salad dressing and mayonnaise differ only moderately in definition (Sheldrake 2003). Salad dressings tend to have lower oil content, are more highly flavored, and may contain starch to give the required consistency, resulting in either spoonable or pourable products, whereas mayonnaise products are generally spoonable, less flavored and have no added starch because of their relatively high oil content.

To meet the ever-changing demands of large consumer populations with different taste preferences and habits, a versatility of salad dressing and mayonnaise products made with varying ingredients are developed and appear on the market each year. Animal based ingredients such as egg yolk, milk protein, whey concentrates, whey peptide fractions, and casein have been widely used in the formulation of dressing and mayonnaise products. However, in the last few years, concerns have grown regarding the supplies of food that will be required to feed the expanding world population and about increasing hunger. The increasing pressure for a reduction in or removal of animal based ingredients is one of the primary trends currently driving product innovation in developed countries, which is aimed at meeting the demands of consumers seeking meals with a better balance of animal and plant based ingredients, products with low cholesterol levels, and more recently, allergen-free ingredients.

There is a growing push, therefore, to identify and develop appropriate and inexpensive vegetable protein sources that can provide an adequate supply of energy with desired functionality. Most plant protein sources have lower calorie content and little to no saturated fat compared to animal proteins; they are therefore associated with a significantly lower risk of coronary artery disease and

stroke (Hu 2003). Pulse flours and their fractions, being an excellent and inexpensive source of protein, are promising ingredients that can be used to partially or fully replace egg yolk in the formulation of salad dressing and mayonnaise products while furnishing higher amounts of fiber in the diet. Several scientific research studies in recent years have focused on new products development using pulses as ingredients, due to their unique nutritional and functional properties. None of these studies have, however, been carried out on the development of salad dressings supplemented with pulse flours and pulse fractions. As salad dressings are nutritionally weak food products, the supplementation of pulse flour and fractions could enhance their nutritional value. In addition, their natural abundance in protein and carbohydrates, which can both physically and chemically interact with other components present in salad dressings, provides them with the potential to positively influence the appearance, microstructure, texture, rheology and sensory properties of salad dressings.

2.2 Pulses

2.2.1 Chemical Composition of Pulses

The proximate chemical composition of pulses (Table 2.1) varies depending on pulse type and variety. Pulses contain high amounts of carbohydrate ranging between 53-65%. They are also a high source of protein and contain between 18-32% proteins which is on average two to three times higher than cereal grains, starchy roots and tubers (Phillips 1993). Fat content of pulses, on the other hand, is rather low with chickpea containing up to 7% fat, lupins up to 10% and the other pulses generally containing less than 3%.

The major proteins found in pulses are water-soluble albumins and salt-soluble globulins, with prolamins and glutelins present in small concentrations. Several techniques have been developed to extract the proteins in pulses into protein concentrates (>65% protein, dry weight basis) and protein isolates (>90% protein, dry weight basis) including air classification, membrane separation, alkaline/isoelectric precipitation and acid/salt extraction (Mondor et al. 2009; Boye et al. 2010c; Boye and et al. 2009). Some of these products are commercially available today, such as Propulse^{TM} (a natural food grade pea

protein isolates) from Nutri-Pea limited, and Fababean protein concentrates from Parrheim Foods Inc. The proteins from legumes are generally rich in most of the amino acids that are essential for human health, especially lysine. They are mostly poor in the sulfur containing amino acids (i.e., methionine and cysteine), which makes pulses a natural complement to cereal products, which are rich in sulfur amino acids and lack lysine (Dalgetty, Baik & Swanson, 2003).

Table 2.1 Proximate composition of various pulses.

Component	Fat (%)	Protein (%)	Starch (%)	Amylose (% of starch)	Ash (%)	Moisture (%)	Total fiber (%)	Reference
Field pea	1.0-1.7	20.2-27.4	41.6-49.0	20.7-33.7	2.3-3.4		5.8-8.7 (ADF)* 8.4-11.2 (NDF)*	(Wang 2004)
Chickpea	4.4-6.9	17.9-30.8	33.1-43.9	20.5-29.2	2.7-3.8		3.0-13.5 (ADF)* 4.2-13.6 (NDF)*	(Wang 2004)
Beans	0.7-2.3	19.7-34.3	31.8-45.3	19.9-29.6	3.2-4.7		5.5-9.3 (ADF)* 7.3-12.8 (NDF)*	(Wang 2004)
Lentils	1.0-1.3	21.3-30.2	41.5-48.5	22.5-28.3	2.3-3.5		4.5-7.4 (ADF)* 7.0-9.5 (NDF)*	(Wang 2004)
Yellow peas	2.01±0.28	21.09±0.28			2.42±0.01	14.19±0.03		(Boye et al. 2010a)
Green lentils	0.82±0.003	23.03±0.08			2.39±0.03	10.68±0.01		(Boye et al. 2010a)
Red lentils	0.53±0.003	25.88±0.12			2.34±0.02	9.27±0.11		(Boye et al. 2010a)
Desi chickpea	5.23±0.15	20.52±0.24			3.04±0.01	9.26±0.04		(Boye et al. 2010a)
Kabuli chickpea	7.34±0.54	16.71±0.15			2.76±0.01	12.06±0.15		(Boye et al. 2010a)
Kidney bean	0.83	23.58			3.83		24.9	USDA[2]
Beans[1]	4.4-6.9	17.9 -30.8	33.1-43.9		2.7-3.8			(Wang 2004)

[1] Includes red, light red and white kidney beans, navy, black turtle, cranberry, dutch brown, great northern, pinto, small red and pink beans; [2] USDA – http://www.ars.usda.gov/main/site_main.htm?modecode=12-35-45-00; * ADF refers to acid detergent fiber, NDF refers to neutral detergent fiber

In addition to protein, pulses contain high amounts of carbohydrates, particularly starch (Table 2.1). Some pulse starch fractions such as pea starch (containing 50% to 80% starch) are now commercially available and are being used in the manufacture of a wide variety of food products. The abundance of resistant starch (RS) and slowly digestible starch (SDS) in pulses is responsible for their low glycemic index (www.pulsecanada.com); these starches have been linked with health benefits such as reduced risk of colon cancer and diabetes, and providing a substrate for growth of probiotic organisms (Hoover & Zhou 2003; Hoover et al. 2010). Pulses also offer good sources of dietary fiber (Table 2.1). In

food processing, pulse fibers are of interest as they can modify or enhance the texture of food products through fat or water retention (Tosh & Yada 2010). The soluble sugar fraction of pulses includes monosaccharides (ribose, glucose, galactose and fructose) and disaccharides (sucrose and maltose). The major oligosaccharides of pulses belong to the α-galactoside group (with α-D-1,6-linkage). Raffinose, stachyose and verbascose, which are galactosides derived from sucrose, are an important group of sugars in pulses (Oomah et al. 2011).

Most pulses contain relatively low amounts of fat (1.0-7.2%). The fatty acid composition of pulse lipids varies among varieties with the predominant fractions being unsaturated fatty acids, specifically, oleic and linoleic acid (Dalgetty et al. 2003). Although these fatty acids are unsaturated and considered good for the health, the sometimes undesirable beany flavour of legumes occurs due to the formation of aldehydes, ketones and alcohols resulting from enzymatic and non-enzymatic actions associated with these fatty acids (Walker & Kochhar 2007).

The mineral content of pulses varies between 2.5% and 4.2%. Examples of minerals available in pulse seeds include calcium, iron, phosphorus, potassium, zinc and selenium. When compared with most common foods, pulses are a rich source of vitamin B, folic acid and nicotinic acid. The vitamin B_1 (thiamin) content in pulses is equal or higher than those of cereal grains and pulses also contain small amount of vitamin B_2 (riboflavin) (Walker & Kochhar 2007).

2.2.2 Functional Properties of Pulses and Their Fractions

Pulse flours and their fractions may be added to foods to increase their nutritional value while providing desirable functional attributes. Functional properties influence the physicochemical quality and performance of food products during preparation, processing, storage, and consumption (Kinsella 1979). They, thus, contribute to the texture and organoleptic characteristics of foods and are essential in the manufacture of products such as confectioneries, beverages, dressings, bakery and meat products to name a few. Functional properties of most interest in food processing include solubility, water binding, fat binding, bulk density, gelation, thickening, emulsification, foaming, and flavour binding. For salad dressing and mayonnaise applications, emulsification

properties are of most interest. Table 2.2 below shows the emulsifying properties of some pulse flours and pulse fractions.

Table 2.2 The emulsifying properties of pulse flour and pulse fractions.

Pulse variety	Emulsifying capacity	Emulsifying activity	Emulsifying stability	Reference
Red kidney bean	55.0 ±1.8 (ml/100ml)		52.4 ±1.8 (ml/100ml)	Siddiq et al. (2010)
Small red kidney	60.5 ±1.9 (ml/100ml)		62.3 ±2.2 (ml/100ml)	Siddiq et al. (2010)
Cranberry bean	53.4 ±2.1 (ml/100ml)		52.4 ±2.0 (ml/100ml)	Siddiq et al. (2010)
Black bean	45.6 ±1.8 (ml/100ml)		48.2 ±1.7 (ml/100ml)	Siddiq et al. (2010)
Cow pea (dehulled)	101 ±1.98 (ml oil emulsified g^{-1} of sample)	58.4 ±0.62 (%)	54.6 ±0.28 (%)	Ghavidel et al. (2006)
Lentil (dehulled)	83 ±1.14 (ml oil emulsified g^{-1} of sample)	55.6 ±0.98(%)	52.6 ±0.46 (%)	Ghavidel et al. (2006)
Green gram (dehulled)	73 ±1.64 (ml oil emulsified g^{-1} of sample)	63.0 ±0.29(%)	57.4 ±0.48 (%)	Ghavidel et al. (2006)
Bengal gram (dehulled)	251 ±1.16 (ml oil emulsified g^{-1} of sample)	54.9 ±0.21(%)	53.2 ±0.13 (%)	Ghavidel et al. (2006)
Chickpea protein isolates (IEP)*		63.7 ±1.0 (%)	94.3 ±0.9 (%)	Paredes-Lopez et al. (1991)
Chickpea protein isolates (MI)*		63.7 ±1.0 (%)	94.3 ±0.9 (%)	Paredes-Lopez et al. (1991)

*IEP refers to the protein extraction using the isoelectric precipitation method; MI refers to protein extraction by micellization (salt extraction).

2.2.3 Value-Added Products Made with Pulse Flours and Fractions

Due to their versatility, pulse flours and fractions are increasingly being explored in the production of a wide variety of food products. Table 2.3 provides an example of some of the novel ways that pulse ingredients are being used in foods. Some of the major application areas include breads, crackers, sausages, pastas, and yogurts, etc. The challenges associated with the incorporation of pulse flours and ingredients into foods are being addressed through research and examples of some of these studies are provided in Table 2.3.

2.3 Emulsions

2.3.1 Overview of Food Emulsions

An emulsion is a mixture of small droplets of one liquid dispersed in another immiscible liquid. Emulsion-based food products include milk, mayonnaise, salad dressing, butter, low-fat spreads, sauces, and cream liqueurs. An emulsion may be

an oil-in-water (O/W) emulsion or a water-in-oil (W/O) emulsion. A schematic of an O/W and W/O emulsion is shown in Fig. 2.1. A detailed description of the chemical forces that stabilize emulsion systems are presented in Table 2.4.

Table 2.3 Examples of some value-added products containing pulse flours and fractions

Ingredients	Applications	Characteristics	References
Pulse flours			
Small red, black, pinto, and navy bean flour	Tortilla	Tortillas with acceptable texture and improved nutritional profile were produced at the 25% substitution level. Dough rheology, firmness, cohesiveness and rollability of the tortillas were, however, negatively affected as bean flour content increased.	(Anton et al. 2008)
Chickpea, blackeye bean, lentil flour	Low-fat meatball	Legume flour supplementation at the 10% level slightly increased the toughness of the meatballs. Sensory evaluation, however, showed the supplemented meatballs to have acceptable characteristics. The study concluded that legume flour can be successfully used in meatballs as extenders.	(Serdaroglu et al. 2005)
Lentil flour mixed with corn flour	Extruded snack	Extruded snacks were successfully made, however, the functionality of the extrudates decreased with the addition of lentil flours. An interaction effect between extrusion conditions and material properties was found which would require optimization to yield products with acceptable quality.	(Lazou & Krokida 2010)
Extruded chickpea flour	Weaning food	The authors found that weaning foods prepared with the combination of extruded chickpea flour (78.8%) and nixtamalized extruded maize (21.2%) showed high protein quality and digestibility which could be used to support the growth of infants.	(Milán-Carrillo et al. 2007)
Chickpea flour and soy protein concentrate	Cheddar Cheese	The product prepared from blends in which 25, 20, 27.5 and 27.5% of solids-non-fat was supplied from Cheddar cheese, whey protein concentrate, soybean protein concentrate and chickpea flour, respectively, was the most acceptable and had fine consistency.	(El-Neshawy et al. 1988)
Chickpea, green and red lentil, yellow pea, pinto and navy bean flour	Cracker snack	At a 100% substitution level, pulse based crackers showed similar physical and nutritional characteristics to products on the market and were scored highly in consumer acceptance tests. The products generally exhibited a light colour, good flavour and crisp texture.	(Han et al. 2010)
Pea ingredients (flour, starch, and fiber)	Bologna sausage	Adding pea ingredients to low-fat formulations decreased cooking and purge losses, indicating that the binders (i.e., pea ingredients) improved water retention in the sausage.	(Pietrasik 1999)
Split pea or faba bean flour	Pasta	The cooking quality of pasta was impacted when substituted with the pulse flours at the 35% level. Supplementation decreased the optimal cooking time for low temperature dried pasta and resulted in lower water uptake and higher cooking losses.	(Petitot et al. 2010)
Chickpea flour	Sponge or layer cake	At 50% and 100% substitution levels, lowered cake volume and a firmer texture were observed for chickpea flour fortified cakes compared with the ones made with regular wheat flour.	(Gómez et al. 2008)
Pulse fractions			
Modified pea starch	Low fat or fat free ice cream	Sensory attributes, such as coldness and firmness, for the low fat samples were not significantly different from those for ice cream with regular fat content. Scores for viscosity, smoothness and mouth coating were, however, generally lower for the low fat ice cream prepared using modified pea starch.	(Aime et al. 2001)
Field pea hulls	Bread	Field pea hull (containing 55.1% crude fiber) was successfully incorporated at the 15% substitution level. The authors found that pre-hydration of the fiber for 20 hours before blending of the flours increased loaf volume and bread quality.	(Sosulski & Wu 1988)
Dietary fiber from pea cotyledon	Sausage	Supplementation of pork sausage at the 4% level did not change elasticity, cohesiveness, and springiness of the sausage compared with controls. Fiber addition, however increased gumminess and chewiness.	(Cardoso et al. 2008)
Lentil flour	Yogurt	Supplementation of yogurt with lentil flour (1-3%) enhanced acid production during fermentation suggesting a prebiotic effect. Syneresis increased at 1-2% supplementation, however, the 1-2% lentil flour supplemented yogurt showed comparable sensory properties to yogurt prepared with 1-2% skim milk powder.	(Zare et al. 2011)
Dietary fiber from field beans	Sponge cake	At~5% supplementation, slight changes of physical characteristics were observed, including reduced pH levels of the cake batter and a reduction of cake volume. The fortified cake, however, had 5.7g/100g more fiber than the regular cake without changing the sensory characteristics.	(Sreenath et al. 1996)

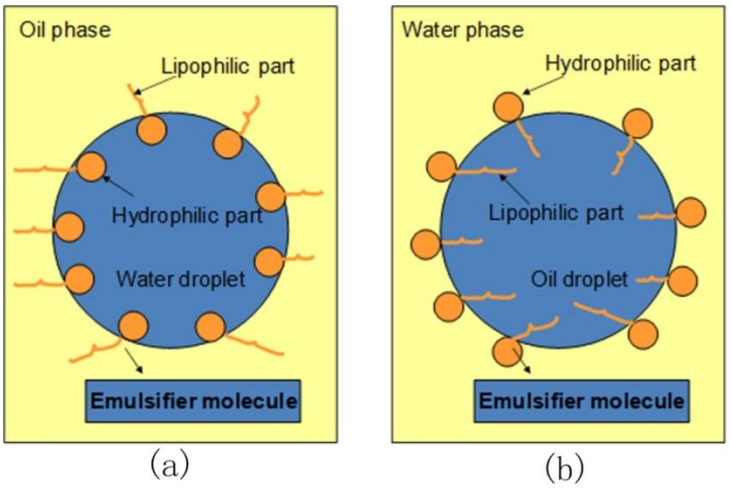


Fig. 2.1 Schematic of (a) water in oil (W/O) emulsion; (b) oil and water (O/W) emulsion.

Table 2.4 The physical and chemical interactions encountered in emulsions.

Interaction	Occurrence	Effect on emulsion
Electrostatic interaction	Between molecular species that possess a permanent electrical charge (e.g., ions and polar molecules)	(1) The organization of water molecules by dipole-dipole, dipole-ion, and ion-ion interactions; (2) the conformation and interactions of biopolymers in aqueous solution; (3) the overall properties of emulsions (since the majority of the food ingredients in emulsions are either ionic or dipolar)
Van der Waals interaction	Between all types of molecules (either ionic, polar, or nonpolar), and is weaker than electrostatic interaction	(1) Mostly determines the interactions between nonpolar molecules; (2) determines the structure and physicochemical properties of organic liquids
Steric overlap interaction	The large repulsive force generated when two molecules are close	(1) Influences the packing of molecules in liquids and solids
Hydrogen bonds	Between a lone pair of electrons on an electronegative atom and a hydrogen atom on a neighboring group	(1) Determines the unique properties of water in emulsion; (2) causes appreciable alignment of the molecules in emulsions
Hydrophobic interaction	The attractive force between the non-polar groups as the non-polar groups are separated by water	(1) Immiscibility of oil and water; (2) adsorption of emulsifier molecules to an interface; (3) aggregation of protein molecules; (4) formation of emulsifier micelles

Source: McClements (2005g).

2.3.2 Role of Ingredients

Ingredients used in food emulsions such as salad dressing and mayonnaise products interact with each other either physically or chemically and determine the quality of the final products. The use of different types of emulsifiers,

thickeners, and fat replacers has been studied by some researchers and has been tested by food manufacturers with the aim of maintaining the overall physicochemical and organoleptic properties of the salad dressing and mayonnaise product. The nature and role of individual raw materials commonly used in various dressing and mayonnaise products are further examined below.

Oil

Oil contains different types of molecules, including acylglycerols, fatty acids, and phospholipids. Oil plays an important role in food emulsions since it contributes to the body (viscosity and cling), texture (creamy and smooth mouthfeel), lubricity (slipperiness), appearance (sheen), and flavor (intensity and duration) of products, in addition to enhancing shelf life (McClements & Demetriades 1998; Stauffer 1999). Oil is also responsible for dissolving different ingredients such as vitamins, coloring agents, antioxidants, and surfactants. It is a major source of energy and nutrients. The types of oil commonly used in the formulation of dressing and mayonnaise include soybean, canola, and sunflower oil, and sometimes cottonseed and olive oil (Martin & Wil 2000). Consumer concerns about the adverse health effects associated with overconsumption of lipids have led to a trend within the food industry toward the development of reduced fat products. The use of fat substitutes has become a hot topic for food scientists. However, they face a considerable challenge given that no single ingredient can mimic the characteristics of fat and oil. Therefore reducing the fat content of dressings and mayonnaise products can have a profound influence on overall quality.

Water

Water is one of the most important components in almost every type of food. Several ingredients in dressing and mayonnaise products are soluble in water, including protein, polysaccharides, salts, vitamins, colors, antioxidants, and surfactants. The interaction between the macromolecules (such as proteins and polysaccharides) and water, which is governed by the pH of the aqueous solution, determines the solubility, partitioning, volatility, conformation, and chemical reactivity of the majority of food ingredients in dressing and mayonnaise

emulsions (McClements 2005b). The incorporation of water can reduce the viscosity and the elastic modulus of the food system.

Water activity (a_w) is the measurement used to indicate the amount of "free water" in a sample, i.e., the water molecules that are not chemically or physically bound in the sample. Free water can serve as a medium for microbial reproduction, migration and contamination. Therefore, a_w is an important parameter for evaluating the quality and safety of salad dressing and mayonnaise products. Values for a_w of 0.95 and 0.93 have been reported for mayonnaise samples containing ca. 39% oil and 78% oil, respectively (Chirife et al. 1989). The combination of high a_w (0.85 to 0.89/0.93) and low pH (3.3 to 4.1) can inhibit the growth of both yeast and lactobacillus organisms in food products (Martin & Wil 2000).

Emulsifiers

Emulsions are thermodynamically unstable systems due to the density difference and the energetically unfavorable contact between oil and water. The incorporation of emulsifiers and/or thickening agents is a critical part of emulsion preparation. Emulsifiers and thickening agents may be grouped together as stabilizers (McClements & Demetriades 1998). The difference between an emulsifier and a thickener lies in the different properties they impart to emulsion systems. Emulsifiers, are used to prevent the oil and water droplets in an emulsion from separating and coalescing after they are intentionally disrupted to form a continuous phase during homogenization. Emulsifiers are surface-active molecules that decrease the interfacial tension between oil and water phase, and provide a protective coating around the emulsion droplets, thus, preventing droplet aggregation. The mechanisms involved in preventing droplets from aggregating vary among the different emulsifiers used; they include electrostatic, steric, hydration, and thermal fluctuation interactions (McClements 2008).

The main classes of food emulsifiers include dairy proteins (whey protein, caseinates), vegetable proteins (pea and soya proteins), phospholipids (lecithin), fat derived emulsifiers (mono-and diglycerides, and esters of mono- and diglycerides), carbohydrate derived emulsifiers (starch ester, sucrose ester, and

polysorbates), and hydrocolloids (gum arabic) (Sheldrake 2003). These emulsifiers are mainly nonionic (e.g., monoacylglycerols, sucrose ester of fatty acids), anionic (e.g., fatty acids), or zwitterionic (e.g., lecithin) (McClements & Demetriades 1998; McClements 2005b). Further information on the different types of emulsifiers and their specific functions in emulsion systems can be found in the literature (Dickinson & McClements 1995; Faergemand & Danisco 2003; McClements 2005b). Brief summaries are provided below.

Proteins

The kinetics of protein adsorption at the oil–water interface involve two major steps: first, the native protein molecules are diffused and penetrate at the interface; secondly, these adsorbed molecules are rearranged to achieve a state of minimum free energy by unfolding and exposing the hydrophobic/hydrophilic groups to the surface and binding with the lipid/aqueous phase. The large free energy associated with the large interfacial area which is thermodynamically unstable could therefore be diminished in the presence of proteins in the emulsion.

Egg yolk in liquid, frozen and dried states, or in the whole egg form has been used in the formulation of salad dressing and mayonnaise products. Their performance differs depending on the form in which they are used (Harrison & Cunningham 1985; Yang & Lai 2003). When whole egg is used, the final product is much stiffer than when egg yolk is used as an emulsifier, possibly because the presence of denatured egg albumin at the interface forms a matrix in the aqueous phase and provides more emulsification capacity. The yield stress of whole egg-emulsified salad dressing or mayonnaise, therefore, increases (Stauffer 1999). Egg yolk has a complex composition, with a protein: lipid ratio of 1:2. Lipoproteins, phopholipids and cholesterol are the major components of egg yolk. The majority of proteins in yolk are organized into micellar and granular structures together with polar and non-polar lipid molecules (Kiosseoglou 2003). As shown in Fig. 2.2, apolipoprotein, phospholipids/lecithin, and cholesterol are bound to each other through non-covalent bonds and form large lipoprotein complexes.

Some workers pre-heated proteins to promote a certain degree of denaturation before incorporating them into emulsions (Riscardo et al. 2003).

Guilmineau & Kulozik (2007) found that pre-heating egg yolk at 68 ℃ for up to 11 min prior to emulsification resulted in a reduction (up to 40%) in the average oil droplet size in mayonnaise compared with an emulsion stabilized with non-heated egg yolk. The rheological properties were also affected due to the presence of thermally unfolded proteins, which were more active in interaction. When lupin protein isolates were treated at different temperatures (50–90 ℃) for varied time periods (10 and 40 min), the rheological and textural parameters as well as the protein surface hydrophobicity increased with increasing temperature and heating time, and the Sauter diameter of the oil droplets decreased (Raymundo et al. 1998). However, from an economic point of view, proteins are frequently used in their native forms in the preparation of dressing and mayonnaise emulsions.

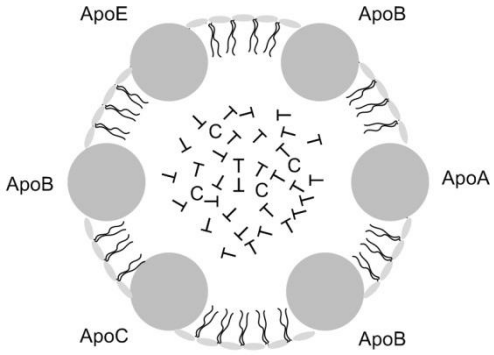


Fig. 2.2 Lipoprotein structure (chylomicron).
(ApoA, ApoB, ApoC, and ApoE - apolipoproteins; T - triacylglycerol; C - cholesterol; the ovals between apolipoproteins represent phospholipids).

The composition of emulsifiers around oil droplets is dependent on the competitive adsorption and/or displacement of different protein components; this distribution could lead to an improvement or deterioration in the performance of the emulsifier mixture. Clark et al. (1992) found that emulsions were stronger and the quality of the final emulsion products improved when a mixture of different types of proteins was used. The competitive adsorption between egg yolk lipoprotein and whey protein at oil–water interfaces was evaluated by Aluko et al. (1998). Emulsions containing varying ratios of whey protein isolate (WPI) and LDL or granule lipoprotein produced emulsions with smaller particle sizes in

comparison with emulsions prepared with WPI alone. Both whey protein (Aluko et al. 1998) and caseinate (Mine & Keeratiurai 2000) may be displaced by LDL or granule lipoproteins in emulsions, a finding attributed to the high penetrating ability of yolk lipoprotein molecules. In contrast, other researchers (Polyakov et al. 1997; Mine & Keeratiurai 2000) have reported a deterioration in emulsifying performance when a mixture of proteins were used compared with the emulsions stabilized by single emulsifier owing to a disorganization and competitive adsorption of different types of proteins at the interfaces. As reported by Riscardo et al. (2003), the performance in terms of viscous and viscoelastic functions of emulsions containing binary emulsifiers, including egg yolk/pea protein and egg yolk/caseinate blends, is dependent on the weight ratio of emulsifier in the binary blends.

In addition to the important emulsification capacity of lipoproteins from egg yolk, the performance of dairy proteins such as whey protein isolate and casein (Álvarez Cerimedo et al. 2010; Riscardo et al. 2003; Turgeon et al. 1996), vegetable proteins, such as soybean protein (Diftis et al. 2005; Puppo et al. 2000), lupin protein (Franco et al. 1998; Raymundo et al. 2002), pea protein (Franco et al. 2000), and wheat protein (Ghoush et al. 2008), have been extensively investigated in salad dressings applications. Emulsifying capacity is generally determined by the relative adsorption of proteins, which depends on their concentration, hydrophobicity, ability to unfold and possible packing configurations at the interface (Parker 1987). Emulsifiers with better emulsifying capacity are able to reduce the average size of the oil droplets, which affects the texture and rheology of salad dressing and mayonnaise products.

Phospholipids and lecithin

Lecithins are natural substances which are derived from various foods such as soybeans, egg yolks, corn, canola, sunflower, and wheat germ (Http 2). Phospholipids are the functional ingredients of all forms of lecithin. Lecithins do not have a uniform chemical structure; instead they are a group of similar but clearly differentiated components, classified as phospholipids (Whitehurst 2004). The structure of lecithin from egg yolk consists of triglycerides and phospholipids.

It is different from the lecithin derived from soybean, which is composed mainly of four types of phospholipids, i.e., phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidic acid (PA), and may contain relatively large amounts of triglycerides (Hancer, Patist et al. 2002). The molecular structure of phospholipids, i.e., the major component of lecithin, is partly hydrophilic and partly hydrophobic, which makes lecithin an effective emulsifier at the oil and water interface.

Small molecule surfactants

Small molecule surfactants are comprised mainly of lipid-based ingredients, such as fat-derived monoglycerides and their derivatives, as well as carbohydrate based components, such as starch esters, sorbitan esters and their derivatives, including polysorbates (Tweens), and sucrose esters. The main function of these ingredients is generally not emulsification; instead they are often used to control fat morphology and crystallization, promote shelf life through interactions with starch, and destabilize emulsions by competitive displacement of proteins from the oil–water interface (Dickinson 1992). Monoglycerides and their derivatives account for about 75% of world production of food emulsifiers and are considered the most important group of emulsifiers. The hydroxyl group in monoglycerides is usually replaced by other branches, such as the acetyl group, lactic acid, succinic acid, citric acid, diacetyltartaric acid, or the polyglyceryl group. This modification is aimed at improving the emulsifying or other functional properties of these chemicals. Polysorbate 60 is a common ingredient used in commercial dressing formulations; the addition of a maximum level of 0.3% is permitted to enhance "home emulsification".

Others

Mustard powders contain almost equal amounts of protein, carbohydrate and oil. They are also rich in mucilaginous material, which is composed primarily of polysaccharides (Cserhalmi al. 2000). The protein and carbohydrate bind with oil in the mustard powder structure, contributing to its emulsifying capacity at the oil–water interface. The emulsifying properties of mustard powders are not only dependent on their chemical and physical properties, the method of incorporating

mustard in food emulsions also determine their emulsifying performances (Yang & Lai 2003).

Thickeners

Gums

A thickener is a chemical component or mixture of components that can impart long-term emulsion stability by thickening a food system (i.e., reducing the movement of the system) and by forming viscous, ordered networks in the continuous phase to prevent oil separation (Dickinson & Stainsby 1988). The body/texture and mouthfeel of a food product is improved as a result of the addition of these ingredients. Thickeners function in emulsions either as a bulking agent (such as starch) or by forming networks (such as pectins). Polysaccharide macromolecules (gums), casein micelles, fat crystals, starch and modified starch all belong to the category of thickeners. Most of the gums used as thickeners are hydrophilic, except for gum arabic and propylene glycol alginate (PGA), which are amphoteric and are able to form a film at the oil–water interface (Akiyama et al. 1984; Fennema 1985; Pettitt et al. 1995; Yilmazer et al. 1991).

Generally, biopolymer gums are obtained from trees, plants, tree gum exudates, fermentation of bacterial polymeric products, biosynthesis, and chemical modification (Sikora et al. 2008). Biopolymer gums are usually highly hydrated and extended molecules or molecular aggregates with a long polysaccharide chain which has numerous side branches of sugars or oligosaccharides. Their highly branched structure contributes to water solubility. The ability of such gums to increase the viscosity of emulsions depends principally on their molecular weight, degree of branching, conformation, and flexibility (Huang et al. 2001). Gums are often added to salad dressings and mayonnaise products for emulsion stability, control of pourability, cling improvement, and suspension of solid or spice particles (Ma & Barbosa-Canovas 1995a). Most biopolymers help to stabilize droplets against coalescence principally through a combination of physical and chemical interactions, including electrostatic and polymeric steric interactions, hydrogen bonding, hydrophobic association, and cation-mediated crosslinking. In addition to their

stabilizing ability, these gums also contribute to the technical and functional properties of emulsions in terms of aqueous solubility, thickening ability, gelling and gel stabilizing ability, and most importantly sensory creation ability (Phillips & Williams 2009; Sikora et al. 2008). Recently, many gums including xantham, guar gums, gum arabic, and pectin have been recognized as providing health benefits on account of their role in reducing blood cholesterol levels and their prebiotic effects (Glueck et al. 1994; Behall 1997; Phillips & Williams 2009).

The behavior of various gums in formulated dressing and mayonnaise is significantly influenced by pH, ionic strength, concentration and temperature of the gum solution. The gums used in the formulation of dressing products should be stable in an acidic environment. The tendency toward hydrolysis at low pH can decrease the viscosity of gum solutions and impair their performance. Xanthan, PGA, and hydroxypropyl methylcellulose are gums that are frequently used in low-fat or fat-free dressings. They exhibit high resistance to hydrolysis during storage at a low pH level. The viscosity of different gums may be low (e.g., arabic, ghatti), medium (PGA, tragacanth, xanthan) or high (guar, and locust bean gum) (Yilmazer et al. 1991).

Cellulose is a group of important gums with substituted groups which improve its solubility. The most common cellulose gums used in low fat dressing and mayonnaise are those substituted by carboxymethyl, methyl, and hydroxyproply plus methyl groups. Other cellulose gums, such as microcrystalline cellulose, usually have different uses in processed foods. In addition to the traditionally used gums, gums derived from flaxseed (Stewart & Mazza 2000), hsian tsao leaf (Lai & Lin 2004), and *Lepidium perfoliatum* seed (Koocheki et al. 2009) have been studied for development as potential new alternatives. Canada's *Food and Drug Regulations* have no provisions pertaining to the maximum permitted levels of different gums in formulated dressing products, which means that their use is governed by good manufacturing practices (Health Canada, 2011). Recently, permission to use gum arabic modified with Octenyl Succinic Anhydride (OSA) as an emulsifier has been assessed according to Health Canada guidelines, and the maximum level use of 1% has been approved in French

dressing and salad dressing (Health Canada Amends, 2012). No related regulations regarding the maximum levels of using these gums in salad dressings and mayonnaise are specified according to USFDA (2012) and EU legislations (EEC, 1992).

Starch and modified starch

Starch is composed mainly of linear amylose and branched amylopectin. Starch granules retain their integrity in the native form; they can be used to provide the desired structure to finished dressing and mayonnaise products. Pre-gelatinized starches have been subjected to hydration, swelling, crosslinking during heating, and retrogradation upon cooling. The specific time and temperature for gelatinization differ depending on the source of the starch.

Modified starch refers to starches which have been subjected to different treatments aimed at achieving broader applications in the food industry. Waxy maize starch, which is composed of nearly 100% amylopectin, can be modified by crosslinking with sodium trimetaphosphate or stabilizing by hydroxypropyl substitution. Crosslinked starch has hydroxyl groups on adjacent chains joined by covalent bonding. Stabilized starch has hydroxyl groups formed of ester or ether bonds with other small molecules. The purpose of crosslinking is to prevent hydrolysis under the acidic environment characterizing salad dressing. Starch gel tends to become soft if hydrolysis occurs. Modifying starch can interfere with recrystallization of the side chains of the starch molecules and it helps to maintain the creamy texture of dressings during storage in the refrigerator (Stauffer 1999). Starch modification, therefore, facilitates the formation of stable gels at low pH and shear and at increased or decreased gelatinization temperatures, and leads to gels with flexible alteration in mouthfeel, i.e., either much softer or firmer. Maltodextrins and dextrins, polysaccharides produced by starch hydrolysis, are often used as bulking agents or fat mimetics which impart body and mouthfeel to food products.

Other fat mimetics

In addition to gum and starch, the solid fibrous products from different sources such as potato, oat and wheat can also be used as thickeners. They have

the ability to bind water, reducing available free water, and therefore enhance the viscosity of emulsions (Sheldrake 2003). Unlike starch, these fibrous products are resistant to enzymatic breakdown within the gut.

A variety of new fat substitutes have been developed to satisfy demands associated with the current trend toward reduced fat foods. Some commercial fat replacers that are protein-based, carbohydrate-based, or fat-based produce the desirable mouthfeel of fat without having the energy content of fat. Several of these products include Simplesse™, Kelcogel™, Litesse™, Olestra™, Caprenin™, etc.

Acidifying ingredients

The acidification of salad dressing and mayonnaise is often achieved by the addition of acidifying ingredients, including vinegar, lemon juice, lime juice and organic acid acidulants (citric acid, acetic acid, lactic acid, tartaric acid and malic acid), or mineral acid acidulants (phosphoric and hydrochloric acids). The pH value of food products influences their susceptibility to microbial growth. However, pH control alone is not sufficient to preserve dressing or mayonnaise from spoilage. An acidic environment with a pH value of 3.0 to 4.5, together with the preservative effect of undissociated acetic acid (typically added in the form of vinegar, lactic acid, or other weak acids), is important for ensuring the microbiological stability of such products. The weak acids used in salad dressings differ in their preservative abilities. For example, citric acid reduces the pH level but has no antimicrobial activity, whereas undissociated acetic acid inhibits the growth of lactobacilli. The solubility of undissociated acid in the aqueous phase is also an important factor, since the portion that solubilizes in the oil phase has no preservation effect. The presence of more than 0.2% undissociated acetic acid in the aqueous phase can control the growth of pathogens (Martin & Wil 2000). The growth of *Salmonela* and *L. monocytogenes* in a typical mayonnaise sample was inactivated by 4 log in 3 days where the products had a pH below 4.1 and 0.7% acetic acid in the aqueous phase. By contrast, a mayonnaise product with 1.4% acetic acid in the aqueous phase was found to be organoleptically unacceptable (Glass & Doyle 1991).

Sugar and salt

The presence of salt and sugar in dressing and mayonnaise products not only functions as seasoning, but also helps to reduce water activity and therefore inhibit spoilage organisms. In some commercial dressing products, corn syrup (such as 15.5% high-fructose corn syrup) is sometimes used as a sugar source because of its enhanced flavor.

The types and concentrations of salt in dressing and mayonnaise products contribute to the structure and overall textural attributes of these foods (Harrison & Cunningham 1986). Calcium, sodium, potassium, chloride, carbonate and phosphate are common salts used in dressing products. Harrison & Cunningham (1986) reported that the addition of various types of salt significantly affected the structure and performance of liquid egg yolk in mayonnaise. Mart ínez et al. (2007) also found that salt content (from 0 to 2.3%, w/w) had a significant effect on the rheological properties of salad dressing-type emulsions stabilized by binary blends of egg yolk and different types of amphiphilic molecules (e.g., Tween 20, sucrose laurate and pea protein). The addition of salt produced a progressive increase in viscous and viscoelastic parameters of dressings; a markedly higher increase was observed when proteins (i.e., egg yolk and pea protein) were used as the predominant emulsifiers in the blends, a finding that may be attributable to an increase in interdroplet interactions and increased viscosity of the continuous phase induced by salt addition.

Spices and flavoring ingredients

Flavor plays an important role in determining consumer purchasing intention. Mustard is the principal flavor ingredient in mayonnaise. A greater diversity of flavoring ingredients is used in salad dressings. Spices or dried vegetable pieces, including pureed or dried celery, onion, carrot, garlic, paprika, green and red peppers as well as parsley are commonly used in commercial dressing products. Some non-standard dressings on the market consist of very complex mixtures, which sometimes include dairy products (e.g., buttermilk powder, modified milk ingredients, Parmesan and Romano or blue cheese), as well as other flavoring ingredients, such as anchovy paste, monosodium glutamate

(MSG), and sodium inosinate. Disodium inosinate is a common food additive, which is used as a flavor enhancer in commercial dressings; it is used in synergy with MSG to provide the savory taste known as umami; it is also added in conjunction with ingredients that are natural sources of glutamic acid, such as Parmesan cheese, tomatoes or yeast extract (Http 3). The solubility and hydrophobicity of spices and flavoring and coloring agents determine how and when these ingredients will be tasted; they can therefore influence the overall flavor of products and consumer perceptions. Flavor compounds must be released from and diffuse out of the droplets in dressing emulsions before they can be perceived by the taste receptor. The textural attributes of dressing and mayonnaise thus are able to modulate flavor perception.

Most of the aroma compounds in foods are fat soluble; the partition coefficient indicates that these compounds are associated with the lipid phase at equilibrium (de Roos 1997; Leland 1997). Flavor perception can be modified in reduced-fat or fat-free dressing and mayonnaise relative to full-fat products since the distribution of fat and water in an emulsion influences the balance of each flavor. Additionally, the matrix of other food ingredients markedly affects the rates and pattern of flavor release. McClements and Demetriades (1998) report that the sensation of less polar flavors tends to be tasted by the receptor after the sensation of more polar flavors (e.g., sugar, vinegar, acetic acid, and citric acid). With a decrease of fat content in dressing and mayonnaise, the sensation of the polar flavors tends to become more intense. For the food industry, therefore, it can be a great challenge to develop products of this type with reduced-fat content that will meet consumers' desire for fatty sensation with the required flavor release pattern.

Food preservatives

In addition to pH and undissociated organic acids, preservatives in legally allowed amounts are often used in dressing and mayonnaise products to ensure microbiological stability. Weak lipophillic organic acids are an important group of preservatives commonly used in food emulsions; they include ethylene diamine tetra-acetic acid, (EDTA), sorbic acid, potassium sorbate, calcium sorbate,

benzoic acid, sodium benzoate, potassium benzoate, and calcium benzoate. The permitted levels of these different preservatives vary depending on the legislation in effect in different countries. The permitted levels of sorbic acid and benzoic acid are 2000 and 1000 ppm respectively for emulsified sources with less than 60% fat within the European Community (Casas et al. 2000). Under Canada's *Food and Drug Regulations*, the maximum levels of sorbic acid and benzoate acid are 3350 ppm, and the maximum level of sodium benzoate is 1000 ppm (Health Canada, 2011). Sequestrants (also known as chelating agents) such as EDTA and benzoates have preservation effects. These compounds are able to bind metals such as copper and iron; they can therefore inhibit the degradation of fats induced by the presence of undesirable metals and prevent the formation of unpalatable flavors.

2.3.3 Emulsion Homogenization Techniques and Equipment

Homogenization is the process of converting two immiscible liquids into an emulsion, or of reducing the size of the droplets in an existing emulsion. A mechanical device known as a homogenizer is used to achieve homogenization by applying intense energy in order to disrupt and mix the oil and water phases. Examples of commonly used homogenizers include the Ultra-Turrax homogenizer, the Polytron PT homogenizer, the Warring blender, colloidal mills, and high-pressure valve homogenizers (Table 2.5). In most cases, a coarse emulsion is prepared by premixing the emulsion ingredients which have been dosed separately and then feeding the resulting emulsion into the homogenizer for fine emulsification. Homogenization is an important step in the production of salad dressings, since the device and process selected have considerable influence on the bulk physicochemical, rheological, and sensory properties of the emulsion. The type of homogenization treatment used can, thus, determine the acceptability of the final product. The speed and duration used with different types of homogenizers as reported in previous studies are summarized in Table 2.6. The choice of a homogenization device depends on the volume or amount of sample, the nature of the starting materials, the desired physicochemical properties, and the overall quality attributes of the final products, as well as the cost and the

operating context (i.e., laboratory, pilot plant, or industrial food processing) (McClements 2005a).

Table 2.5 Examples of equipment type and processing conditions used in the production of salad dressing and mayonnaise type emulsions.

Number	Homogenization techniques	Speed and time duration	Reference
1	Ultra Turrax T-25	8000 rpm for 5 min	(Sun et al. 2007).
2	Ultra Turrax T-8 (pre-homogenization) Ultrasonic liquid processing (final homogenization)	20000 rpm for 1 min (pre-homogenization) 20 min (final homogenization)	(Álvarez Cerimedo et al. 2010)
3	Two-stage high-pressure valve homogenizer	270 bar (first stage) 340 bar (second stage)	(Singh et al. 2003)
4	Pilot plant colloidal mill	2830 rpm for 5 min	(Riscardo et al. 2003)
5	Waring blender (pre-homogenization) Colloid Mill (final homogenization)	high speed for 120 s with gap setting of 0.2 mm (pre)	(Stewart & Mazza 2000)
6	Hand-held homogenizer (pre-homogenization) Two-stage high-pressure valve homogenizer (final homogenization)	high speed for 2 min (pre) four times at 5000 psi	(Hu et al. 2003)
7	Ultra Turrax T-50	5000 rpm for 7 min (oil was slowly added during the first 3 min)	(Dolz et al. 2008)
8	Ultra Turrax T 50	6000 rpm for 7 min	(Romero et al. 2009)
9	Pilot plant colloidal mill	rotation speed at 2830 rpm with rotor-stator gap of 1mm for 5min	(Mart ńez et al. 2007)
10	Rotor stator turbine	6000 rpm for 3.5 min	(Moros et al. 2002)
11	Ultra-Turrax T-50	5000 and 8000 rpm for 3 or 5 min	(Franco et al. 1995)
12	Pilot plant colloidal mill	2830 rpm for 3, 5 or 10 min (with a rotor-stator distance of 1 mm)	(Franco et al. 1995)
13	Propeller-type mechanical stirrer (pre-homogenization) Ultra-Turrax T-50 (final homogenization)	8000 rpm, 9500 rpm, 13500 rpm, 20500 rpm and 24000 rpm	(Paraskevopoulou et al. 2007)
14	Ultra Turrax T-25	8000-20500 rpm for 3-10 min	(Franco et al. 1998)
15	emulsor high pressure homogenizer	pressure was kept at 3–4 $\times 10^3$ kPa	(Wendin & Hall 2001).
16	high shear mixer (pre-homogenization) two-stage high pressure homogenizer (final homogenization)	2500 to 10000 psi (first stage of high pressure homogenization); 500 psig (second stage of high pressure homogenization)	(Breitbart et al. 2000)

Table 2.6 The common homogenization techniques used in the manufacture of salad dressing and mayonnaise.

Homogenizer	Principle	Characteristics	Advantages and disadvantages
Ultra Turrax, Polytron PT	Rotor-stator principle (mixing, shear and elongational stresses, turbulence, and cavitation)	(1) Produce emulsions of medium and high viscosity (2) particle size can be reduced to a threshold of *ca* 4-10 μm (3) often used in food research lab and food industry (4) shear rate ranges from 20,000 to 100,000s^{-1} (5) stator geometries consist of slotted, disintegrating, fine emulsor, square hole, and standard emulsor (different design determines the flow behavior and the resulted emulsion products)	Operation in open vessel: (1) difficult to maintain adequate flow which results in unevenly distributed particle sizes when both viscosity of materials and vessel size increases; (2) uneven distribution may create zones of localized heat leading to thermal degradation.
Colloid mill	Rotor stator principle	(1) Produce emulsions of medium and high viscosity (2) produce emulsions with a minimum droplet size of 1 μm (3) often used in pilot plant and industrial production (4) the gap distance between the rotor and stator can be adjusted from 50 to 1000 μm to change the intensity of shear stress (5) the flow rate can be varied between 4 and 20,000L h^{-1}	Does not have the issue above such as uneven distribution of particle size and degradation (materials can be continuously fed into colloid mill); The liquids to be homogenized usually are fed in the form of a coarse emulsion
Warring blender, turbines and propeller-type stirrers	high speed blender (a combination of longitudinal, rotational, and radial velocity gradients)	(1) Produce emulsions of low or intermediate viscosity (2) produce emulsion with a minimum droplet size of 2 μm (3) blades, propellers, and turbines are common stirrers used	May generate localized heat which could result in thermal degradation of emulsion samples
High pressure valve homogenizer	Intense shear, cavitation, turbulent and laminar flow	(1) Produce emulsions of low or intermediate viscosity (2) produce emulsions with a minimum droplet size of 0.1 μm (3) one-stage valve and two-stage valve homogenizers are two types of high pressure homogenizers used	It is a secondary homogenization technique (i.e., a coarse emulsion should be produced before being further reduced in the high pressure system)

The above table is summarized based on Atiemo-Obeng & Calabrese (2004); Utomo et al., (2009); Rodgers & Cooke (2001); McClements (2005a), McClements (2008); Fellows (2000); Sugiura et al. (2002); and Http 1.

2.3.4 Characterization of Emulsions

Different analytical instruments and experimental techniques are used to characterize salad dressing and mayonnaise products. In research laboratories and in the food industry, characterization is an important step for monitoring the properties of food emulsions before, during, and after production, and for elucidating the relationship between colloid characteristics and their bulk physicochemical properties. The quality of food emulsions can be controlled by adjusting the processing and compositional variables in order to develop products with pre-defined characteristics (McClements 2005e; McClements 2007).

Appearance

The appearance of food emulsion products, such as dressings, plays an important role in determining consumer purchasing intention. A number of characteristics contribute to the overall appearance of such foods, including homogeneity, opacity, and color (Pettitt et al. 1995). Homogeneity is achieved by efficient homogenization and appropriate selection of materials to ensure emulsion stability. Opacity is dependent on the extent of scattering. Opacifying agents such as titanium dioxide (Breitbart et al. 2000) and microgranular protein (Selinger & Laaman 1994) have been explored for their potential to opacify low-fat dressing products.

The overall appearance of an emulsion is due to its interaction with radiation in the visible region of the electromagnetic spectrum (Clydesdale & Ahmed 1978). A combination of scattering and absorption occurs when a beam of light travels through both the aqueous phase and the droplets of emulsions. These interactions are governed by the emulsion composition, droplet concentration, droplet size, refractive index, chromaticity (dye) characteristics, and the microstructure of the dressing and mayonnaise products (McClements & Demetriades, 1998). The tristimulus coordinate system (L^*, a^*, b^*) is used mostly to measure the emulsion color, in which L^* represents lightness, and a^* and b^* are the color coordinates; + a^* represents the red coordinate, -a^* is the green coordinate, + b^* is the yellow coordinate, and – b^* is the blue coordinate.

The sheen of high-fat dressing and mayonnaise, which is attractive to consumers, cannot be achieved by adding gums, starches, or protein derivatives to reduced-fat products. Instead, the addition of a strongly surface-active emulsifier such as one of the polysorbates and a small amount of oil is enough to produce the surface effect and improve the appearance of the products (Stauffer 1999).

McClements et al. (1998; 2002) advanced the theory that the color of emulsions can be predicted based on their composition and microstructure. Good agreement was found between the theoretical predictions and experimental measurements, providing an avenue to explore in order to facilitate the systematic development of food emulsions with desirable appearance. According to their theory and experimental results, the "blueness" of emulsions increases with decreasing droplet concentration and increasing droplet size (Chantrapornchai et al. 1998). The color of emulsions changes from gray to increasingly bright as the droplet size decreases due to an increase in light scattering (McClements & Demetriades 1998).

Rheology

Rheology is the study of the relationship between applied force and the deformation of solids and the flow of fluids. Rheological data can be used to control and monitor the quality of dressing and mayonnaise products, from the perspective of sensory quality, ingredient interaction, and shelf life stability (McClements 2005c).

The mouthfeel of products relates to their density, viscosity, surface tension and other physical properties. The changes that occur when a food moves or flows in the mouth and throat are closely related to the food's rheological properties and consequently have a considerable effect on sensory attributes and consumer perception (McKenna & Lyng 2003). The desirable viscosity for salad dressings ensures that they are readily pourable and recover their initial high viscosity at rest; dressings should also cling well to the salad and not flow quickly to the bottom of the salad bowl. The rheological behavior and textural properties of dressing and mayonnaise are controlled by several factors: (1) the use of different ingredients, especially texturizing agents; (2) homogenization devices; (3) the

speed of the devices during operation, which changes the way the ingredients interact in the system; (4) the order and speed of oil and vinegar addition, which are also cited as a factor influencing the quality of the final product, such as the homogeneity, consistency, and stability.

The rheological behavior of a food emulsion is closely related to its deformation, disintegration and flow in response to a force applied for a certain period of time (Zhang et al. 2008). McClements (2005c) described the rheological flow behaviors of different types of food emulsions as pseudoplastic, dilatant, thixotropic, and rheopectic. Dressing and mayonnaise samples can be tested using a range of different stresses or shear rates that represent the effect of such actions as tasting, pouring, shaking, and stirring. The examples of shear rates and the real life processes they mimic are summarized in Table 2.7 (Figura & Teixeira 2007).

Salad dressing and mayonnaise products exhibit viscoelastic properties which are time- shear-, temperature-, and pressure-dependent. Steady-state flow tests (viscosity *vs.* shear rate), oscillation tests (storage and loss moduli *vs.* strain or frequency or temperature), as well as creep and recovery tests (strain or compliance *vs.* time) are commonly used to study the structural organization and interactions in products and to monitor quality.

Steady state flow test

The steady-state flow test is a measurement of viscosity *vs.* shear rate. Apparent viscosity at different shear rates can be obtained during testing. The shear values are applied successively, and the data are sampled under equilibrium conditions (i.e., when the shear rate reaches equilibrium) during the steady-state flow test, which differs from the stepped state flow test and continuous flow tests. Measurements of viscosity *vs.* shear rate can provide information about the strength of colloidal interactions between the droplets of an emulsion (McClements & Demetriades 1998). The shear rate range choices as discussed earlier are summarized in Table 2.7.

Table 2.7 Shear rates applied during rheology tests and experience in reality (Figura & Teixeira 2007).

Shear rate (s^{-1})	Processes
10^{-6}~10^{-4}	sedimentation of small solid particles
10^{-4}~10^{-1}	sedimentation of larger solid particles
10^{-2}~10^{-1}	flow caused by surface tension
10^{-1}~10^{1}	flow caused by gravity
10^{0}~10^{3}	extrusion processing
10^{1}~10^{2}	roller drum processing; pouring; dipping; chewing and swallowing
10^{0}~10^{3}	flow-through pipe
10^{1}~10^{3}	stirring and mixing
10^{2}~10^{4}	painting, brushing
10^{3}~10^{5}	wet milling
10^{5}~10^{6}	homogenization (high pressure nozzle)

Several mathematical models have been developed for use in describing the rheological characteristics of samples during the steady-state flow test. The most frequently used models include the power law model, the Herschel Bulkley model and the Carreau model.

The power law model (also called Ostwald-de Waele model) is defined as follows:

$$\eta = m \times (d\gamma / dt)^{n-1} \quad , \qquad \text{(Eq. 1)}$$

where n is the flow behavior index (dimensionless), η is the shear viscosity (Pa.s), m is the consistency coefficient (Pa.s^n), η is the viscosity (Pa.s), and $d\gamma / dt$ is the shear rate (s^{-1}). This model should only be applied after it has been proven experimentally that the relationship between log (τ) and log $(d\gamma / dt)$ is linear over the shear rates used. For an ideal liquid, n=1 (Newtonian fluid); for emulsions which exhibit shear thinning behavior, n<1 (pseudoplastic fluid); and for emulsions which exhibit shear thickening behavior, n>1 (dilatants fluid). Salad dressing and mayonnaise are generally shear-thinning fluids, in which the viscosity decreases with an increase in shear rate, which points to changes in the spatial distribution of particles as the shear rate increases. The pseudoplasticity of dressings (n value) enhances their sensory qualities, including mouthfeel and

flavor release, and at the same time guarantees a high degree of mixability, pourability and pumpability (Katzbauer 1998). By comparing the consistency coefficient (*m* value), information can be obtained on the inherent viscosity of and colloidal interactions within emulsions. To study the time-dependent thixotropy behavior of dressing and mayonnaise samples, the steady-state flow test can be performed by measuring the stress as a function of increasing and decreasing shear rate (ramp-up and ramp-down time mode). The degree of thixotropy can be obtained by measuring the hysteresis loop between the up and down curves of a flow curve (Koh et al. 2008; Laca et al. 2010).

When it is necessary to consider the force to be overcome to initiate viscous flow (i.e., yield stress), the Herschel-Buckley model can be used which better describes the flow behavior of dressing emulsions with a yield stress.

$$\sigma = \sigma_0 + m(d\gamma / dt)^n , \qquad \text{(Eq. 2)}$$

where σ is the shear stress (Pa), and σ_0 is the yield stress (Pa), *m* is the consistency index (Pa.s^n), *n* is the flow behavior index (dimensionless), and $d\gamma / dt$ is the shear rate (s^{-1}).

The Casson model (as represented by the equation below) is sometimes used to quantify emulsions with a yield stress and high shear viscosity; it is typically used for inks and molten chocolate.

$$\tau^{0.5} = k_0 + m(d\gamma / dt)^{0.5}, \qquad \text{(Eq. 3)}$$

where τ is the shear stress (Pa), *m* is the consistency index (Pa.s^n), $\sigma_0 = k^2{}_0$ is the yield stress (Pa).

The Carreau model is presented by the following equation:

$$\frac{\eta - \eta_\infty}{\eta_0 - \eta_\infty} = \left[\frac{1}{1 + (\gamma / \gamma_c)^2} \right]^s , \qquad \text{(Eq. 4)}$$

where γ_c is a critical shear rate for onset of the shear-thinning region (s^{-1}), and *s* is a parameter related to the slope of this intermediate region (dimensionless), η is the viscosity (Pa.s), γ is the shear rate (s^{-1}), η_0 is the zero-shear rate-limiting rate viscosity (Pa.s), and η_∞ is the higher high-shear rate-limiting viscosity (Pa.s). The Carreau model is often used to fit flow curves with a shear-thinning behavior with

three different regions (Fig. 2.3). The first region (first Newtonian plateau region), which is found at low shear rates, indicates a tendency to reach a constant viscosity on the viscosity *vs.* shear rate curve, i.e., η_0. The intermediate region of the flow curves exhibits a power law decrease in viscosity, and the third region (the second Newtonian plateau region) shows a high shear rate limiting viscosity (η_∞). The values of these fitting parameters (η_0 , η_∞ *s*, γ_c) can therefore be compared for salad dressing and mayonnaise samples prepared using various formulations (Mart ńez et al. 2007).

A significant difference exists between the power law model and the Carreau model in the range of shear rates applied. The Carreau model is often used when measurements are carried out across a shear rate range covering many orders of magnitude. However, when measurements are performed in the intermediate region (Fig. 2.3) and are sufficiently lower than those of the second Newtonian plateau but higher than those of the first Newtonian plateau, the power law model is usually applied.

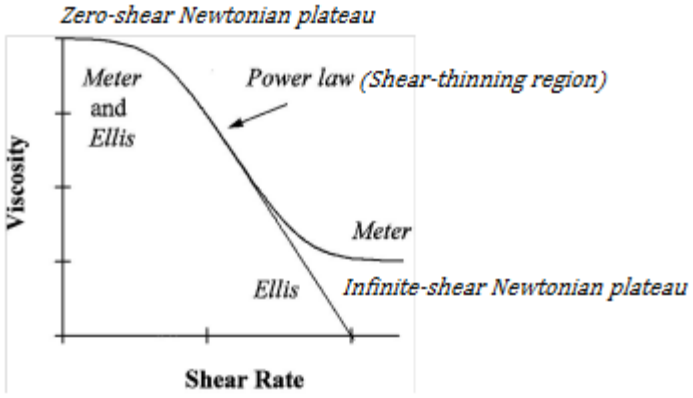


Fig. 2.3 A typical viscosity profile of a pseudoplastic material fitted to the Carreau model. The viscosity decreases from a constant value (η_0) at low shear rates to another constant value (η_∞) at high shear rates.

The Cross model, as shown in the following equation, is a relative of the Carreau model and is often used to fit the η *vs.* $d\gamma/dt$ profile.

$$\eta = \eta_{\infty} + \frac{\eta_0 - \eta_{\infty}}{1 + (\tau / \tau_i)^n}, \qquad \text{(Eq. 5)}$$

where η is the viscosity (Pa.s), η_0 is the zero-shear rate-limiting rate viscosity (Pa.s), and η_{∞} is the higher high-shear rate-limiting viscosity (Pa.s), τ is the shear stress (Pa), n is the power index (dimensionless), and τ_i is the shear stress (Pa) when the viscosity is midway between the low and high shear rate limits (McClements 2005c).

Dynamic oscillation test

A dynamic oscillation test is often used to study the magnitude of the shear modulus (storage modulus G′ (Pa) which represents elastic properties, and loss modulus G″ (Pa) which represents viscous properties) and the phase angle (δ) as a function of frequency, stress, and temperature. Unlike the rotary test, where the sample is subjected to an applied rotary motion, a sinusoidal stress is applied and the resulting sinusoidal strain is measured or vice versa (i.e., a sinusoidal strain is applied and the resulting sinusoidal stress is measured) during the oscillation test. The sinusoidal mode is achieved by fixing the lower plate of the rheometer and allowing the upper plate (the cone or plate) to move clockwise and counter-clockwise. The magnitude of the deforming loads during the oscillation test is normally small; it is usually chosen by performing an amplitude sweep test prior to the oscillation test. The stress or strain should be chosen within the linear viscoelastic region (LVE) where the properties and the structure of materials are not affected during the amplitude sweep test.

The complex dynamic viscosity can be calculated by the following equation (Paraskevopoulou et al. 1997).

$$\eta^* = \left[(G')^2 + (G'')^2 \right]^{1/2} / \omega, \qquad \text{(Eq. 6)}$$

where η^* is the complex dynamic viscosity (Pa.s), G′ is the storage modulus (Pa), G″ is the loss modulus (Pa), and ω is the frequency (s^{-1}).

In some cases, the crossover point between G′ and G″ can be observed during the oscillation test, which gives an idea of how long the structure of the dressing products can last in the mouth (Koh et al. 2008). The plateau modulus or

linear viscoelasticity (G_N^0) values (Pa) can be obtained during the oscillation test by using an approximation procedure described by the following equation (Wu 1989):

$$G_N^0 = [G']_{\tan\delta \to \min imum} \quad \text{where } \tan\delta = G''/G', \qquad \text{(Eq. 7)}$$

The plateau modulus is defined as the extrapolation of the entanglement contribution to G′ at high frequencies; it can be considered as a measure of the intensity of the entangled structural network developed between the adsorbed and non-adsorbed protein molecules due to extensive flocculation (Franco et al. 1997).

Creep and recovery test

The creep and recovery test is used to study the possible internal structure of a system and the structural variations associated with the introduction of changes in its composition due to the stress-controlled effect of a food emulsion system (Dolz et al. 2008). During the creep test, a constant stress is applied to a material and the changes in its dimensions with time are monitored. During the recovery stage when the stress is removed, the time dependence of the material deformation is measured as a function of a pre-established time period. The strain/compliance *vs.* time curves can be obtained during the test. The *compliance* (*J*) (Pa^{-1}) which is equal to the ratio of the strain to the applied stress is a better parameter for characterizing rheological characteristics because the magnitude of the applied stress is taken into account (McClements 2005c).

The behavior of a viscoelastic material falls between two classical extremes (i.e., Hookean solid and Newtonian fluid); the material does not instantaneously adopt its new dimension when force is applied to it, nor does it return to its original state when the force is removed. A typical curve obtained during a creep and recovery test for a viscoelastic material is shown in Fig. 2.4. As can be observed, the strain or compliance rate (i.e., the slope) decreases with time and ultimately reaches the maximum value in the creep zone. In the recovery zone, *J* initially recovers from its elastic component instantaneously, which can be observed as a sharp decrease at the beginning of the recovery stage. *J* recovers from its viscous component slowly with time, exhibiting a smooth and slowly decreasing curve.

The extent of strain recovery or recoverable strain, which is expressed as $Q(t)\%$, can be calculated from the recovery zone (Fig. 2.4). $Q(t)\%$ is a quantity used to estimate the elasticity of the material as defined in the equation below (Zhang et al. 2008). The higher the extent of strain recovery, the greater the elasticity of the sample.

$Q(t)\% = [\Delta(\chi_{c(t)})] \times 100$, in which, $\Delta(\chi_{c(t)}) = \dfrac{\chi_{(1)} - \chi_{(2)}}{\chi_{(1)}}$, (Eq. 8)

where $Q(t)\%$ is the recoverable strain, $\chi_{(1)}$ and $\chi_{(2)}$ are the strains (dimensionless) at the equilibrium points of the creep and the recovery zones respectively (Fig. 2.4) based on a test with time duration of (t_1+t_2)s.

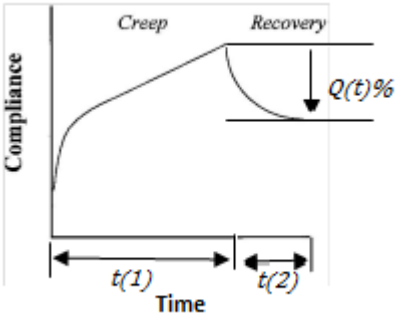


Fig. 2.4 A typical creep and recovery test graph for viscoelastic materials.

$Q(t)\% = [\Delta(\chi_{c(t)})] \times 100$, in which, $\Delta(\chi_{c(t)}) = \dfrac{\chi_{(1)} - \chi_{(2)}}{\chi_{(1)}}$, where $\chi_{(1)}$ and $\chi_{(2)}$ are the strains at the equilibrium of the creep and the recovery zones respectively, based on a test with duration time of (t_1+t_2)s.

Emulsion stability

Emulsion stability is the ability of a food emulsion to resist changes in its properties caused by physical and/or chemical processes. Physical changes mainly refer to creaming, sedimentation, flocculation, coalescence, and phase inversion. Chemical processes in a food emulsion are determined by a series of chemical structure alterations as a result of oxidation, lipolysis, proteolysis, or polymerization.

Emulsion stability is technically determined based on either the amount of oil that separates from emulsions during centrifugation (Fomuso et al. 2001; Koh

et al. 2008), or the separated volume of aqueous phase observed in a graduated syringe over a period of time (Turgeon et al. 1996). Emulsion stability can be evaluated using a QuickSCAN Analyzer, which is equipped with a pulsed near infrared light source (λ =850nm) and two synchronous detectors. The backscattering profile (BS%) or a transmission profile (T%) can be obtained as a function of time and tube length by scanning the sample every 40 μm or so over the entire length of the sample at different time intervals. The destabilization kinetics of emulsions can be measured by plotting the average values of ΔBS% within the 10–35 mm tube zone to evaluate clarification kinetics as a function of time; and the ΔBS% value in the 35–60 mm tube zone can be used to record the creaming kinetics at the top phase (Pan et al. 2002). The clarification kinetics can be determined by measuring the ratio of serum height to total sample height (in percentage) at 5% transmission as a function of time from this equation: $h_t(\%) = \frac{h_w}{h_{total}} \times 100$ (Eq. 9), where h_w is the serum height (cm), h_{total} is the total tube length (cm), and h_t is the ratio at quiescent storage time t (s) (Cerdeira et al. 2007). The creaming kinetics can be measured by recording the variations of peak thickness at a threshold value *vs.* time, since the variation in peak width at a fixed height during the studied time can be related to the kinetics of migration of small particles (Mengual et al. 1999; Lizarraga et al. 2008). Additionally, the kinetics of coalescence, flocculation and sedimentation can also be monitored using the QuickSCAN Analyzer. McClements (2005d, 2007) has reviewed various techniques and methodologies used for the characterization of emulsion stability.

The emulsion stability of dressing and mayonnaise products, which generally have a shelf life of 9 to 12 months, is influenced by several factors, including the emulsifying capacity of various emulsifiers, the proportion of each ingredient, methods of homogenization, pH, the charge and the size of the dispersed droplets, and the viscosity, as well as the transportation and storage conditions to which the food emulsions are subjected.

Microstructure

Microcopy provides information on the structure, dimensions, and organization of the components in food emulsions that cannot be acquired with the unaided eye. The different types of microscopes used include the following: conventional optical microscope, laser scanning confocal microscope, transmission electron microscope (TEM), scanning electron microscope (SEM), and atomic force microscope (AMF) (Aguilera & Stanley 1999; McClements 2005e). The conventional optical microscope contains a series of lenses which direct the light through the specimen and magnify the resulting image. The laser scanning confocal microscope focuses an extremely narrow laser beam at a particular point in the *x-y* plane; the microscope images are recorded either by moving the specimen or moving the laser beam (McClements 2005e). The electron microscope provides information on emulsion structures by using electron beams; it normally has a magnification range of 20 – 500,000X (Aguilera & Stanley 1999). The two basic types of electron microscopes are TEM (in which transmitted electrons are captured) and SEM (in which secondary electrons are captured). SEM is often used to provide images of the surface topography of specimens, whereas TEM is an important tool in studying the biological structure (such as the quaternary structure of food proteins), or the interactions between food components (Aguilera & Stanley 1999). AFM is typically used to provide information on structures at the atomic and molecular levels (Aguilera & Stanley 1999; McClements 2005e).

In addition to the interactions among ingredients, the microstructure of food emulsions (i.e., the droplet characteristics) is largely dependent on the characteristics of the emulsifiers used, i.e., the ratio of emulsifier to dispersed phase, their adsorption speed and efficiency, and the maximum reduction in interfacial tension, as well as the effectiveness and the amount of emulsifiers used to protect the droplets against coalescence (McClements 2005a). The microstructure of food emulsions is also greatly influenced by the homogenization techniques applied.

Flavor

The term flavor refers to the volatile and non-volatile components in foods that are sensed by receptors in the nose (aroma) and by the tongue and inside of the mouth (taste) as a function of time. The flavor of a food emulsion is therefore a combination of aroma, taste, and mouthfeel (Taylor & Linforth 1996; McClements & Demetriades 1998). Dynamic headspace analysis and quantification by gas chromotography-mass spectrometry (GC-MS) are the most commonly used methods to identify the volatile flavor release in dressing and mayonnaise emulsions. The chemical-impact compounds which are non-volatile can be analyzed by HPLC and ion chromatography (Marsili 2007). The ultimate identification of the flavor profile of a food emulsion product is achieved by sensory evaluation testing, which involves having human panelists assess flavor quality and intensity. Sensory tests based on diverse principles are often of crucial importance in food research, during product development, and for quality control purposes, since laboratory techniques cannot completely model the complex human sensory systems (Meilgaard et al. 2007).

There are two basic methods of sensory evaluation: affective and analytical (Marsili 2007). The affective method (also called consumer testing) evaluates consumers' preference/acceptance and opinions about a product. It entails the participation of a large number of untrained consumers. The paired comparison test and the nine-point hedonic scale are the most frequently used methods in consumer testing. By contrast, the analytical method often involves 8 to 14 highly trained panelists. Two important analytical methods consist of discriminative tests (i.e., difference and threshold) and descriptive sensory tests. Difference testing may involve the use of paired comparison tests, triangle tests, and duo-trio tests. Descriptive testing uses ranking and scaling techniques to describe and/or quantify the differences between samples while also assessing the levels of various taste attributes. One of the most frequently used descriptive testing methods is quantitative descriptive analysis (QDA) (Piggott 1984). The panelist QDA results for samples are often compared in sensory profiling/spider graphs with a view to making product formulation adjustments that yield products with

more desirable and improved sensory attributes (Marsili 2007). When the measurements are evaluated dynamically, each attribute is evaluated and quantified over a period of time (Meilgaard et al. 2007).

Particle size and particle charge

Most of the important properties of food emulsions (e.g., shelf life, appearance, texture, and flavor) have a close relationship with droplet size characteristics. Therefore, it is extremely important to reliably control, predict, measure, and report the size of the droplets in emulsions (Dickinson 1992; McClements 2005e).

Common techniques used to determine the droplet size distribution (DSD) include static light scattering (laser diffraction), dynamic light scattering, electrical pulse counting (Coulter Counter), sedimentation measurement, ultrasonic spectrometry, photon correlation spectroscopy, and NMR techniques (Christiansen et al. 2006; McClements 2007).

To monitor the particle changes in a food emulsion based on volume or weight properties according to equivalent sphere theory, DSD can be described in terms of $D[4,3]$ and $D[3,2]$. $D[4,3]$ refers to the volume weighted mean (μm); and $D[3,2]$ refers to the surface weighted mean or Sauter mean diameter (μm), which is inversely proportional to the specific surface area of droplets. Both parameters can be calculated using the following equation:

$$D[x,y] = \frac{\sum n_i d_i^x}{\sum n_i d_i^y}, \qquad \text{(Eq. 10)}$$

where d_i is the droplet diameter () and n_i is the number of droplets of d_i diameter.

$d(v,0.1)$, $d(v,0.5)$, $d(v,0.9)$ are values of particles size (μm) in food emulsions corresponding to the cumulative distribution at 10%, 50% and 90%, respectively. For example, $d(v,0.1)$ represents a size value below which 10% of the cumulative distribution lies. The uniformity parameter, (U), which relates to the deviation of droplet size (d_i) from the median of the distribution, is an index of polydispersity and can be calculated using the following equation (Mart ínez et al. 2007; Romero et al. 2009):

$$U = \frac{\sum V_i \left| d(v,0.5) - d_i \right|}{d(v,0.5) \sum V_i}, \quad \text{(Eq. 11)},$$

where V_i is the volume associated with particles of diameter d_i and $d(v, 0.5)$ is the median of the distribution.

The span (S) which indicates the width of the distribution regardless of the median size (Palazolo et al. 2004) can be expressed as: $S = \frac{d(v,0.9) - d(v,0.1)}{d(v,0.5)}$, (Eq. 12).

The analytical techniques that can be used to measure electrical charges (ζ-potential) include microelectrophoresis and electroacoustic methods (McClements 2007). The charges present on the dispersed particles are important in maintaining the stability of a reduced-fat emulsion with small particle sizes; however, they are less important in high viscosity food emulsions such as high fat mayonnaise (Harrison & Cunningham 1985).

2.4 Formulation of Novel Salad Dressing Products

The optimization of product formulation is of great importance during the design of any novel food product. The effect of different ingredient concentrations on the characteristics of the products (e.g., appearance, rheology, emulsion stability, flavor, and particle size) should be considered. Additionally, the techno-functional and the sensory properties of the designed products should be compared with properties of similar commercial products. Response surface methodology (RSM) is a useful statistical method that can be used for such studies. Details of the method have been extensively reviewed (Lundstedt, 1998; Myers & Montgomery, 2002; Douglas 2001). RSM can be used to: (1) study the effect of controllable parameters on the dependent responses of interest; (2) predict and improve the performance of a manufacturing process to produce products with enhanced quality and acceptability; (3) determine optimal processing parameters by desirability function. Thus, using this tool, novel salad dressings based on ideal formulations having physical properties similar to those of targeted commercial dressings could be developed.

Connecting Statement to Chapter 3

In order to expand the use of pulse flours in different food applications, it is necessary to consider the effects of differing processing treatments. Thermal processing is frequently applied to food formulations and is especially important in the case of pulses due to the existence of anti-nutritional components and the time required for preparation. Functional properties, which determine the performance of pulse flours in various food applications, are of primary importance and are essential in the manufacture of various food products. The work described in this chapter is a comprehensive evaluation of the effects of thermal processing (roasting and boiling) on trypsin inhibitor activity, functional properties (including protein solubility, fat absorption capacity, water holding capacity, gelling capacity, emulsifying properties, and foaming properties), and the scanning electron microstructure of flours prepared from different varieties of lentil, chickpea and pea. This chapter addresses the first objective discussed in the "Objectives of study" section of Chapter 1. The results of this study have been presented as follows:

Ma, Z., Boye, J. I., Simpson, B. K., Prasher, S. O., Monpetit, D., & Malcolmson, L. (2011). Thermal processing effects on the functional properties and microstructure of lentil, chickpea, and pea flours. *Food Research International, 44*(8), 2534-2544.

Ma, Z., Boye, J. I., Simpson, B. K., Prasher, S. O., Monpetit, D., & Malcolmson, L., "Influence of Thermal Processing on the Functional Properties and Microstructure of Lentil, Chickpea, and Pea Flours." *The 2010 Canadian Institute of Food Science and Technology/ Agriculture and Agri-Food Canada (CIFST/AAFC) Conference*, Winnipeg, Manitoba, Canada, May 30 to June 1, 2010 (Poster Presentation).

Chapter 3. Thermal Processing Effects on the Functional Properties and Microstructure of Lentil, Chickpea, and Pea Flours

Abstract

Pulses are rich in nutrients. The existence of anti-nutritional components and the length of time required for preparation have, however, limited their frequency of use compared to recommended intake levels. Anti-nutritional components in pulses can be largely removed by heat treatment. Additionally pre-treatment of pulses with heat and processing of seeds into flour could further enhance their use by decreasing processing and preparation times. In this study, trypsin inhibitor activity, functional properties, and microstructural characteristics of flours prepared from different varieties of lentil, chickpea, and pea as affected by roasting and boiling were evaluated. Both thermal treatments resulted in significant reduction (p<0.05) in trypsin inhibitor activity ranging from -95.6% to -37.8%. Scanning electron microscopy (SEM) results showed that the roasted pulse flours had similar microstructure (i.e., starch granule and protein matrix structure) to the raw samples. For the pre-boiled flours, amorphous flakes were observed by SEM with no presence of intact starch granules. This is likely due to gelatinization of starch during cooking. Interestingly, flours treated by boiling exhibited significantly higher (p<0.05) fat binding capacity, water holding capacity, and gelling capacity, while protein solubility was significantly reduced compared to the raw and roasted pulse flours. Overall, thermal treatments either had no impact or impacted to different extents the emulsifying and foaming properties of the flours. Our results suggest that thermally-treated pulse flours may have very good potential to be used as value-added food ingredients for food applications due to their improved nutritional value and, in some instances, superior functionality.

3.1 Introduction

The importance of pulses and their health-promoting benefits are widely known. Pulses are an excellent and inexpensive source of protein, complex carbohydrates, fiber, and minerals. Consumption of pulses has been associated

with many health benefits, including the reduction of the risks of type 2 diabetes and cardiovascular disease and prevention of the onset of various cancers (Roy et al. 2010).

Pulses remain underexploited, however, partially due to the presence of undesirable beany flavors (Walker & Kochhar 2007), the deficiency of sulfur amino acids in pulse proteins, the presence of antinutritional compounds (Salunkhe 1982), such as trypsin inhibitors, and the length of time required for preparation. Decreases in trypsin inhibitor content after thermal processing has been extensively reported (Hernandez-Infante et al. 1998; Marquez & Alonso 1999; Vidal-Valverde et al. 1994; Wang et al. 2003; Wang et al. 2009). Moreover, in addition to increasing the nutritional value of pulses, thermal processing also reduces the unacceptable beany flavor, making pulses more palatable.

Thermal treatment may also have marked impacts on product functionality (e.g., solubility, foaming, gelling, water binding and fat binding properties). Functional properties affect processing applications, food quality and acceptance, and how ingredients are used in foods and in food formulations (Mahajan & Dua 2002). Generally, these properties are contributed by the protein components of foods and are affected by composition, structure, conformation, interactions with other food components, and the environment (Kinsella & Melachouris 1976). In pulse flours, however, complex carbohydrates and other components such as pectins and mucilages may also contribute to the overall effect observed; in particular, the starch component of pulse flours has been regarded as a valuable source in the food industry owing to its versatile functionalities (Singh 2001).

Protein denaturation occurs during thermal treatment, and the nature and type of the proteins as well as the degree to which they are denatured are important factors which can influence the functionality of pulse flours (Wu & Inglett 1974). Additionally, the structure and physicochemical properties of starch in pulse seeds are altered to varying extents during heat treatment. Depending on the type of starch present and the degree of modification, heat treatment may result either in gelatinization or retrogradation (including new crystallization or recrystallization and perfection of the small crystalline regions) of the starch

granule (Chung et al. 2009; Donovan et al. 1983), a phenomenon that may also influence functionality.

Lentil (*Lens culinaris* L.), chickpea (*Cicer arietinum* L.), and pea (*Pisum sativum* L.) are the most common pulses consumed in many countries. Unfortunately, consumption of pulses as human food in many western countries is relatively low compared to many other parts of the world. The development of new ready-to-use pulse ingredients could stimulate production while potentially increasing pulse consumption in the west. Researchers have emphasized extending the consumption and use of grain legumes as functional ingredients in the form of flours which could be used in various food applications such as baked goods, snacks, soups, beverages, salad dressings, and dips amongst others (Kon & Burtea 1979).

Thermal treatments, including moist heating, dry and wet heating, autoclaving, boiling, and drum-drying processes, reportedly reduced nitrogen solubility, emulsifying properties, foaming properties, and gelling capacity in the flours of soy, peanut, cowpea, yam bean, winged bean, and chickpea but also increased water-holding and fat-binding capacities (Abbey & Ibeh 1988; Bencini 1986; McWatters & Holmes 1979; Narayana & Narasinga Rao 1982; Obatolu et al. 2007; Prinyawiwatkul et al. 1997). Most of these studies were performed by applying thermal treatment to the whole seeds before grinding them into flours, and the pulse species used were also limited making it difficult to obtain and compare data on the effects of thermal treatments on the different pulses. The present study was, therefore, undertaken in order to systematically compare the influence of two different thermal treatments (roasting and boiling) on the trypsin inhibitor activity (TIA), functional properties (i.e., solubility, color, fat and water absorption capacity, gelling, foaming and emulsifying properties), and microstructure of flours prepared from various varieties of pulses grown in Canada. Pulse varieties included in this study were Desi chickpea, Kabuli chickpea, red lentil, green lentil, and yellow pea with and without decortication. The varieties were selected based on their relative economic importance in Canadian production.

3.2 Materials and Methods

3.2.1 Materials

Flours of green lentil (with and without hulls), red lentil (with and without hulls), dehulled Kabuli chickpea were provided by the Canadian International Grains Institute (Winnipeg, MB, Canada). Dehulled Desi chickpea and dehulled yellow pea flours were commercial products and were provided by Diefenbaker Seed Processors Ltd. (Elbow, SK, Canada), and Parrheim Foods Inc. (Saskatoon, SK, Canada), respectively. All other materials and chemicals used were purchased from regular suppliers and were of analytical grade. Millipore filtered water was used for all experiments.

The Kabuli chickpea seeds were dehulled by increasing the moisture to 14%, drying at 70 ℃ for 20 min prior to dehulling using a dehuller/splitter, made by SK Engineering & Construction India Pvt Ltd. (Gurgaon, India). Lentils seeds were not tempered, and were directly dehulled using a Buhler pilot scale dehuller and splitter (Buhler, Markham, Ontario) operated at 530 rpm. The whole and dehulled seeds were first milled using a Jacobson 120-B lab scale hammer mill (Minneapolis, MN, USA) with a 1.5 mm screen, and then pin milled using a Hosokawa Alpine 100- UPZ pin mill (Runcorn, Cheshire, England) at 18,500 rpm.

3.2.2 Thermal Processing Methods

For roasting, pulse flours were evenly spread thinly on aluminum dishes, and were roasted for 1 min in an oven (Double model OD302, Fisher & Paykel Appliances Ltd., Huntington Beach, CA, USA) preheated to 80 ℃. After cooling to room temperature the flours were stored in air-tight plastic containers at 4 ^{o}C until analyzed. For boiling (hydrothermal processing), the pulse flours (10 % w/v) were dispersed in Millipore water under agitation for 1 h at 20 ℃, boiled in a water bath at 90 ℃ for 20 min, stored overnight in a freezer at −40 ℃, freeze-dried in a VirTis model 50-SRC-5 freeze-drier (VirTis Co., Inc., Gardiner, NY, USA), and then ground with a domestic coffee grinder (model BA-800, Hudson's Bay Co., Toronto, ON, Canada). Samples were stored at 4 ℃ in airtight containers and sealed plastic bags until further analysis.

3.2.3 Proximate Analysis

The pulse flours prior to heat treatment were analyzed to determine their proximate composition using official methods. Protein content was determined with a LECO apparatus (LECO FP-428, LECO Corp., St. Joseph, MI, USA) using the AOAC Dumas method (1995) and a nitrogen conversion factor of 6.25. Fat content was determined with a SER 148 Solvent Extractor (Velp Scientica srl, Milan, Italy) equipped with six Soxhlet posts according to the official method of the AACC (2003). Moisture was determined according to the AACC official method (1983) by drying the samples overnight at 100 ℃ in a Fisher Isotemp Vacuum Oven (Fisher Scientific Co., Montreal, QC, Canada). Ash content was determined according to the AACC official method (2003), crude fiber was analyzed according to the AOCS official method Ba 6a-05 (1998) and total carbohydrate content was calculated by difference. All determinations were done in triplicate, and average values were calculated (Batista et al. 2006; Nunes et al. 2003; Musampa et al. 2007; Wilson 1980).

3.2.4 Trypsin Inhibitor Activity (TIA)

The TIA was determined based on the methods of Kakade, Simons et al. (1969) and Hamerstrand et al. (1981) with some modifications as follows: briefly, 1 g of sample was extracted with 50 mL of 0.01 N NaOH while stirring for 3 h at room temperature, 2.0 mL aliquots of the diluted sample extract were added to the triplicate sets of test tubes for testing, the sample suspension was incubated with trypsin solution in a water bath at 37 ℃ for 10 min, and the samples were centrifuged at 2060 *g* before the absorbance was measured at 410 nm. Dilution factors of about 10, 15, 10, 50, and 20 were necessary for raw red lentils, green lentils, yellow peas, Desi chickpeas, and Kabuli chickpeas, respectively, and 2 for roasted and boiled pulse flours. The dilution factors used were selected based on 1 mL aliquots of each solution producing trypsin inhibitions between 40% and 60%. The differential absorbance (AI) due to the inhibitor per milliliter of diluted sample extract was subtracted from the trypsin standard. The TIA is expressed as milligrams pure trypsin inhibited, determined as follows: TIA (mg/g) = (2.632 × dilution factor × AI)/(sample weight × mL extract taken)

3.2.5 Scanning Electron Microscopy

A thin layer of each of the raw, roasted, and boiled flours of lentil, chickpea, and pea was deposited on a double-sided adhesive carbon tape mounted on an aluminum specimen holder, and any unattached particles were removed. The specimen holder was sputter-coated with approximately 10 nm gold using a sputter coater (model 108, Kurt J. Lesker Co., Clairton, PA, USA) and then transferred to a scanning electron microscope (model S-3000N, Hitachi, Tokyo, Japan). Samples were examined at a voltage of 5 kV.

3.2.6 Functional Properties

Protein Solubility. Protein solubility was determined at pH levels of 3, 5, and 7 using the method of Betschart (1974) with slight modifications as described by Boye et al. (2010a). The amount of protein in the supernatant was determined by the method of Bradford (1976) with a Cary 300 Bio UV-visible spectrophotometer (Varian Canada, Inc., St-Laurent, QC, Canada). The percent solubility was calculated as the percentage ratio of protein in the supernatant to that of the total protein in the initial sample.

Color Measurements. Color measurements were determined with a Minolta Chroma Meter (CR-300, Konica Minolta Co. Ltd., Osaka, Japan). D65 (day light condition) was chosen as the measurement light source. A white tile was used to calibrate the instrument, where Y=92.2, x=0.3162, and y=0.3324 (based on the CIE chromaticity coordinates which can be automatically converted to L^*, a^*, and b tristimulus coordinates by the Minolta Chroma meter). Measured values were expressed as L^*, a^*, and b^*, where the L^* value is for lightness to darkness (0 = Black and 100 = White), and a^* and b^* are for the color-opponent dimensions, in which a^* is a measure of redness (+ve) to greenness (-ve), with a higher positive a^* value indicating more red, and b^* is a measure of yellowness (+ve) to blueness (-ve), with a higher positive b^* value indicating more yellow.

Fat Absorption Capacity. The fat absorption capacity (FAC) was determined in triplicate according to the procedure of Lin et al. (1974) with slight modifications as reported by Boye et al. (2010a).

Water-Holding Capacity. The water-holding capacity (WHC) was determined according to the official procedure AACC (1983).

Gelling Capacity. The gelling capacity of the pulse flours was determined according to the method of Sathe et al. (1982) with slight modifications. An appropriate amount of each pulse flour was weighed in a test tube containing 5 mL deionized water to make suspensions at concentrations of 5%, 10%, and 15%. The samples were vortexed, and the tubes were sealed and heated at 100 ℃ in a boiling water bath for 1 h. The tubes were then cooled rapidly under running tap water and further cooled overnight at 4 ℃. The tubes were inverted to determine if the suspensions had formed a gel and were characterized as described by Boye et al. (2010a).

Emulsifying Properties. Emulsifying properties were determined by the method of Pearce & Kinsella (1978) with some modifications as described by L'Hocine et al. (2006). A Polytron PT 2100 homogenizer (Kinematica AG, Littau-Luzern, Switzerland) and a Cary 300 Bio UV-visible spectrophotometer (Varian Canada, Inc., St-Laurent, QC, Canada) were used during the test.

Foaming Properties. The procedure proposed by Waniska & Kinsella (1979) with some modifications was followed for measuring the foaming properties of the pulse flour samples, as described by L'Hocine et al. (2006).

3.2.7 Statistical Analysis

All analyses were conducted in triplicate. Values given in tables and figures are the means of three determinations. Error bars indicate the standard deviation. The statistical significance of difference was evaluated by one-way analysis of variance (ANOVA) using the PRISM software, version 3.02 (GraphPad Software, Inc., San Diego, CA, USA). Significant differences between means were determined by Tukey's multiple comparison test at the 5% significance level by means of comparing different varieties of untreated pulse flours as well as the same variety of pulse flour under different treatments.

3.3 Results and Discussion

3.3.1 Proximate Analysis

Proximate compositions of the seven different types of pulse flours are presented in Table 3.1. Protein contents varied significantly among the flours. The highest value was obtained for the dehulled red lentil (26.98%), and the lowest

was obtained for the dehulled Kabuli chickpea (19.66%). For the green and red lentil, protein contents of the flours obtained from the non-dehulled seeds were significantly lower than those of dehulled seeds. Fat contents of the flours were generally low, with the exception of the dehulled Kabuli chickpea flour which had significantly higher fat content (5.75%), followed by the dehulled Desi chickpea flour (4.67%). Ash contents for all the pulses ranged between 2.27% and 3.10%, and moisture contents ranged between 3.54% and 7.92%. Crude fiber contents varied between 2.06% to 12.16%.

Table 3.1 Proximate composition of raw flours.

Component (%)	Dehulled green lentil	Green lentils with hull	Dehulled red lentil	Red lentil with hull	Dehulled Desi chickpea	Dehulled Kabuli chickpea	Dehulled yellow pea
Protein	24.83^{a} ±0.02	24.39^{b} ±0.1	26.98^{c} ±0.07	25.79^{d} ±0.03	24.47^{b} ±0.08	19.66^{e} ±0.06	22.36^{f} ±0.09
Fat	0.93^{a} ±0.1	0.84^{a} ±0.05	0.81^{a} ±0.07	0.48^{a} ±0.06	4.67^{b} ±0.06	5.75^{c} ±0.4	0.94^{a} ±0.1
Ash	2.27^{a} ±0.1	2.27^{a} ±0.02	2.44^{a} ±0.03	2.36^{a} ±0.1	3.10^{b} ±0.06	2.72^{c} ±0.1	2.83^{c} ±0.1
Moisture	4.93^{a} ±0.5	4.87^{a} ±0.09	3.94^{b} ±0.3	4.15^{b} ±0.2	6.55^{c} ±0.1	3.54^{b} ±0.2	7.92^{d} ±0.1
Crude fiber	2.69^{a} ±0.04	3.38^{b} ±0.05	2.39^{c} ±0.01	3.73^{d} ±0.04	3.78^{d} ±0.03	12.16^{e} ±0.12	2.06^{f} ±0.01
Carbohydrate [a]	67.04	67.63	65.83	67.22	65.95	68.33	61.21

[a] Calculated by difference

Mean values bearing different lower case letters a, b, c in the same row for each pulse are significantly different (p<0.05) as per Tukey's multiple comparison test.

3.3.2 Trypsin Inhibitor Activity

Differences in the TIA of the pulse flours before and after thermal treatment are shown in Fig.3.1. For the raw flours, significantly higher TIA was found for the dehulled Desi chickpea flour (10.14 ± 0.16 mg/g), followed by the dehulled Kabuli chickpea (6.21 ± 0.23 mg/g). The lowest TIA value was observed for the dehulled yellow pea (1.14 ± 0.18 mg/g). These results are in good agreement with results previously reported by Marquez & Alonso (1999), Vidal-Valverde et al. (1994), and Wang et al. (2003), who found that chickpea contained higher levels of trypsin inhibitor than lentil or pea. No significant differences were found between TIA values of flours for red and green lentil with or without hulls, whereas significantly higher TIA levels were observed in green lentil compared to red lentil.

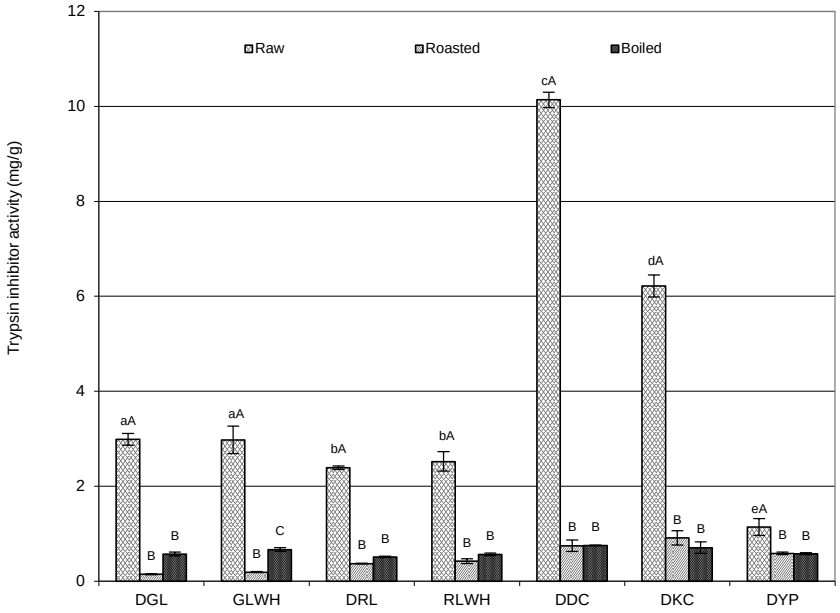


Fig. 3.1 Trypsin inhibitor activity (TIA) of pulse flours.
This figure shows TIA before and after thermal treatments. (DGL: Dehulled green lentil; GLWH: Green lentil with hulls; DRL: Dehulled red lentil; RLWH: Red lentils with hulls; DDC: Dehulled Desi chickpea; DKC: Dehulled Kabuli chickpea; DYP: Dehulled yellow pea). For the raw flour, mean values bearing different lower case letters within the same series are significantly different (p<0.05) as per Tukey's multiple comparison test. For all graphs, mean values bearing different capitalized letters A, B, C for the same type of pulse and treatments under the same parameter are significantly different (p<0.05) as per Tukey's multiple comparison test.

Both thermal treatments resulted in significant reductions in the TIA of all the pulse flours. Maximum TIA reduction (95.6%) was found for the roasted dehulled green lentil, and the lowest (37.8%) was observed for the boiled dehulled yellow pea flour. The effect of roasting did not differ significantly from that of boiling except for the red lentil with hulls, which had a significantly lower TIA value after roasting compared with that after boiling. These results are consistent with the findings of Wang et al. (2009), who observed that soaking and boiling decreased TIA values, ranging between 61.2% and 82.6% for different lentil varieties,. The results are also in good agreement with the findings of Jourdan et al. (2007), who reported that the TIAs of common bean was reduced between 80% and 95% after soaking and cooking in a 90 ℃ water bath for 15 min and were completely eliminated after cooking at 90 ℃ for 40 min. Cooking of yellow field pea for 30 min destroyed an average of 84.3% TIA (Wang et al. 2003).

Furthermore, dry heating of whole chickpea seeds under pressure at 120 ℃ and 1 atm for 15 min reportedly reduced the TIA by 27%, whereas soaking in water in addition to boiling reduced the TIA by 100% (Frias et al. 2000).

3.3.3 Microstructural Properties of the Flours

Microstructure of the raw and thermally treated pulse flours were examined by scanning electron microscopy (Fig. 3.2). In the raw samples, a uniform microstructure was observed for all the different types of pulses and cultivars studied (Figs. 3.2a, 3.2d, 3.2g). Starch granules could be clearly observed, varying in shape from ovoid to spherical, with heterogeneous sizes ranging from 19 to 35 μm in length and from 14 to 22 μm in width. The discernible globular or irregular particles attached to or located between the starch granules were the protein bodies or fragments of protein matrix disrupted during milling. Particles might also have included mineral and fiber components, as reported by other workers (Aguilera et al. 2009; Sotomayor et al. 1999). The roasted flours (Figs. 3.2c, 3.2f, 3.2i), had similar micrographs to the raw flours, though the starch granules were slightly smaller than in the raw flours, with sizes in the roasted flours ranging from 19 to 26 μm in length and 14 to 19 μm in width. No major differences were observed between the micrographs of the roasted flours for the different flours samples. The flours subjected to boiling and freeze-drying, however, had very different microstructures compared to the raw and roasted samples (Figs. 3.2b, 3.2e, 3.2h). As expected, no starch granules could be seen, and more amorphous flakes were observed, a phenomenon that might be due to the mixture of protein and starch due to pre-gelatinization, resulting in a homogenous network made of cross-linked protein and starch molecules. The particle sizes of these amorphous flakes are dependent on the grinding technique, and no significant differences were observed between the samples subjected to boiling.

The microstructures of the pulse flours observed after boiling in this study are different from those reported by other researchers (Aguilera et al. 2009; Marconi et al. 2000). The large divergence between the present study and others might be due to differences in the thermal processing conditions used. In most reported studies, soaking and heat treatments were applied directly to whole seeds

instead of flours, as was done in the present case. Thus, in the previously reported studies, soaking and cooking treatments resulted in swelling and enlargement of some of the starches as well as a more flattened surface (Aguilera et al. 2009). Some of the starches had more amorphous extracellular material after cooking, or endocorrosion and breakages occurred in the starch granules after fermentation (Sotomayor et al. 1999). However, the integral starch granule structure was still maintained, and the protein matrix adhering to the starch granules was still visible, although alterations to the protein structure were found (Blaszczak et al. 2007). Gelatinization and cross-linking is more likely to occur in flours than in whole seeds during thermal treatment as the proteins and starches are all exposed and are intimately mixed. In the case of whole seeds, however, the starch granules and proteins are encapsulated inside the seed walls, which can restrict cross-linkages with other molecules. This may explain why the integrity of the starch granules in the microstructure of the whole seeds is maintained after cooking.

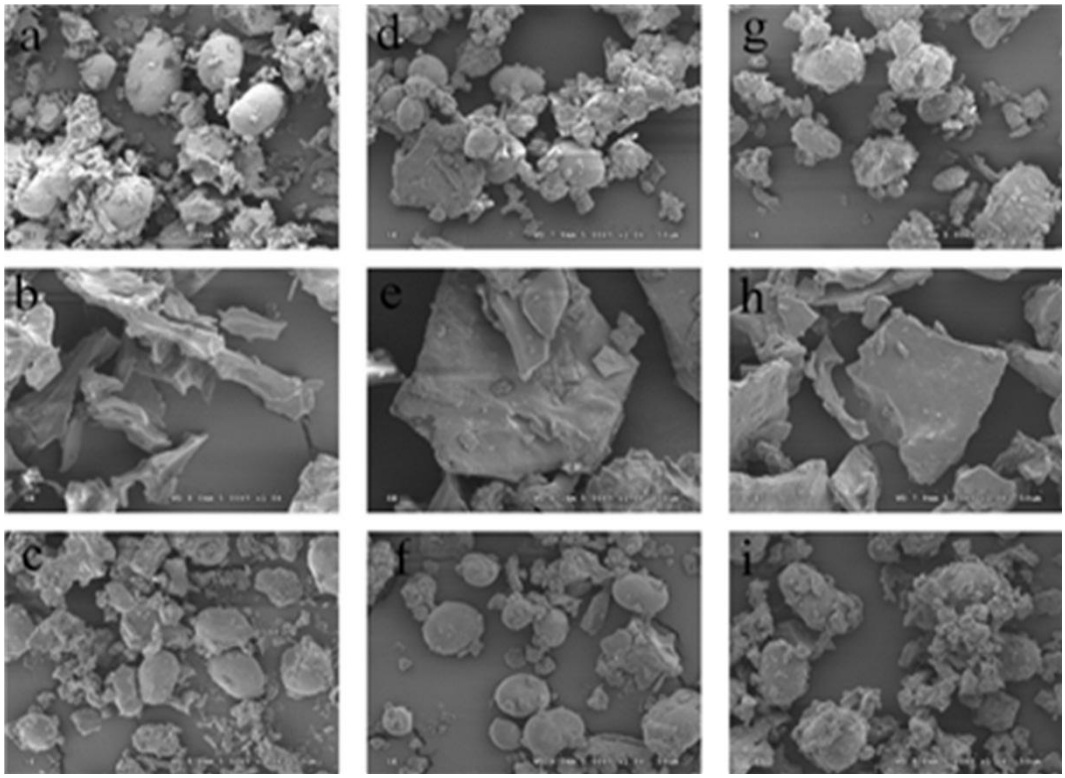


Fig. 3.2 Scanning electron micrograph of lentil, chickpea and pea flours.
Arrows show starch granules. (a) raw dehulled green lentil; (b) boiled dehulled green lentil; (c) roasted dehulled green lentil; (d) raw dehulled Kabuli chickpea; (e) boiled dehulled Kabuli chickpea; (f) roasted dehulled Kabuli chickpea; (g) raw dehulled yellow pea; (h) boiled dehulled yellow pea; (i) roasted dehulled yellow pea.

3.3.4 Functional Properties

Protein Solubility. The protein solubility results (Fig. 3.3a) for the raw samples was found to be pH-dependent. The raw flours exhibited higher protein solubility at neutral conditions (pH 7) compared to acidic pH of 3 and 5. Protein solubility ranged between 53.67% and 61.04% at pH 7 with no significant differences observed between flours. The lowest solubility values were observed at pH 5 and were less than 10% for all the raw flours (data not shown), and no significant differences were observed between the raw samples or between the raw samples and the other thermally treated flours. At pH 5 the pulse proteins are in the neighborhood of the isoelectric point, at which protein-protein interactions disfavor solubility when compared to the other pH levels studied. At pH 3, the protein solubility obtained for the raw dehulled green lentil was significantly higher than the values obtained for the other raw flours (Fig. 3.3a). The next highest values were obtained for the dehulled red lentil and the red lentil with hulls, and the lowest value was obtained for the raw dehulled Kabuli chickpea (0%), for which this pH was reported as the isoelectric point (Carbonaro et al. 1997). There were no significant differences in solubility between the raw samples and the roasted samples except for the dehulled green lentil and the dehulled Desi chickpea, which had decreased protein solubility at this pH. The results showed a direct effect of boiling on solubility of the pulse proteins. According to the Tukey's test, boiling dramatically decreased protein solubility at pH 3 for all the samples except for the dehulled Kabuli chickpea. There was no significant difference observed at pH 7 for the roasted pulse flours compared to the untreated flours, with the exception of the green lentil with hull, which exhibited significantly lower solubility (Fig. 3.3b). A significant reduction in protein solubility at pH 7 was also found for all the boiled flours. A negative effect of wet cooking and dry heating treatments on the solubility of pulse flours has been reported for winged bean flours, cowpea flours, chickpea flours, soybean and peanut flours, Bengal gram, black gram, green gram, and lentil flours at pH levels from 2 to 12 after various heat processes (Bencini 1986; McWatters &

Holmes 1979; Nagmani & Prakash 1997; Narayana & Narasinga Rao 1982; Prinyawiwatkul et al. 1997).

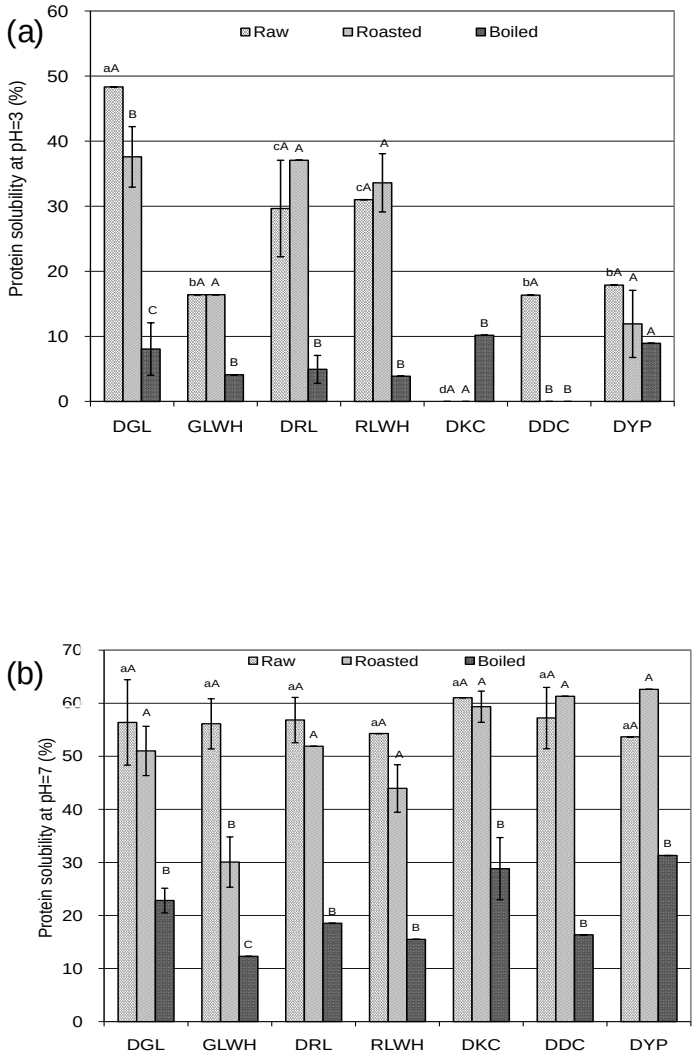


Fig. 3.3 Protein solubility of pulse flours at (a) pH 3 and (b) pH 7 before and after thermal treatment.
(DGL: Dehulled green lentil; GLWH: Green lentil with hull; DRL: Dehulled red lentil; RLWH: Red lentils with hull; DDC: Dehulled Desi chickpea; DKC: Dehulled Kabuli chickpea; DYP: Dehulled yellow pea). For the raw flour, mean values bearing different lower case letters within the same series are significantly different (p<0.05) as per Tukey's multiple comparison test. For all graphs, mean values bearing different capitalized letters A, B, C for the same type of pulse and treatment under the same parameter are significantly different (p<0.05).

The decreased protein solubility could be ascribed to biochemical changes in protein structure during heating. Extensive cross-linking between protein and starch molecules during heating, and particularly during boiling may have caused the formation of aggregates which rendered the protein insoluble. Both intramolecular hydrogen bonds and non-polar bonds are cleaved and can reform during heating and subsequent cooling, and the resultant changes in conformation could make proteins less soluble. The insolubility could also be due to disulfide-sulfhydryl interchange reactions (Neucere 1972). In the present study, the boiling (wet) treatment had a harsher effect than the roasting (dry) treatment and led to a significantly lower protein solubility value. These results are in accordance with those of Pour-El & Peck (1973), who found similar results after subjecting defatted soy flake samples to both dry heat (130 ℃) and wet heat (steaming at 100 ℃) treatments for varying periods of time.

Color Measurements. The Hunter color values (L^*, a^*, b^*) of the raw and thermally treated flours are presented in Table 3.2. Among the raw pulse flours studied, the dehulled Kabuli chickpea showed the highest L^* parameter value, 62.77 ± 0.89, indicating the flour's lighter color compared to the other pulse flours. All the raw flours showed negative a^* values except for the dehulled red lentil and the red lentil with hull. Significantly lower a^* value (-0.93 ± 0.03), was obtained for the raw dehulled green lentil, indicating its greener hue compared to all the other samples. The highest a^* value (3.15 ± 0.03) was obtained for dehulled red lentil, indicating its redder hue. The dehulled Desi chickpea had the highest b^* value, which was significantly different from the values observed for the other raw pulse flours, indicating its yellower hue compared to the other flours.

Significant color changes were observed in the samples after roasting and boiling. After roasting, the L^* values for the treated pulse flours generally increased significantly compared to the values for the raw pulse flours, indicating increased lightness after roasting. The exceptions were the dehulled green lentil, whose L^* value was significantly decreased, and the dehulled Desi chickpea and dehulled Kabuli chickpea, which had similar L^* values. The boiling process increased the L^* value significantly for most of the samples in comparison with

the raw and roasted flours, with the exception of the non-dehulled flours (green lentil and red lentil), which had similar or decreased *L** values.

Table 3.2 Color measurement of pulse flours before and after thermal treatments

Samples		De-hulled green lentil	Green lentil with hull	De-hulled red lentil	Red lentil with hull	De-hulled Desi chickpea	De-hulled Kabuli chickpea	De-hulled yellow pea
Raw	L	55.25^{aA} ±0.36	53.23^{bA} ±0.05	57.40^{cA} ±0.28	51.87^{dA} ±0.44	59.01^{eA} ±0.39	62.77^{fA} ±0.89	49.55^{gA} ±0.49
Roasted	L	53.98^{aB} ±0.26	56.76^{bB} ±0.56	58.55^{cB} ±1.11	55.91^{bB} ±1.04	58.40^{cA} ±0.22	63.49^{dA} ±0.18	56.74^{bB} ±0.48
Boiled	L	72.49^{aC} ±0.27	52.28^{bA} ±0.48	61.69^{cC} ±0.29	49.03^{dC} ±0.35	64.79^{eB} ±0.47	70.82^{fB} ±0.86	58.79^{gC} ±0.21
Raw	a	-0.93^{aA} ±0.03	-0.82^{bA} ±0.03	3.15^{cA} ±0.03	1.69^{dA} ±0.04	-0.50^{eA} ±0.02	-0.72^{fA} ±0.03	-0.46^{eA} ±0.03
Roasted	a	-0.91^{aA} ±0.02	-0.74^{bA} ±0.03	3.33^{cB} ±0.05	1.79^{dB} ±0.10	-0.56^{eA} ±0.02	-0.81^{bB} ±0.01	-0.56^{eB} ±0.02
Boiled	a	-1.91^{aB} ±0.04	0.67^{bB} ±0.01	-0.18^{cC} ±0.02	1.47^{dC} ±0.04	-0.97^{eB} ±0.03	-0.18^{cC} ±0.04	-0.42^{fA} ±0.02
Raw	b	9.76^{aA} ±0.01	8.28^{bA} ±0.12	10.35^{cA} ±0.05	8.28^{bA} ±0.09	13.21^{dA} ±0.18	11.87^{eA} ±0.13	7.99^{bA} ±0.09
Roasted	b	9.92^{aA} ±0.06	9.46^{bB} ±0.15	10.87^{cB} ±0.15	9.13^{dB} ±0.37	13.45^{eA} ±0.08	12.66^{fB} ±0.10	9.88^{aB} ±0.11
Boiled	b	14.34^{aB} ±0.11	9.67^{bB} ±0.08	11.30^{cC} ±0.07	8.48^{dA} ±0.09	16.69^{eB} ±0.08	15.53^{fC} ±0.22	12.94^{gC} ±0.05

For the raw flour, mean values bearing different lower case letters within the same raw are significantly different (p<0.05) as per Tukey's multiple comparison test. For all samples, mean values bearing different capitalized letters A, B, C for the same type of pulse and treatment under the same parameter are significantly different (p<0.05) as per Tukey's multiple comparison test.

The dehulled red lentil and non-dehulled red lentil had increased *a** values after roasting, as did the dehulled green lentil, non-dehulled green lentil, and dehulled Kabuli chickpea after boiling. The dehulled red lentil, non-dehulled red lentil, and dehulled Desi chickpea had decreased *a** values after boiling, as did the dehulled Kabuli chickpea and dehulled yellow pea after roasting.

The trend for the *b** value was similar to that observed for the *L** value (i.e., both increased significantly after roasting and boiling and exhibited an increased yellow hue), except for the dehulled green lentil, dehulled Desi chickpea after roasting, and non-dehulled red lentil after boiling which all had similar values. Moreover, the boiled pulse flours had an increased *b** value, with the exception of the non-dehulled green lentil and non-dehulled red lentil, which had similar and decreased values, respectively, compared to the roasted pulse flours. The altered color of the thermally treated flours may be advantageous in terms of incorporating them into certain foods, such as cookies and extruded snacks, in which a golden or brownish color may be desirable (Prinyawiwatkul et al. 1996).

The Hunter color values reported for Indian cultivars of field pea (*P. sativum* L.) and pigeon pea (*Cajanus cajan* L.) were 78.81 to 84.17 for *L**, −1.53 to −7.15 for *a**, and 15.81 to 18.86 for *b** for field pea, and 77.89 to 78.17 for *L**, −2.84 to 3.53 for *a**, and 16.83 to 18.24 for *b** for pigeon pea (Maninder et al. 2007), and the values for flours from Indian cultivars of Kabuli and Desi chickpea were 81.64 to 85.41 for *L**, −0.72 to −1.10 for *a**, and 14.1 to 20.7 for *b** (Kaur & Singh 2005). It was also reported that soaking, boiling, and fermentation processes increased the lightness of whole cowpea seeds and gave them a greener and bluer hue (with higher *L**, lower *a**, and lower *b** values) (Prinyawiwatkul et al. 1996).

Fat Absorption Capacity. The FAC is an important functional property, as it helps to improve mouthfeel and the retention of flavor (Kinsella & Melachouris 1976). No significant difference was found among the seven types of flours. FAC for all the raw flours ranged between 169.91% and 176.94% (Fig. 3.4). There was a general tendency for FAC to decrease after the roasting process, and this effect was statistically significant for the green lentil with hull and dehulled yellow pea after roasting. The significantly highest FAC was observed for the boiled samples. Thus, FAC of the boiled dehulled green lentil flour (290.08%) was higher than that of the raw flour (169.91%), a 70.7% improvement, and the FAC for the dehulled Desi chickpea increased from 176.01% to 215.57% after the boiling process, a 22.5% improvement. This result for the flours after boiling was consistent with the findings of Abbey & Ibeh (1988), who reported that the FAC of cowpea flour increased from 2.9 to 3.2 g/g after autoclaving, a 10.3% improvement. Similar results were also reported by Del Rosario & Flores (1981), and Nwanekezi et al. (1994) on mung bean flour, African yam bean flour, and Bambara groundnut flour.

Improved FAC has been attributed to enhanced hydrophobic properties of proteins and the superior fat-binding performance of non-polar amino acid side chains (Kinsella & Melachouris 1976). It could therefore be inferred that more non-polar residues from the interior of the protein molecules were unmasked after boiling. Additionally, the physical structural differences and the variation in

particle size distribution of the boiled flours may have induced greater porosity allowing greater entrapment of fat compared to the raw and roasted flours.

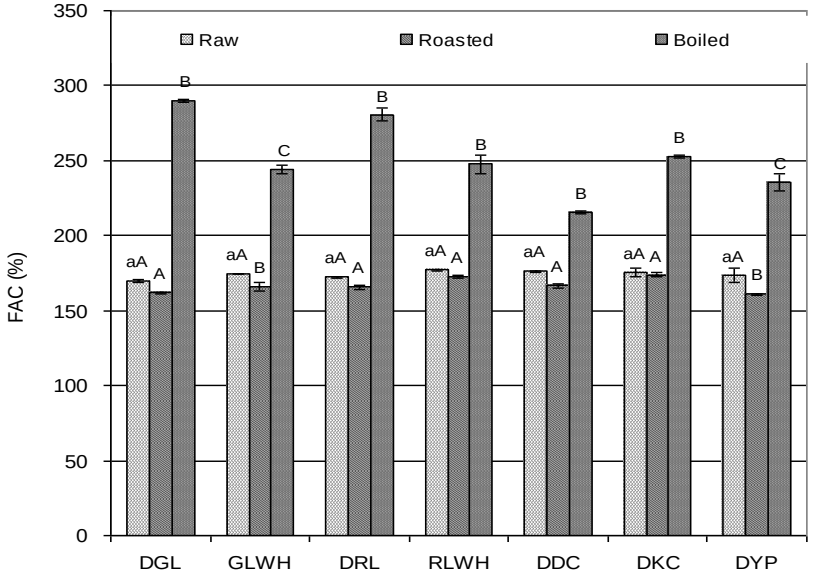


Fig. 3.4 Fat absorption capacities of pulse flours before and after thermal treatments.
(DGL: Dehulled green lentil; GLWH: Green lentil with hull; DRL: Dehulled red lentil; RLWH: Red lentils with hull; DDC: Dehulled Desi chickpea; DKC: Dehulled Kabuli chickpea; DYP: Dehulled yellow pea). For the raw flour, mean values bearing different lower case letters within the same series are significantly different (p<0.05) as per Tukey's multiple comparison test. For all graphs, mean values bearing different capitalized letters A, B, C for the same type of pulse and treatment under the same parameter are significantly different (p<0.05).

Water Holding Capacity. WHC, which is distinct from water sorption ability, is defined as the ability to physically hold water against gravity (Kinsella 1979) and is defined as the amount of water that can be absorbed per gram of sample. The WHC of flours is a very important functional property in many different food applications. The highest WHC among all the raw samples was found for the dehulled Kabuli chickpea flour (Fig. 3.5). WHC of the roasted flours did not differ much from that observed for the raw pulse flours. However, all the boiled samples exhibited exceptionally high WHC values compared to the raw and roasted samples (i.e., from 1.3843 mL/g for boiled dehulled yellow pea flour to 1.7908 mL/g for the boiled dehulled Kabuli chickpea flour). The largest increase in WHC (146.5%) was observed for the dehulled green lentil and the smallest increase (71.8%, from 1.0425 to 1.7909 mL/g) was noted for the dehulled Kabuli chickpea. These results are supported by Lin et al. (1974), who

found that heat denaturation of sunflower proteins improved their water-imbibing capacity. As well, winged bean flour (Narayana & Rao 1982), chickpea flour (Bencini 1986), and lentil flour (Nagmani & Prakash 1997) exhibited significantly increased WHC after autoclaving, cooking in water, $NaHCO_3$, and citric acid, dry heating under pressure. This effect might again be due to physical structural differences of the boiled flours allowing greater porosity and fluid entrapment and/or greater water binding properties of subunits or amino acid residues exposed as a result of denaturation (Catsimpoolas & Meyer 1970). In addition, starch gelatinization and the swelling of crude fiber during heating might also contribute to increase WHC (Aguilera et al. 2009). Flours with high WHC could be good ingredients in bakery applications (e.g., bread formulation), since a higher WHC enables bakers to add more water to the dough, thus improving the handling characteristics and maintaining freshness in bread (Wolf 1970).

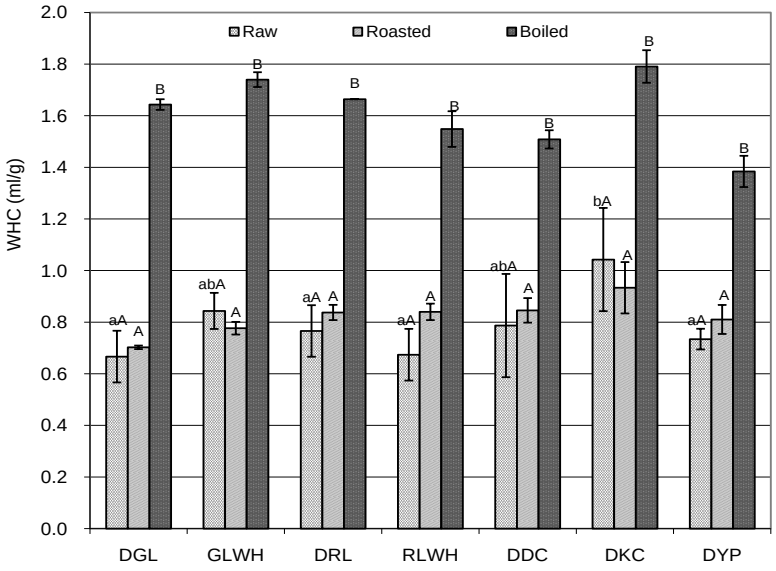


Fig. 3.5 Water holding capacity of pulse flours before and after thermal treatments.
(DGL: Dehulled green lentil; GLWH: Green lentil with hull; DRL: Dehulled red lentil; RLWH: Red lentils with hull; DDC: Dehulled Desi chickpea; DKC: Dehulled Kabuli chickpea; DYP: Dehulled yellow pea). For the raw flour, mean values bearing different lower case letters within the same series are significantly different (p<0.05) as per Tukey's multiple comparison test. For all graphs, mean values bearing different capitalized letters A, B, C for the same type of pulse and treatment under the same parameter are significantly different (p<0.05).

Gelling Capacity. Gelling capacity is very useful in food systems such as puddings and sauces that require thickening and gelling. Gelation may be

described as a process in which denatured molecules cross-link to form aggregates stabilized by a variety of bonds including, electrostatic interactions, hydrogen, hydrophobic and/or disulfide bonds. Sathe, et al. (1982) attributed variations in the gelling capacity of various pulse flours to different relative ratios of constituents such as proteins, carbohydrates, and lipids as well as the interactions among all of the constituents making up the pulses. Protein and starch from pulses could form network separately or interactively in the raw flours. As earlier studies had reported the least gelation concentration of legume flours to be between 10% and 14% (w/v), three concentrations, namely 10%, 15%, and 20% (w/v), were chosen in the present study to compare the gelling capacity before and after thermal treatment.

Table 3.3 Gelling capacity of pulse flours before and after thermal treatments

Samples	Pulse Conc.(%w/v)	De-hulled green lentil	Green lentil with hull	De-hulled red lentil	Red lentil with hull	De-hulled Desi chickpea	De-hulled Kabuli chickpea	De-hulled yellow pea
Raw	10	−	−	⊕	−	⊕	⊕	−
	15	⊕	⊕	⊕	⊕	⊕⊕	⊕⊕	⊕
	20	⊕⊕	⊕⊕	⊕⊕	⊕⊕	⊕⊕	⊕⊕	⊕⊕
Roasted	10	⊕	−	⊕	−	⊕	⊕	−
	15	⊕⊕	⊕	⊕⊕	⊕	⊕⊕	⊕⊕	⊕
	20	⊕⊕	⊕⊕	⊕⊕	⊕⊕	⊕⊕	⊕⊕	⊕⊕
Boiled	10	⊕	⊕	⊕	⊕	⊕	⊕	⊕
	15	⊕	⊕	⊕⊕	⊕⊕	⊕⊕	⊕⊕	⊕⊕
	20	⊕⊕	⊕⊕	⊕⊕	⊕⊕	⊕⊕	⊕⊕	⊕⊕

−No gel; ⊕Weak gel; ⊕⊕ Firm gel

As shown in Table 3.3, all the raw flours formed a firm gel at a concentration of 20% (w/v). Moreover, the dehulled Desi and Kabuli chickpea flours also formed firm gels at a concentration of 15 % (w/v). At a concentration of 10%, all the other pulse flours formed a weak gel, with the exception of the dehulled green lentil, non-dehulled red lentil and non-dehulled green lentil flours, which formed no gel, and the dehulled yellow pea, which formed a viscous solution.

Gelling capacity of the pulse flours increased slightly after roasting. In addition to the dehulled Desi and Kabuli chickpea flours, the dehulled green and red lentil flours also formed firm gels at a concentration of 15% (w/v). All the boiled pulse flours tended to have better gelling capacity compared to the raw and roasted samples. Thus, all the boiled flours formed weak gels at a concentration of 10% (w/v) and a firm gel at a concentration of 15% (w/v). These results are in good agreement with work done on chickpea flours prepared by soaking and cooking for 20, 30, and 50 min, followed by drum-drying (Bencini 1986). However, other results were also found in the literature, where the least gelation concentration of cowpea flour increased, indicating a poorer gelation capacity after whole seeds had been soaked and boiled for 45 min (Prinyawiwatkul et al. 1997) or autoclaved at 121 ℃ for 15 min (Abbey & Ibeh 1988), as was also the case with chickpea and lentil flours after whole seeds had been soaked and boiled for 70 and 30 min, respectively (Aguilera et al. 2009). The disparity in the results may be mainly due to the conditions used for the thermal treatments. For the results that showed decreased gelling capacity (Abbey & Ibeh 1988; Aguilera et al. 2009; Prinyawiwatkul et al. 1997), thermal pretreatment was applied to the whole seeds before they were ground into flour, a process that was different from precooking the ground flours. In the case of the precooked flours, the starch and protein molecules come into intimate contact and are initially mixed together in solution facilitating the formation of potential networks during the thermal pretreatment; cross-linking is therefore much easier to occur than for the raw flours. In other words, protein denaturation and starch pre-gelatinization occurring during the heat pretreatment of the precooked flours could have facilitated the formation of a stronger gel matrix at a lower concentration compared to the raw and roasted flours. The pre-roasted flours behaved differently than the precooked flours in terms of gelling capacity, because the roasted flours were preheated under dry conditions, and there was not enough water to support pre-gelatinization. These results were also confirmed by the microstructures. Additionally, the differences occurred in the structural characteristics of the raw and thermally treated flours may also affect the absorption of water and swelling

which were involved during gelatinization process. When whole seeds are precooked (Prinyawiwatkul et al. 1997; Aguilera et al. 2009), starches and proteins are encapsulated in the cell, limiting pre-gelatinization during precooking.

Emulsifying Properties. Emulsifying properties are represented by the emulsifying activity index (EAI) and emulsifying stability index. The EAI reflects the ability and capacity of a protein to aid in the formation of an emulsion and is related to the protein's ability to absorb to the interfacial area of oil and water in an emulsion. The ESI normally reflects the ability of the proteins to impart strength to an emulsion for resistance to stress and changes and is therefore related to the consistency of the interfacial area over a defined time period (Pearce & Kinsella 1978). Emulsifying properties are very important properties that proteins and other amphoteric molecules contribute to the development of traditional or novel foods. Carbohydrates such as starch and fiber may also enhance emulsion stability by acting as bulky barriers between the oil droplets, preventing or slowing down the rate of oil droplet coalescence (Aluko et al. 2009).

The emulsifying stability results did not vary much between samples, as illustrated in Fig. 3.6a. No significant differences in results were observed between the pulse flours from different cultivars or between the raw flours and the flours after both types of thermal treatments, with the exception of the roasted dehulled yellow pea, whose ESI value was higher than that of the untreated flours. For EAI, better emulsifying activities were observed for the dehulled Kabuli chickpea and dehulled yellow pea after roasting and boiling, in the dehulled green lentil after roasting, and in the non-dehulled green lentil, dehulled red lentil after boiling (Fig. 3.6b). The increased EAI might be due to the dissociation and partial unfolding of globular proteins, leading to exposure of hydrophobic amino acid residues, which consequently increased the surface activity and adsorption at the oil and water interface (Nir et al. 1994). Moreover, interactions between proteins and carbohydrates in pulse flours may also impact the EAI to different extents. Similar EAI improvement was also observed when peanut flours were heated directly at 100 ℃ for 3 min (Prinyawiwatkul et al. 1993). Whereas some workers have attempted to correlate protein solubility with emulsifying properties, this

does not always hold as reported in the studies of Prinyawiwatkul et al. (1997) and Aluko & Yada (1993). The non-dehulled red lentil flour behaved different from the other samples in that it showed decreased EAI after roasting and boiling (in spite of multiple replicate analyses). The reason for this remains unclear. Obatolu et al. (2007), however, also observed that roasting resulted in significant reductions, from 50.7% to 20%, in the emulsifying capacity of yam bean. Prinyawiwatkul et al. (1997), Narayana et al. (1982) and Aguilera et al. (2009), also respectively, observed decreased EAI after cowpea seeds were boiled for 45 min, winged bean flours were autoclaved for 10 min, and whole chickpea seeds and whole lentil seeds were boiled for 70 min and 30 min.

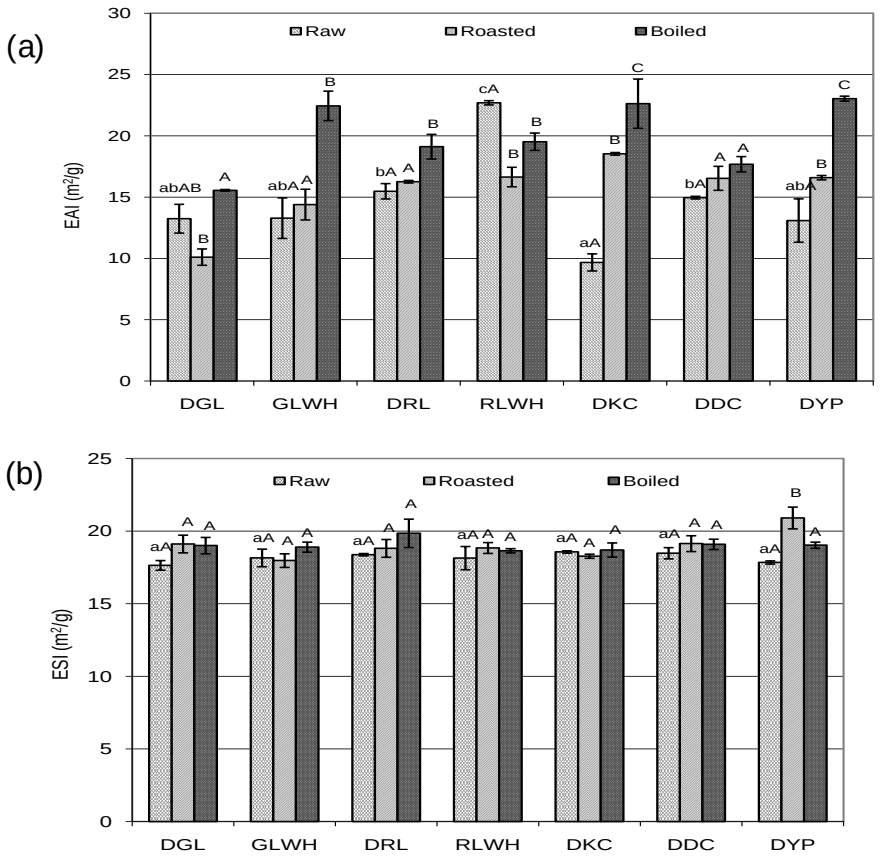
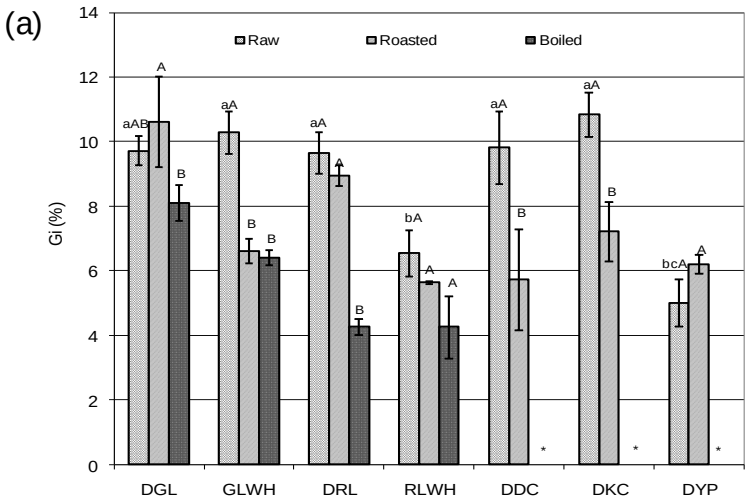


Fig. 3.6 (a) Emulsifying activity index (EAI) and (b) emulsifying stability index of pulse flours before and after thermal treatments.
(DGL: Dehulled green lentil; GLWH: Green lentil with hull; DRL: Dehulled red lentil; RLWH: Red lentils with hull; DDC: Dehulled Desi chickpea; DKC: Dehulled Kabuli chickpea; DYP: Dehulled yellow pea). For the raw flour, mean values bearing different lower case letters within the same series are significantly different (p<0.05) as per Tukey's multiple comparison test. For all graphs, mean values bearing different capitalized letters A, B, C for the same type of pulse and treatment under the same parameter are significantly different (p<0.05).

Variations in the emulsifying properties after thermal treatment reported by different workers could potentially be due to the different heating conditions used. In most reported studies heat treatment was applied to the whole seeds (Prinyawiwatkul et al. 1997; Aguilera et al. 2009; Obatolu et al. 2007), whereas in this study the flours were dispersed in solution and then heated. Furthermore, emulsifying properties can be affected by protein content, protein structures, lipid content, and ratio of amylose and amylopectin in starch (Kaur & Singh 2005; Patel & Kilara 1990; Singh 2001; Singh et al. 2007). Pulse flours with superior emulsifying properties could be very useful in food systems such as salad dressing, beverages, and meat analogs.

Foaming Properties. Foam formation and stability generally depend on the interfacial film formed by proteins which keeps air bubbles in suspension and slows down the rate of coalescence. Foaming properties are dependent on the proteins as well as on other components such as carbohydrates. Foaming properties measured in this study included Gi (which is the percent of gas entrapped in the foam), FE (which is the percent foam expansion) and R5 (which represents the percent of liquid retained in the foam after 5 min). High Gi and FE values indicate high foaming capacity and foam expansion, respectively, while a high R5 value indicates high foam stability. Better foaming capacity implies greater incorporation of air bubbles, whereas foam stability is described as the ability of the protein and other components to form a strong and cohesive film around air bubbles and greater resistance of air diffusion from the bubbles.



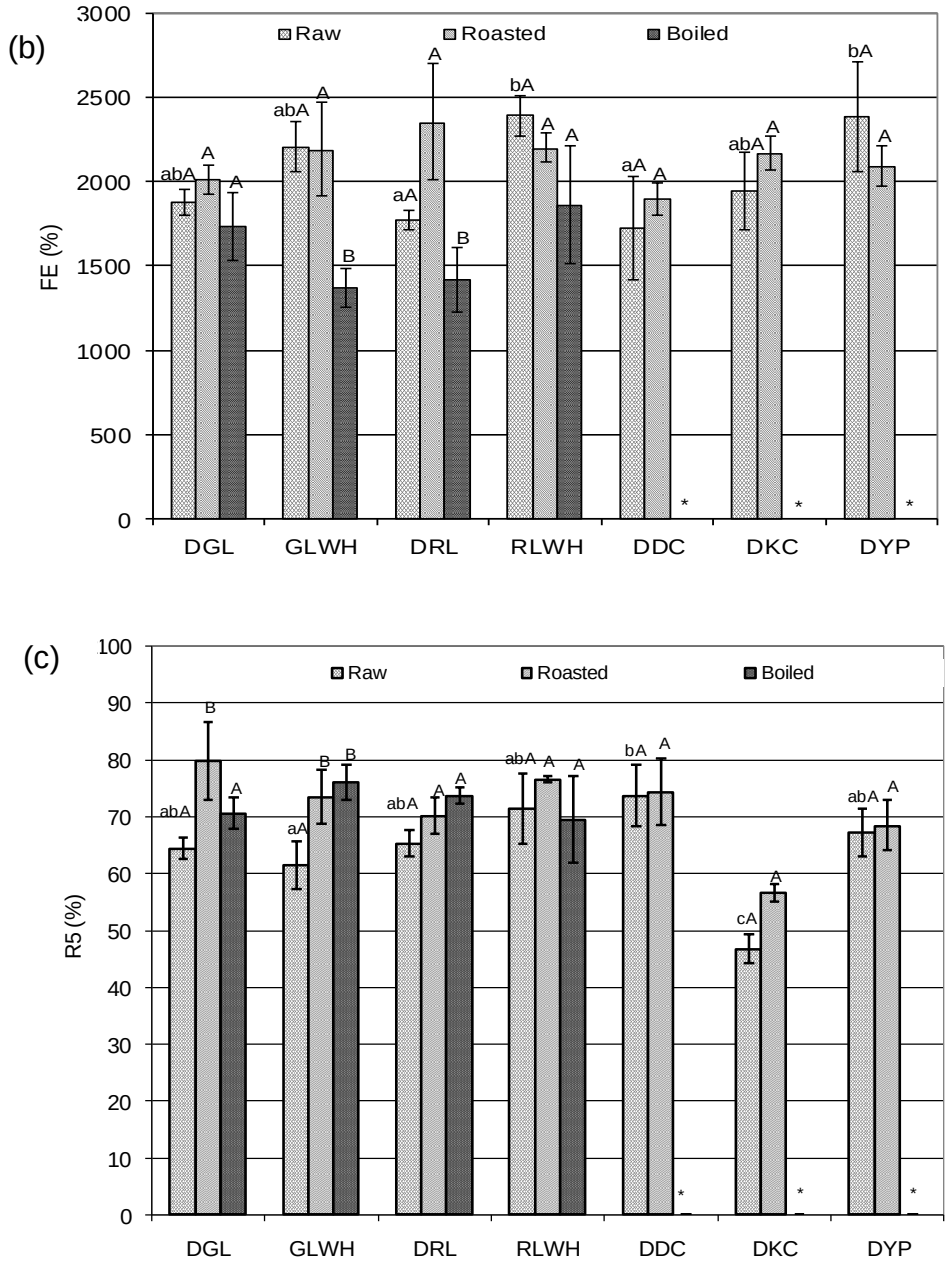


Fig. 3.7 (a) Foaming capacity (Gi), (b) foam expansion (FE) and (c) foaming stability (R5) of pulse flours before and after thermal treatments.
(DGL: Dehulled green lentil; GLWH: Green lentil with hull; DRL: Dehulled red lentil; RLWH: Red lentil with hull; DDC: Dehulled Desi chickpea; Dehulled Kabuli chickpea; DYP: Dehulled yellow pea).* Sample was unable to foam up to 70 mL mark under the experimental condition. Mean values bearing different lower case letters a, b, c within the same series of untreated pulse flours are significantly different (p<0.05) on application of Tukey's multiple comparison test. Mean values bearing different capitalized letters A, B, C within the same type of pulse with different treatment under the same parameter are significantly different (p<0.05).

The foaming properties of the flours before and after the thermal treatments are presented in Fig. 3.7. The effect observed was not consistent in all the samples and depended very much on the type of pulse. In general, the thermal treatments either had no impact on the foaming properties or impacted them to different extents. The raw flours of the non-dehulled red lentil and dehulled yellow pea exhibited significantly lower Gi values compared to those of the other raw flours (Fig. 3.7a). The non-dehulled green lentil, dehulled Desi and Kabuli chickpea exhibited decreased Gi values after roasting, whereas the other roasted samples had similar results without statistically significant differences compared to the raw ones. All the samples showed significantly decreased Gi values after boiling, with the exception of the non-dehulled red lentil. No measurable foam was formed up to 70 mL under the experimental conditions in the case of the dehulled Desi and Kabuli chickpea, and dehulled yellow pea after boiling. With regard to FE values (Fig.3.7b), no significant difference was observed in the flours after the roasting process. All the samples exhibited significantly decreased FE values (%) after boiling, with the exception of the dehulled green lentil and non-dehulled red lentil. The decreased Gi and FE values after boiling are consistent with results obtained by Bencini (1986), who found that the whippability of chickpea flours was lower after heat treatment. Similar results were found after thermal treatment of glandless cottonseed flour, winged bean flour, chickpea flour, and lentil flours from Spanish cultivars (Narayana & Narasinga Rao 1982; Aguilera et al. 2009; Lawhon et al. 1972). The decrease in Gi and FE after boiling could be ascribed to the aggregation of disordered proteins that occurred during protein denaturation, which may have reduced the concentration of effective adsorbing species (Stainsby, 1986) and the decreased solubility observed for these samples.

Foaming capacity and foam expansion are related to the proteins' ability to rapidly diffuse to the interface, reorient, and form a viscous film without excessive aggregation or coagulation, whereas foaming stability is influenced by intermolecular cohesiveness and viscosity of the film as well as a certain degree of elasticity permitting localized contact deformation (Kinsella 1981). The R5 values, representing foaming stability (Fig.3.7c), increased for the dehulled green

lentil after roasting and the non-dehulled green lentil after roasting and boiling, with others including dehulled red lentil, red lentil with hull, dehulled Desi chickpea, dehulled Kabuli chickpea and dehulled yellow pea flours exhibited increased values after roasting or boiling however no significant differences were observed.

The molecular flexibility that is desirable for facilitating foam formation does not ensure stability, which is generally contributed by intermolecular interactions and cohesiveness. During thermal processing, viscosity might increase as a result of partial surface denaturation of the proteins, which in turn could impart rigidity to the interfacial film for foam stabilization and therefore cause increased R5 values in certain pulse flours (Kinsella 1981). Additionally, conformational changes in proteins induced by interactions such as hydrogen bonding and electrostatic and hydrophobic interactions, which are very important factors for foam stabilization, can occur during thermal processing which may contribute to increase the R5 value (Kinsella 1981; Stansby 1986). However, due to differences in the types of proteins and starch molecules in the different pulse types and pulse varieties (Boye et al. 2010a; de Almeida Costa et al. 2006), the viscoelastic properties of the flours after thermal treatment can be expected to differ which may explain the variance in the foaming properties observed. Thus, whereas roasting and boiling appears to increase foaming stability for some pulse flours, it is impossible to say that this effect is consistent for all the pulse flours.

3.4. Conclusions

In conclusion, pre-cooking of pulse flours either by roasting or boiling may be a potential way to enhance their use in food formulation as this may shorten the times required for processing and preparation while enhancing nutritional value. Thermal treatment can, however, significantly influence the functionality of the flours. Scanning electron microscopy studies provided information on differences in the microstructure of the flours as a result of thermal treatment. Compared to data presented in the literature where whole seeds were boiled prior to grinding, heating of flour solutions prior to drying results in distinctly different microstructures, with a clear absence of intact starch granules probably as a result

of pre-gelatinization. The differences in microstructure translated into significant difference in nutritional and functional properties with the thermal treatment of the flours, particularly the wet treatment, resulting in superior functionalities such as reduced TIA and increased fat and water absorption capacity, gelling and emulsifying activity. Opportunities may, therefore, exist to explore the potential of thermal treatment to enhance the functionality of pulse flours for different food application while improving their nutritional quality.

Connecting Statement to Chapter 4

In Chapter 3, a significant reduction ($P<0.05$) in trypsin inhibitor activity and significantly higher fat binding capacity, water holding capacity and gelling capacity were induced by thermal treatments. In addition to the presence of anti-nutritional compounds in pulses, another factor that limits the use of pulses is their undesirable beany flavour. The volatile flavour profile of these value-added ingredients is of great concern and is often considered an important quality trait in determining the acceptability of food products. The work described in this chapter centres on a comparison of the volatile compounds in navy bean, red kidney bean, green lentil and yellow pea; and an evaluation of the changes in total volatile counts, and the relative peak area (i.e., of chemicals in the same family) induced by thermal processing (roasting flour, roasting seed, pre-cooking seeds, pre-cooking slurry, pre-cooking-freeze-drying, and pre-cooking-spray-drying) using a headspace-solid phase microextraction-gas chromatography-mass-spectrometry method. This chapter addresses the second objective discussed in the "Objectives of study" section of Chapter 1. The results of this study will be presented as follows:

Ma, Z., Boye, J. I., Azarnia, S., Simpson, B. K., Prasher, S. O., Changes in the volatile flavor profile of lentil, pea, navy bean and red kidney bean as affected by different thermal processing treatments. *Journal of Food Science,* (to be submitted).

Chapter 4. Changes in the Volatile Profile of Lentil, Pea, Navy Bean and Red Kidney Bean as Affected by Different Thermal Processing Treatments

Abstract

The objective of this study was to identify and quantify the volatile composition of navy beans, red kidney beans, green lentils and yellow peas, and determine the flavor changes induced by thermal processing. The volatile profile of the following samples were studied: roasted flours, ground roasted seeds, pre-cooked seeds, pre-cooked slurries, pre-cooked–freeze-dried, and pre-cooked–spray-dried flours. A headspace solid-phase microextraction gas chromatography–mass spectrometry (GC/MS) method was used for the analysis of volatile compounds in the pulse samples. The total area counts (TAC) of the volatile compounds in each type of pulse subjected to different types of thermal processing were compared. In general, the TACs of volatile compounds in pre-cooked samples were reduced significantly ($P<0.05$) compared with the raw samples, likely due to the loss of volatile and/or hydrophilic compounds induced by cooking and drying. GC/MS data furthermore revealed several major differences in relative peak area (RPA) for the same chemical family as a function of different types of thermal processing. The chemical compounds identified in the raw samples underwent either an increase or a decrease in level as a result of the type of thermal treatment. A variety of alkylpyrazine compounds were also produced. The results indicate that volatile compounds of pulses are significantly affected by the type and processing conditions. Product developers may be able to use this information to address flavor-delivery challenges in novel food applications.

4.1 Introduction

Pulses are a good and inexpensive source of proteins, complex carbohydrates, fiber and minerals and are gaining increasing recognition around the world as healthy foods that can provide an adequate supply of energy and protein to meet various dietary needs (Boye & Maltais 2011). The growing trend towards convenience and ready-to-eat foods has stimulated interest in processing pulse seeds to obtain flours and different fractions. Opportunities exist for using

pulses and their fractions as supplements in the development of various food products, such as weaning food, meat products, bakery products, soups, purees, extruded snacks, pasta, yogurt and salad dressing. However, the presence of antinutritional compounds (such as trypsin inhibitors) and some undesirable beany flavors are challenges that need to be addressed in food product development. Significant reductions in antinutritional activities, as affected by various thermal treatments, have been reported in a number of studies (Vidal-Valverde et al. 1994; Wang et al. 2003; Iwuoha & Umunnakwe 1997; Del Rosario et al. 1984; Jourdan et al. 2007; Ma et al. 2011). Thus, the potential of thermally treated pulse ingredients with enhanced nutritional properties for use in different food products could be increasingly realized. The flavor profile of these value-added ingredients, however, remains an issue of interest as flavor is considered an important quality trait in determining the acceptability of food products.

The fresh flavors of legumes are associated with naturally occurring compounds, normal metabolism of the plants and the flavors produced by enzymatic degradation during harvesting, storage and processing. For example, volatile C_6 and C_9 aldehydes and alcohols are the principal chemicals produced upon physical disruption of the tissues of edible plants; this occurs as a result of aerobic oxidation of linoleic and linolenic acids in the presence of lipoxygenase and alcohol oxidoreductase (Galliard et al. 1976; Lumen et al. 1978) or by autoxidative decomposition of these fatty acids. The flavor associated with cooked legumes (e.g., green, beany and earthy odor) is due to enzymatic, non-enzymatic and chemical reactions induced during thermal processing depending on the temperature applied (Sessa 1979). Chemical compounds identified so far in pulses, include aldehydes, alcohols, ketones, and heterocyclic compounds (Self et al. 1963; Azarnia et al. 2011b; Barra et al. 2007; Oomah et al. 2007; Jakobsen et al. 1998). These play an important role in determining the flavor profiles of pulses and may be significantly altered during cooking. The appearance of novel chemical compounds and the changes in their concentrations during thermal processing could also have a significant impact on the taste and flavor of the finished food product.

Headspace solid-phase microextraction (HS-SPME) has been used in combination with gas chromatography (GC) and mass spectrometry (MS) for flavor analysis of different food matrices (e.g., vegetables, fruits, juices, soft drinks and alcoholic beverages) (Kataoka et al. 2000). HS-SPME combines sampling, extraction, concentration and sample introduction in a single solvent-free step through adsorption/absorption of volatiles onto an adsorbent fiber coated with an appropriate stationary phase (Vas & Vékey 2004). Absorption/adsorption is based on equilibrium partitioning of the analytes between the solid phase of the SPME fiber and the liquid or solid sample matrix (Pawliszyn 1995). The approach has proven efficient and effective as a method for the sensitive detection of volatile, semi-volatile, polar and non-polar compounds. In the coupled system, the SPME fiber with the concentrated analytes is transferred to and desorbed into a hot GC injector port and eluted by the mobile phase for subsequent chromatographic analysis and MS detection (Vas & Vékey 2004; Azarnia et al. 2011b; Kataoka 2005).

Very few published studies have focused on the volatile components of different pulse varieties, and changes in volatile profiles associated with different types of thermal processing have not been investigated in a systematic manner. The aim of the present study, therefore, was to identify and compare the volatile profiles of selected pulses grown in Canada (i.e., green lentils, yellow peas, red kidney beans, and navy beans) and to study the effects of thermal processing, such as cooking and drying (i.e., roasted flour, roasted seeds, pre-cooked seeds, pre-cooked slurry, pre-cooked–freeze-dried, and pre-cooked–spray-dried samples), on changes in the volatile compounds of these pulses.

4.2 Materials and Methods

4.2.1 Materials

The following pure commercial standards were purchased from Sigma-Aldrich (Oakville, ON, Canada): alcohols (1-butanol, 1-pentanol, 1-pentanol, 3-hexanol, 1-penten-3-ol, 2-methoxy-4-vinylphenol, 1-nonanol, 2-ethyl-2-hexanol, 2-methoxy-ethanol, 2-ethyl-1-butanol), aldehydes (benzaldehyde, hexanal, octanal, 3-methyl-butanal, 2-methyl-butanal), ketones (acetone, 2-pentanone, 2-heptanone,

2-butanone), aromatic compounds (toluene, benzothiazole, 2-methyl furan, *p*-xylene, *o*-xylene), alkanes (n-dodecane, nonadecane, n-undecane, n-tetradecane, trichloromethane), terpene (d-limonene, 3-carene, camphene), ester (ethyl acetate), sulphur compounds (dimethylsulfide), and nitrogen compounds (2-methyl-pyrazine, ethyl pyrazine, 2-ethyl-3-methyl pyrazine, 2, 6-demethyl pyrazine, 2, 5-dimethyl pyrazine, 2, 3-diethyl-5(6)-methyl pyrazine).

Green lentil seeds (Laird variety) were provided by Pulse Growers in Saskatchewan (Saskatoon, SK, Canada); yellow pea (Eclipse) was provided by the Crop Development Centre (Saskatoon, SK, Canada); red kidney bean was provided by Ferguson Bros. of St. Thomas Ltd. (ON, Canada); and navy bean was obtained from Hensall District Co-operative Inc. (Hensall, ON, Canada). Dry seeds were stored at 4 °C until analysis.

4.2.2 Preparation of Standards

Standard solutions were prepared as described by Azarnia et al. (2011b). Briefly, individual stock solutions of each commercial standard listed in section 2.1 were prepared in 15-mL screw-top amber vials (Supelco, Oakville, ON, Canada) as follows: 10 mL of each commercial standard was individually diluted in 10 mL of methanol (solution 1). Subsequently 1 mL of solution 1 was diluted in 9 mL of methanol (solution 2). Ten microliters of solution 2 was then diluted in 1 mL of 6M saturated NaCl solution. The prepared standard was then analyzed by HS-SPME-GC/MS. Pure 2-pentanone standard was used as a daily reference standard; it was injected at the beginning and after each sixth injection of sample. The reproducibility of the method was evaluated by calculating the relative standard deviation (RSD) of the area count of the standard during each injection. RSD values between 2.4% and 4.0% were ensured.

4.2.3 Sample Preparation

The four different varieties of pulse seeds were processed using seven different thermal treatments as described below. (1) Raw flours were prepared by grinding dry seeds in a domestic coffee grinder in a Black & Decker SmartGrind Model CBG100S coffee bean grinder (The Black & Decker Corporation, Towson, MD, USA) for 40 s and then passing them through a 106-µm sieve; the sieved

material was collected for further analysis and is referred to as "R". (2) The sieved material (R) was then spread thinly in a sealed aluminum dish and roasted for 1 min in an electric double oven (model OD302, Fisher & Paykel Appliances Ltd., Huntington Beach, CA, USA) at 100 ℃; the roasted flour sample is referred to as "RF". (3) Roasted seeds were prepared by roasting the whole seeds in a sealed aluminum dish for 20 min in the same oven as above at 100 ℃, and then ground and sieved using the same method as for raw flours; the roasted seed is referred to as "RS". (4) Cooked whole seeds were prepared by soaking dry seeds of pea, navy and red kidney beans in cold deionized water overnight (16 h) at 4 ℃ using a ratio of 1:3 (seeds:water). Soak water was drained before cooking. The lentil seed was cooked directly without prior soaking. Seeds were cooked in boiling double distilled (DD) water using a ratio of 1:3 (seeds:water) for 20 min, 20 min, 30 min, and 40 min for yellow pea, lentil, navy bean and red kidney bean, respectively. The pre-cooked seeds were weighed and then mashed 2 to 3 times inside the SPME vial using a spatula; they are referred to as "PCSE". (5) The slurry of pre-cooked seeds was prepared by continuously blending PCSE with DD water using a ratio of 1:2 (water:seeds) in a commercial blender (Warring, New Hartford, CT, USA) for 2 min to obtain a homogeneous dispersion. The pre-cooked slurry is referred to as "PCSL." (6) The freeze-dried sample was prepared by evenly spreading PCSL in a pan, freezing overnight in a freezer at -40 ℃; then freeze-dried in a VirTis model 50-SRC-5 freeze dryer (VirTis Co., Inc., Gardiner, NY, USA). The pre-cooked–freeze-dried sample is referred to as "PCFD". (7) The spray-dried sample was prepared by using a BUCHI Mini Spray Dryer (Model 191, Buchi Laboratoriums-Technik, Flawil, Switzerland): the PCSL was passed through a sieve prior to spray drying. The pre-cooked–spray-dried sample is referred to as "PCSD." The seeds of each type of pulse were prepared according to the above-mentioned procedures. Two grams of each prepared sample was individually placed into 10-mL headspace amber vials (Supelco, Oakville, ON, Canada) and stored at 4 ℃ prior to analysis by HS-SPME-GC/MS. All the samples were prepared on the day the HS-SPME-GC/MS analysis was performed.

4.2.4 HS-SPME- GC/MS Analysis

Samples were extracted at 50 ° C for 30 min using a carboxen-polydimethylsiloxane SPME fiber (CAR/PDMS, 85 μm; Supelco, Oakville, ON, Canada) according to the optimized conditions reported by Azarnia et al. (2011b). The SPME extraction parameters were selected as this combination has shown the highest sensitivity in recovering the volatile compounds. The fiber was placed into the split/splitless injector (glass insert SPME, 0.8 ID; Varian, Mississauga, ON, Canada) and the volatile compounds were desorbed at 300 ℃ for 3 min. Analytes were eluted using pure helium carrier gas at a constant flow rate of 1 mL min^{-1} using a VF-5MS capillary column, 30 m × 0.25 mm × 0.25 μm (Varian Inc., Mississauga, ON, Canada). Volatile compounds were determined with a Varian CP-3800 gas chromatograph (Palo Alto, CA). The initial temperature of the GC oven was 35 ℃; it was held for 3 min and then increased to 80 ℃ at a rate of 6 ℃ min^{-1}, and finally to 280 ℃ at a rate of 20 ℃ min^{-1}, and held for 2 min. The total time of analysis was 22.5 min. A Saturn 2000 mass spectrometry detector (Varian Inc., Palo Alto, CA, USA) was used for detection of compounds, and the mass range was 40–400 *m/z*. The total ion current was obtained using an electron ionization source at 70 eV at a scan rate of 1.0 s/scan. Each volatile compound was identified either by the National Institute of Standards and Technology (NIST) database (V.05) through mass spectra library search, or by comparing retention times (RT) and the mass spectra of the compounds with those of the pure commercial standards (as listed in section 2.1). The area count of volatiles for each type of pulse under each processing treatment was obtained from the average of triplicate measurements. The relative peak area (RPA) was calculated using the procedure described by Azarnia et al. (2011b) as follows:

RPA (%) = 100 × peak area of each volatile compound/TAC

The total area counts (TAC) for each sample and the sum of RPA values for the same chemical family were calculated and compared for the different type of pulse subjected to the thermal treatments.

4.2.5 Statistical Analysis

Values given in tables and figures are the means of three determinations. Error bars indicate the standard deviation. Statistical significance was evaluated

by one-way analysis of variance (ANOVA) using GraphPad PRISM version 3.02 software (GraphPad Software, Inc., San Diego, CA, USA). Significant differences between means were determined by Tukey's multiple comparison tests at the 5% significance level.

4.3 Results and Discussion

4.3.1 Effect of Processing on Total Volatile Compounds

The volatile compounds identified in navy bean, red kidney bean, green lentil, and yellow pea under different thermal treatments are presented in Table 4.1 and 4.2, grouped by chemical families. Changes in the TAC values of the samples are shown in Fig. 4.1. Based on the ANOVA test, significant differences (P<0.05) were observed between different pulse types and between different processing treatments, and interactions between types of pulses and treatments were observed. A comparison of the R samples (Fig. 4.1), showed that navy bean had the highest TAC (P<0.05) and red kidney bean the lowest TAC among all the pulse types.

TAC values of the volatile compounds from pre-cooked samples (including PCSE, PCSL, PCFD, and PCSD) were generally reduced compared with the R samples (Fig. 4.1). The reduction in TAC was significant (P<0.05) for all the pulse types, except for red kidney bean PCSD and yellow pea PCFD. The observed results are consistent with previously reported findings that showed significantly decreased total volatile compounds counts in cooked peas and pea slurries (Azarnia et al. 2011b), as well as in cooked French beans (Barra et al. 2007). Whitfield & Shipton (1966) also found that the concentrations of volatiles decreased markedly in blanched peas relative to raw pea samples but there was no change in qualitative composition. Together, these results indicate that during cooking and drying, volatile components are lost or reduced. Some workers have also found that the bonds between protein in pulses and the oxygenated products derived from lipid hydroperoxide decomposition are stronger following protein denaturation induced by wet heating, as more active sites in proteins (such as the α-amino group of lysine and the thiol group of cysteine) are available for binding (Arai et al. 1970; Beyeler & Solms 1974). Flavor compounds may lose their

olfactory effect and become less intense as a result of the formation of these lipoprotein complexes, which may also help to explain the general reduction in TAC for pre-cooked samples. For the roasted seeds, the TACs of volatile compounds were significantly increased (P<0.05) compared with the R samples for navy bean, red kidney bean (Fig. 4.1).

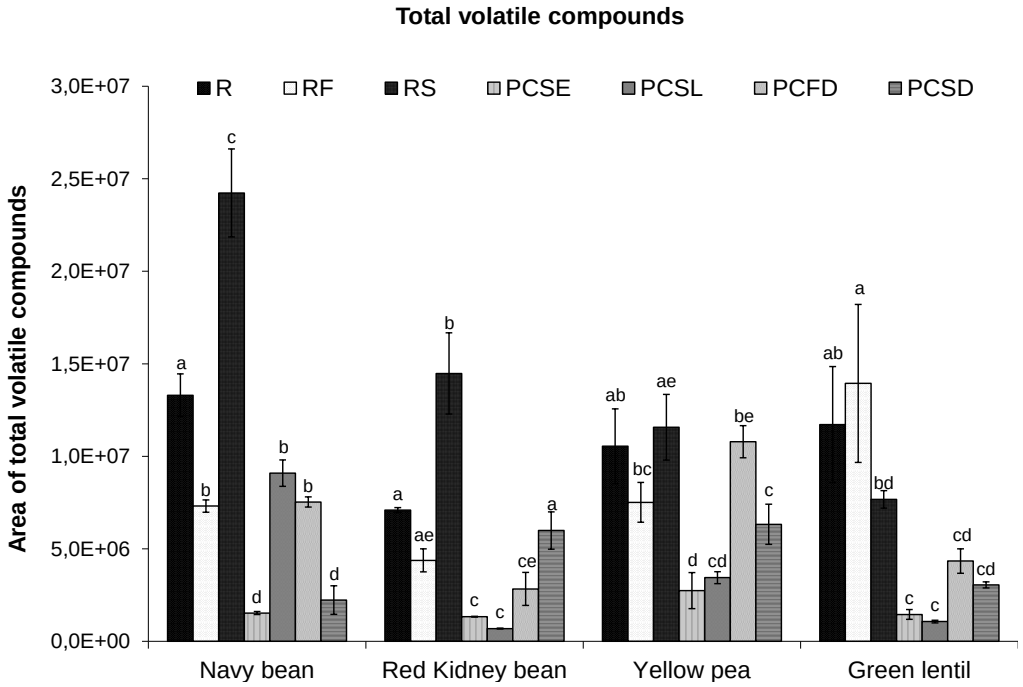


Fig. 4.1 Effect of thermal processing on the total area counts (TAC) of volatile compounds present in navy bean, red kidney bean, yellow pea, and green lentil.
Results are the average value of three replications. For each type of pulse, mean values bearing different letters are significantly different (P<0.05) as per Tukey's multiple comparison test. (R: raw; RF: roasted flour; RS: roasted seed; PCSE: pre-cooked seeds; PCSL: pre-cooked slurry; PCFD: pre-cooked-freeze-dried; PCSD: pre-cooked-spray-dried).

4.3.2 Effect of Processing on Alcohols

The formation of *n*-hexanol, such as 1-hexanol and 3-hexanol, as presented in Table 4.1 and 4.2, is typically achieved by the transformation of *n*-hexanal in the presence of alcohol oxidoreductase (Galliard et al. 1976). The formation of 1-penten-3-ol involves a similar pathway: the oxidation of linolenic acid leads to the formation of 16-hydroperoxide; 1-penten-3-one is then formed by enzymatic isomerization of 16-hydroperoxide; and 1-penten-3-one is finally reduced to 1-penten-3-ol in the presence of alcohol oxidoreductase (Eriksson 1975; Lumen et al. 1978). In our study, 3-hexanol was the most abundant volatile compound, with the highest RPA being found in navy bean, red kidney bean, green lentil and

yellow pea (Tables 4.1, 4.2). This suggests that 3-hexanol, which has a strong green leafy odor, makes an appreciable contribution to the flavor of these pulses; saturated and unsaturated alcohols were quantitatively dominant components and they have been reported to play an important role in pulse flavor (Whitfield & Shipton (1966). The presence of 3-hexanol has been previously reported in green bean, soybean, and winged bean (De Lumen et al. 1978; Kato et al. 1981; Del Rosario et al. 1984).

The total RPA values of alcohols for all the R samples were similar (Fig. 4.2), except that the total RPA of alcohols in the R samples of yellow peas was significantly lower ($P<0.05$) than in the other raw pulse samples. The effect that thermal processing had on the total RPA of alcohols can be seen in Fig. 4.2. A comparison of the raw and pre-cooked pulses (PCSE, PCSL, PCFD, and PCSD) showed a general trend towards a reduction in the concentration of the total RPA of alcohols induced by wet heating. The RPA of alcohols was significantly decreased ($P<0.05$) for all PCSE and PCSD samples, as well as for PCSL of red kidney and green lentil. This observation is supported by the finding reported by Del Rosario et al. (1984) of a decrease in the concentration of nearly all alcohols in both soybean and winged bean headspace samples upon heating. Roasting of the pulses (i.e., RF and RS) caused a significant increase or decrease ($P<0.05$) in the RPA of alcohols relative to the R samples, depending on pulse type. The RF of navy bean and red kidney bean, and the RS of navy bean and green lentil, showed significantly decreased RPA values for alcohols. In contrast, the RS of red kidney bean showed significantly increased RPA values for total alcohol content detected. As reported by Kato et al. (1981), differences between the formation rate and the disappearance rate of the various volatile alcohols may explain the differences observed for different types of pulse during the course of roasting.

Table 4.1 Volatile compounds identified in navy bean and red kidney beans using HS-SPME-GC/MS.

Compounds	Cultivars and various treatments													
	Navy bean							Red kidney bean						
	R	RF	RS	PCSE	PCSL	PCFD	PCSD	R	RF	RS	PCSE	PCSL	PCFD	PCSD
	RPA (%)[a]							RPA (%)[a]						
Alcohols														
1-Butanol	0.12 ±0.03	0.19 ±0.01	0.08 ±0.01	nd	0.13 ±0.03	nd	nd	0.27 ±0.05	0.38 ±0.17	nd	nd	nd	nd	nd
Ethanol, 2-methoxy-	0.12 ±0.02	0.29 ±0.04	nd	1.22 ±0.28	0.13 ±0.02	0.17 ±0.01	0.7 ±0.19	0.31 ±0.04	0.45 ±0.06	0.15 ±0.03	1.17 ±0.05	2.07 ±0.06	0.57 ±0.18	0.37 ±0.09
3-Hexanol	63.71 ±10.95	31.42 ±8.7	2.15 ±1.29	8.6 ±1.41	81.72 ±2.12	73.89 ±2.44	10.96 ±1.89	59.9 ±1.2	41.74 ±9.77	83.07 ±2.2	14.49 ±3.95	9.37 ±1.51	75.45 ±7.71	31.05 ±2.39
3-Pentanol	0.62 ±0.09	0.57 ±0.07	0.28 ±0.03	nd	nd	nd	nd	1.17 ±0.07	nd	0.94 ±0.19	nd	nd	nd	nd
2-Propanol, 1-propoxy-	0.58 ±0.05	0.58 ±0.04	nd	nd	nd	0.78 ±0.06	nd	1.01 ±0.12	1.2 ±0.34	nd	nd	nd	nd	nd
1-Penten-3-ol	0.73 ±0.16	0.78 ±0.03	0.17 ±0.02	nd	nd	0.29 ±0.02	0.97 ±0.16	1.27 ±0.01	1.58 ±0.56	nd	nd	nd	nd	nd
1-Butanol, 2-ethyl	0.32 ±0.05	0.48 ±0.04	nd	nd	nd	nd	nd	0.54 ±0.02	0.62 ±0.35	0.28 ±0.03	nd	nd	nd	nd
2-Nonen-1-ol, (E)-	nd	nd	nd	nd	nd	1.58 ±0.29	4.52 ±1.23	nd	1.74 ±0.49	nd	nd	nd	nd	nd
2-Methoxy-4-vinylphenol	0.2 ±0.05	nd	nd	1.94 ±0.14	nd	1.07 ±0.7	0.73 ±0.22	nd	nd	nd	1.66 ±0.1	nd	nd	nd
2-Pentyn-1-ol	0.16 ±0.04	0.29 ±0.03	nd	nd	nd	nd	nd	0.34 ±0.03	0.52 ±0.09	0.24 ±0.04	nd	nd	nd	nd
1-Octanol, 2-butyl-	2.65 ±1.06	2.77 ±0.63	0.57 ±0.04	1.21 ±0.05	0.17 ±0.02	0.3 ±0.02	5.35 ±0.85	2.98 ±0.85	1.74 ±0.49	nd	2.56 ±0.36	nd	1.36 ±0.63	nd
1-Nonanol	0.88 ±0.13	2.32 ±0.1	nd	nd	nd	nd	nd	0.97 ±0.18	nd	0.58 ±0.05	nd	nd	nd	nd
1-Pentanol	nd	0.79 ±0.17	nd	nd	nd	nd	0.57 ±0.12	nd	nd	nd	nd	nd	nd	nd
1-Pentanol, 4-methyl-2-propyl-	0.46 ±0.24	nd	nd	nd	nd	nd	nd	0.43 ±0.08	nd	nd	nd	nd	nd	nd
2-Hexanol, 2-ethyl-	nd	nd	nd	nd	nd	0.31 ±0.03	nd	nd	nd	nd	nd	nd	nd	nd
2-Hexanol, 2-methyl-	0.32 ±0.07	nd	0.36 ±0.01	nd	nd	0.13 ±0.01	nd	nd	nd	0.6 ±0.18	1.74 ±0.19	nd	nd	nd
Aldehydes														
Benzaldehyde	0.42 ±0.06	1.42 ±0.13	0.25 ±0.01	1.57 ±0.06	0.23 ±0.03	nd	1.77 ±0.33	0.62 ±0.09	0.85 ±0.34	0.27 ±0.03	1.77 ±0.22	nd	nd	nd
Butanal, 3-methyl-	0.41 ±0.04	0.46 ±0.03	1.07 ±0.12	1.67 ±0.37	0.33 ±0.04	nd	1.46 ±0.53	0.98 ±0.06	1.33 ±0.42	0.61 ±0.11	4.86 ±0.88	3.51 ±0.22	nd	1.04 ±0.08
Butanal, 2-methyl-	0.35 ±0.03	0.77 ±0.02	0.53 ±0.1	1.3 ±0.12	0.31 ±0.05	nd	0.87 ±0.06	1.57 ±0.03	2.25 ±0.39	0.58 ±0.1	5.56 ±0.53	4.04 ±0.26	nd	1.08 ±0.12
Hexanal	2.62 ±0.65	6.46 ±0.53	0.56 ±0.02	4.06 ±0.36	1.62 ±0.19	0.48 ±0.05	6.16 ±0.61	3.3 ±0.77	4.4 ±1.33	1.38 ±0.16	1.95 ±0.16	3.84 ±0.62	0.79 ±0.25	27.66 ±3.31
2-Hexenal, 2-methyl-	nd	nd	0.13 ±0.03	0.75 ±0.07	nd	nd	nd	nd	nd	nd	2.15 ±0.67	nd	nd	nd
Heptanal	0.17 ±0.04	0.47 ±0.08	nd	0.73 ±0.04	0.14 ±0.02	0.14 ±0.01	1.15 ±0.64	0.2 ±0.02	0.3 ±0.13	0.12 ±0.02	0.78 ±0.13	1.41 ±0.15	nd	3 ±1.74
Nonanal	nd	nd	nd	1.79 ±0.16	0.39 ±0.04	nd	nd	nd	nd	nd	nd	nd	2.21 ±0.64	10.11 ±1.93
2-Heptenal, (Z)-	nd	0.47 ±0.08	0.12 ±0.01	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Dodecanal	nd	nd	0.82 ±0.03	nd	0.18 ±0.03	nd	nd	nd	nd	nd	nd	nd	nd	3.16 ±1.56
Octanal	nd	nd	nd	1.03 ±0.03	0.17 ±0.02	nd	0.84 ±0.07	nd	nd	nd	nd	nd	nd	0.69 ±0.27
Ketones														
2-Heptanone	nd	nd	nd	0.66 ±0.12	0.12 ±0.01	nd	0.35 ±0.07	nd	nd	nd	0.78 ±0.06	nd	nd	nd
2-Butanone	0.77 ±0.06	0.9 ±0.05	0.37 ±0.06	2.16 ±0.11	0.59 ±0.07	nd	1.14 ±0.23	1.87 ±0.03	2.01 ±0.89	0.9 ±0.15	2.42 ±0.55	2.06 ±0.37	1.21 ±0.3	1.61 ±0.63
Aromatic compounds														
Furan, 2-methyl-	0.55 ±0.05	0.56 ±0.04	nd	1.72 ±0.3	nd	nd	nd	0.84 ±0.05	0.74 ±0.35	nd	1.64 ±0.17	nd	nd	0.74 ±0.23
Furan, 2-ethyl-	nd	nd	nd	4.22 ±0.45	0.57 ±0.14	nd	nd	nd	nd	nd	2.49 ±0.21	3.28 ±0.21	nd	nd
Toluene	0.21 ±0.03	0.36 ±0.03	nd	1.01 ±0.06	0.17 ±0.03	0.17 ±0	0.6 ±0.12	0.48 ±0.01	0.85 ±0.28	nd	1.47 ±0.13	2.69 ±0.21	0.44 ±0.15	nd
Benzothiazole	nd	nd	nd	1.41 ±0.11	nd	0.86 ±0.11	nd	nd	nd	nd	nd	nd	1.51 ±0.45	nd
Phenol, 3-amino-4-methyl-	nd	nd	10.53 ±0.22	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Propyl benzene	nd	nd	nd	nd	nd	nd	nd	0.57 ±0.17	nd	0.42 ±0.13	nd	nd	nd	nd
Styrene	0.6 ±0.15	0.88 ±0.22	1.15 ±0.24	nd	nd	0.18 ±0.01	nd	0.57 ±0.19	0.75 ±0.12	0.26 ±0.04	nd	nd	0.33 ±0.11	0.24 ±0.07
o -Xylene	nd	2.24 ±0.73	nd	nd	nd	nd	nd	nd	nd	nd	0.75 ±0.11	nd	nd	nd
p -Xylene	0.13 ±0.04	0.34 ±0.08	nd	0.84 ±0.28	0.11 ±0.02	0.1 ±0.01	0.31 ±0.09	0.14 ±0.01	nd	nd	0.64 ±0.06	nd	nd	nd

Alkanes														
Decane, 3-methyl-	0.27 ±0.08	0.78 ±0.04	nd	1.63 ±0.17	0.25 ±0.03	0.54 ±0.03	4.39 ±1.55	1.24 ±0.33	4.15 ±0.69	0.24 ±0.02	nd	nd	1.01 ±0.09	nd
Hexane, 2,4-dimethyl-	0.33 ±0.06	0.37 ±0.02	nd	nd	nd	nd	1.11 ±0.22	0.61 ±0.04	0.78 ±0.12	nd	nd	nd	nd	nd
Decane	1.39 ±0.31	nd	nd	0.62 ±0.03	nd	0.14 ±0.01	0.44 ±0.11	1.62 ±0.52	1.07 ±0.45	0.53 ±0.09	1.25 ±0.12	nd	0.3 ±0.14	0.33 ±0.09
Heptane	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.63 ±0.19	nd
Hexane, 3-ethyl-	nd	nd	0.09 ±0.01	1.09 ±0.05	0.16 ±0.01	0.14 ±0.01	nd	nd	nd	1.38 ±0.16	nd	nd	nd	nd
Trichloromethane	0.71 ±0.03	nd	nd	2.05 ±0.14	1.16 ±0.23	0.71 ±0.06	nd	0.99 ±0.03	nd	nd	1.82 ±0.01	8.72 ±0.18	0.91 ±0.43	nd
Hexane, 3-methyl-	0.74 ±0.07	1.00 ±0.12	0.28 ±0.02	nd	0.17 ±0.02	0.36 ±0.02	1.25 ±0.14	1.37 ±0.08	1.71 ±0.73	0.28 ±0.05	nd	nd	nd	1.56 ±0.7
Undecane	1.04 ±0.47	nd	nd	1.35 ±0.12	nd	1.3 ±0.09	nd	nd	nd	nd	nd	nd	2.26 ±0.67	nd
Tetradecane	0.25 ±0.06	0.47 ±0.07	nd	1.49 ±0.09	0.27 ±0.03	1.89 ±0.13	nd	nd	nd	nd	nd	nd	nd	nd
Hexane, 3,3-dimethyl-	nd	2.25 ±0.58	nd	nd	nd	nd	2.00 ±0.63	nd	nd	0.52 ±0.07	nd	nd	nd	nd
Nonadecane	0.22 ±0.04	0.44 ±0.06	nd	nd	0.23 ±0.04	nd	nd	nd	nd	nd	1.86 ±0.2	nd	3.3 ±0.69	nd
Decane, 2,2,3-trimethyl-	nd	1.73 ±0.71	nd	nd	nd	nd	1.31 ±0.55	nd	nd	nd	nd	nd	nd	nd
Decane, 4-methyl-	nd	nd	nd	1.16 ±0.06	nd	0.79 ±0.07	3.22 ±1.16	nd	nd	0.97 ±0.11	nd	nd	1.5 ±0.47	nd
Decane, 2-methyl-	3.33 ±1.08	3.64 ±1.1	0.64 ±0.1	1.9 ±0.28	0.2 ±0.02	nd	2.04 ±0.72	4.13 ±0.12	2.87 ±0.79	0.8 ±0.06	nd	nd	nd	nd
Terpene														
3-Carene	0.17 ±0.04	0.39 ±0.03	nd	nd	nd	0.2 ±0.01	0.46 ±0.13	0.21 ±0.04	0.39 ±0.08	0.11 ±0.03	1.63 ±0.2	nd	0.45 ±0.18	0.32 ±0.08
Camphene	0.23 ±0.05	0.87 ±0.12	0.15 ±0.02	0.62 ±0.02	0.12 ±0.01	0.13 ±0.03	nd	0.25 ±0.07	0.9 ±0.18	0.24 ±0.07	nd	nd	0.39 ±0.14	nd
D-Limonene	nd	13.36 ±3.15	1.14 ±0.53	nd	0.3 ±0.05	0.76 ±0.26	nd	nd	13.88 ±1.08	2.41 ±0.53	nd	3.63 ±0.3	1.51 ±0.59	1.59 ±0.53
Ester														
Ethyl Acetate	1.2 ±0.2	1.16 ±0.03	0.53 ±0.02	nd	nd	0.43 ±0.03	1.7 ±0.51	1.58 ±0.15	1.09 ±0.75	nd	2.08 ±0.03	nd	nd	3.11 ±1.32
Sulphur compounds														
Disulfide, dimethyl	0.35 ±0.06	0.63 ±0.08	0.18 ±0.01	1.48 ±0.07	0.2 ±0.01	0.14 ±0.03	0.7 ±0.22	0.64 ±0.04	1.07 ±0.18	nd	2.92 ±0.15	3.8 ±0.85	0.41 ±0.15	0.51 ±0.18
Diacetyl sulphide	nd	0.79 ±0.11	4.12 ±0.62	1.06 ±0.02	0.79 ±0.05	0.72 ±0.04	nd	nd	1.37 ±0.36	nd	nd	nd	nd	nd
Methanethiol	0.13 ±0.01	0.16 ±0.02	0.64 ±0.16	2.03 ±0.14	0.2 ±0.03	0.16 ±0.01	0.62 ±0.15	0.23 ±0.03	0.32 ±0.09	0.11 ±0.02	1.68 ±0.26	2.26 ±0.17	0.46 ±0.18	0.33 ±0.07
Nitrogen compounds														
Indole	nd	nd	nd	nd	nd	nd	0.99 ±0.53	nd	nd	nd	nd	nd	nd	nd
Pyrazine, 2-ethyl-5-methyl-	nd	nd	3.42 ±0.04	nd	nd	0.64 ±0.26	nd	nd	nd	nd	nd	nd	nd	nd
Pyrazine, 2-methyl-6-propyl-	nd	nd	0.57 ±0.06	1.15 ±0.05	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pyrazine, 2-methyl-5-propyl-	nd	nd	0.55 ±0.12	nd	0.17 ±0.02	0.41 ±0.06	nd	nd	nd	nd	nd	nd	nd	nd
Pyrazine, 2,5-dimethyl-	nd	0.44 ±0.04	nd	nd	nd	0.1 ±0.02	nd	nd	nd	nd	nd	nd	nd	nd
Pyrazine, 2,5-diethyl-	nd	nd	nd	nd	0.18 ±0.02	1.1 ±0.56	nd	nd	nd	nd	nd	nd	nd	nd
Pyrazine, 2,6-diethyl-	nd	nd	0.17 ±0.02	1.24 ±0.07	nd	0.47 ±0.18	nd	nd	nd	nd	nd	nd	nd	nd
Pyrazine, 2,3-diethyl-5-methyl-	nd	nd	1.17 ±0.14	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pyrazine, 3,5-diethyl-2-methyl-	nd	nd	2.47 ±0.25	nd	nd	0.78 ±0.24	nd	nd	nd	nd	nd	nd	nd	nd
Pyrazine, 3-butyl-2,5-dimethyl-	nd	nd	0.92 ±0.21	1.37 ±0.15	0.2 ±0.03	0.53 ±0.07	nd	nd	nd	nd	nd	nd	nd	nd
Pyrazine, 3-ethyl-2,5-dimethyl-	nd	nd	0.84 ±0.32	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Dimethylamine	0.21 ±0.03	0.37 ±0.06	0.12 ±0.01	1.86 ±0.39	0.38 ±0.02	0.33 ±0.05	0.93 ±0.29	0.38 ±0.06	0.65 ±0.09	0.2 ±0.05	3.14 ±0.18	5.63 ±1.33	1.08 ±0.49	0.69 ±0.16
Pyrrole	nd	nd	3.46 ±0.33	nd	nd	nd	nd	nd	nd	0.18 ±0.02	nd	nd	nd	nd
1H-Pyrrole, 1-methyl-	nd	nd	0.53 ±0.04	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1H-Pyrrole, 2-ethyl-	nd	nd	0.15 ±0.02	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1H-Pyrrole, 2,3-dimethyl-	nd	nd	3.24 ±0.33	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1H-Pyrrole, 2,5-dimethyl-	nd	nd	0.08 ±0.01	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1H-Pyrrole, 2,3,5-trimethyl-	nd	nd	9.34 ±0.43	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1-Pentanamine	0.13 ±0.03	0.26 ±0.04	nd	0.66 ±0.07	0.1 ±0.01	nd	0.48 ±0.1	0.15 ±0.01	0.26 ±0.05	nd	nd	1.52 ±0.18	nd	nd
Pyrimidine, 5-methyl-	nd	nd	8.03 ±1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pyrimidine, 4,6-dimethyl-	nd	nd	0.14 ±0.01	nd	nd	nd	nd	nd	nd	0.11 ±0.02	nd	nd	nd	nd

Table 4.2 Volatile compounds identified in green lentil and yellow pea using HS-SPME-GC/MS.

Compounds	Cultivars and various treatments													
	Green lentil							Yellow pea						
	R	RF	RS	PCSE	PCSL	PCFD	PCSD	R	RF	RS	PCSE	PCSL	PCFD	PCSD
	RPA (%)[a]							RPA (%)[a]						
Alcohols														
1-Butanol	nd	nd	0.42 ±0.14	nd	nd	1.26 ±0.12	nd	0.19 ±0.02	nd	nd	nd	nd	nd	nd
Ethanol, 2-methoxy-	0.2 ±0.07	0.13 ±0.06	0.27 ±0.03	0.61 ±0.13	nd	1.44 ±0.29	0.42 ±0.02	0.21 ±0.03	0.26 ±0.04	0.32 ±0.04	0.92 ±0.41	0.7 ±0.26	5.03 ±0.34	0.87 ±0.04
3-Hexanol	67.22 ±9.23	79.86 ±5.86	45.46 ±1.84	50.66 ±3.01	21.16 ±1.04	76.11 ±3.67	32.97 ±4.26	36.06 ±3.42	51.17 ±9.57	40.67 ±4.23	nd	37.84 ±5.24	35.14 ±1.1	6.44 ±1.65
1-Hexanol	0.12 ±0.04	0.1 ±0.03	nd	1.02 ±0.17	1.16 ±0.05	nd	nd	2.12 ±0.46	0.18 ±0.02	nd	nd	nd	nd	0.38 ±0.02
3-Pentanol	0.86 ±0.24	nd	2.31 ±0.16	1.68 ±0.34	nd	nd	0.87 ±0.15	0.58 ±0.11	0.82 ±0.21	nd	nd	nd	nd	0.37 ±0.03
2-Propanol, 1-propoxy-	nd	nd	nd	nd	nd	nd	0.74 ±0.11	0.72 ±0.14	0.81 ±0.09	0.56 ±0.1	3.96 ±1.87	nd	nd	nd
1-Penten-3-ol	0.45 ±0.07	0.34 ±0.08	0.69 ±0.03	0.99 ±0.21	nd	nd	0.69 ±0.07	0.29 ±0.05	0.4 ±0.04	0.26 ±0.07	nd	0.73 ±0.19	nd	0.42 ±0.02
2-Nonen-1-ol, (E)-	nd	nd	nd	nd	nd	nd	9.08 ±2.24	nd	nd	nd	nd	nd	nd	nd
1-Butanol, 2-ethyl	nd	nd	nd	nd	0.95 ±0.13	nd	nd	0.19 ±0.03	nd	nd	nd	nd	nd	nd
1-Octanol, 2-butyl-	0.82 ±0.13	0.69 ±0.25	0.34 ±0.04	2.09 ±0.54	1.58 ±0.28	0.85 ±0.17	0.93 ±0.06	5.2 ±2.2	6.36 ±3.9	nd	15.76 ±5.2	5.26 ±1.53	8.93 ±1.6	0.91 ±0.19
1-Decanol, 2-hexyl-	0.31 ±0.07	0.22 ±0.05	nd	nd	2.23 ±0.34	nd	0.68 ±0.14	0.75 ±0.24	nd	nd	8.32 ±2.25	nd	nd	nd
1-Nonanol	2.36 ±0.71	1.23 ±0.36	nd	nd	nd	nd	nd	1.59 ±0.46	nd	nd	nd	nd	nd	nd
2-Hexanol, 2-methyl-	0.89 ±0.13	0.4 ±0.12	0.98 ±0.19	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Aldehydes														
Benzaldehyde	0.56 ±0.12	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Paraldehyde	nd	nd	nd	nd	nd	0.74 ±0.05	nd	nd	nd	nd	nd	nd	0.25 ±0.02	nd
Butanal, 3-methyl-	0.51 ±0.12	0.36 ±0.1	1.27 ±0.16	nd	3.8 ±0.66	0.73 ±0.1	0.69 ±0.1	0.27 ±0.04	nd	0.38 ±0.06	0.7 ±0.11	0.77 ±0.2	nd	0.54 ±0.07
Butanal, 2-methyl-	0.52 ±0.1	0.41 ±0.1	2.88 ±0.34	2.33 ±0.67	2.75 ±0.51	nd	0.97 ±0.13	0.27 ±0.05	0.38 ±0.07	0.95 ±0.17	0.7 ±0.23	0.74 ±0.09	nd	0.45 ±0.04
Hexanal	5.39 ±0.58	3.44 ±1.73	4.88 ±0.05	1.83 ±0.17	1.88 ±0.24	0.68 ±0.12	27.18 ±3.81	1.12 ±0.4	0.5 ±0.07	1.15 ±0.21	1.88 ±0.48	3.94 ±0.41	0.16 ±0.02	2.87 ±0.2
Nonanal	nd	nd	nd	nd	nd	1.76 ±0.45	2.72 ±0.45	nd	nd	nd	nd	nd	nd	nd
Heptanal	0.38 ±0.08	0.22 ±0.09	0.31 ±0.02	nd	nd	0.21 ±0.04	0.81 ±0.08	0.15 ±0.03	0.21 ±0.02	0.19 ±0.03	0.39 ±0.14	nd	nd	0.77 ±0.09
Dodecanal	nd	nd	nd	nd	nd	nd	0.99 ±0.1	nd	nd	nd	nd	nd	nd	24.07 ±2.84
2-Heptenal, (Z)-	nd	nd	nd	nd	nd	nd	nd	nd	1.14 ±0.5	nd	nd	nd	nd	nd
Octanal	nd	nd	nd	nd	nd	nd	0.52 ±0.04	nd	nd	nd	nd	nd	nd	0.49 ±0.07
Ketones														
2-Pentanone	nd	nd	0.8 ±0.11	nd	nd	nd	nd	0.29 ±0.05	0.39 ±0.04	nd	nd	nd	nd	nd
2-Heptanone	nd	nd	0.8 ±0.11	nd	nd	nd	nd	nd	nd	nd	nd	0.49 ±0.27	nd	nd
2-Butanone	1.04 ±0.33	0.71 ±0.16	nd	3.18 ±0.29	2.28 ±0.07	nd	0.93 ±0.07	1.03 ±0.21	0.94 ±0.08	0.97 ±0.17	1.79 ±0.47	0.91 ±0.22	nd	0.44 ±0.05
Aromatic compounds														
Furan, 2-methyl-	0.89 ±0.24	0.82 ±0.13	0.79 ±0.06	1.87 ±0.45	nd	1.16 ±0.21	nd	0.61 ±0.12	0.41 ±0.04	0.6 ±0.1	0.97 ±0.24	nd	nd	0.25 ±0.03
Furan, 2-ethyl-	nd	nd	nd	2.54 ±0.82	nd	0.7 ±0.07	nd	nd	nd	0.28 ±0.03	2.03 ±0.76	1.04 ±0.07	0.38 ±0.02	nd
Toluene	1.29 ±0.46	1.07 ±0.15	0.96 ±0.02	8.08 ±1.21	8.26 ±0.32	1.95 ±0.28	0.54 ±0.08	0.43 ±0.08	0.4 ±0.12	0.18 ±0.03	0.78 ±0.35	nd	0.14 ±0.01	nd
Benzothiazole	nd	nd	nd	nd	nd	0.85 ±0.09	nd	nd	nd	nd	nd	nd	0.8 ±0.24	nd
Propyl benzene	0.49 ±0.21	0.56 ±0.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Styrene	0.42 ±0.08	0.23 ±0.06	nd	0.87 ±0.2	1.03 ±0.08	0.23 ±0.04	nd	0.57 ±0.12	0.92 ±0.25	0.73 ±0.05	nd	nd	nd	nd
o-Xylene	nd	nd	nd	0.7 ±0.21	0.67 ±0.05	0.22 ±0.02	nd	0.13 ±0.02	0.16 ±0.04	0.13 ±0.02	nd	nd	nd	nd
p-Xylene	nd	nd	0.22 ±0.02	0.55 ±0.14	0.82 ±0.06	nd	nd	0.12 ±0.02	0.16 ±0.04	nd	nd	nd	nd	nd

Alkanes														
Decane, 3-methyl-	nd	nd	nd	nd	1.91±0.18	nd	nd	nd	0.8±0.24	nd	nd	nd	12.1±1.29	0.74±0.29
Copaene	0.39±0.04	0.44±0.2	0.71±0.09	1.95±0.28	2.52±0.26	0.58±0.11	nd	nd	nd	nd	nd	nd	nd	nd
Hexane, 2,4-dimethyl-	1.35±0.44	0.56±0.2	0.97±0.03	1.61±0.55	nd	nd	nd	1.06±0.18	nd	6.3±0.01	0.87±0.35	0.74±0.03	0.22±0.01	nd
Hexane, 2,3-dimethyl-	nd	nd	0.24±0.02	nd	nd	nd	nd	nd	nd	5.25±0.85	nd	nd	nd	nd
Hexane, 2,2,3-trimethyl-	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.1±0.08	0.87±0.05	nd
Decane	0.23±0.04	nd	nd	nd	0.69±0.07	0.18±0.04	nd	10.36±4.36	nd	0.2±0.02	1.16±0.66	0.86±0.11	0.4±0.06	0.81±0.26
Hexane, 3-ethyl-	nd	nd	nd	nd	nd	nd	nd	nd	0.38±0.09	nd	nd	nd	nd	nd
Trichloromethane	0.49±0.12	nd	nd	nd	10.87±0.18	3.00±0.36	nd	0.42±0.06	nd	nd	1.86±0.12	4.89±0.97	1.52±0.14	nd
Hexane, 3-methyl-	1.11±0.71	0.62±0.2	1.71±0.11	nd	nd	nd	0.74±0.08	0.93±0.2	nd	0.48±0.06	nd	nd	nd	0.47±0.03
Undecane	nd	nd	nd	nd	nd	nd	nd	10.36±4.36	4.31±1.89	nd	nd	3.77±0.69	1.66±0.18	nd
Tetradecane	0.7±0.11	0.57±0.22	0.73±0.08	1.99±0.37	4.5±1	0.76±0.2	0.37±0.05	0.55±0.17	0.64±0.29	nd	nd	nd	nd	nd
Hexane, 3,3-dimethyl-	nd	nd	nd	nd	nd	nd	nd	nd	0.35±0.11	4.72±1.17	5.81±0.07	11.99±1.98	12.6±1.89	nd
Nonadecane	0.64±0.19	nd	nd	1.6±0.21	nd	0.46±0.14	nd	nd	nd	nd	0.87±0.17	0.71±0.05	1.34±0.03	nd
Decane, 4-methyl-	nd	nd	nd	nd	nd	nd	0.75±0.03	20.23±8.29	10.08±3.87	2.16±0.37	17.38±3.93	6.68±0.36	0.63±0	1.28±0.49
Decane, 2-methyl-	nd	nd	nd	nd	nd	nd	nd	4.92±1.47	nd	7.92±1.08	13.08±4.82	2.93±1.3	6.94±1.2	nd
Decane, 2,2,3-trimethyl-	nd	nd	nd	nd	nd	nd	nd	4.91±1.03	8.23±2.9	nd	4.14±0.2	2.22±0.14	3.24±0.66	0.3±0.03
Terpene														
3-Carene	0.56±0.07	0.28±0.08	0.67±0.14	2.98±0.69	4.06±0.56	0.47±0.07	0.38±0.11	0.17±0.03	0.32±0.07	nd	nd	nd	nd	nd
Camphene	0.27±0.08	0.23±0.07	0.61±0.03	nd	nd	0.77±0.3	nd	nd	0.46±0.09	0.16±0.02	nd	nd	nd	nd
D-limonene	3.1±1.22	4.03±0.79	5.31±1.21	1.84±0.37	2.99±0.42	nd	1.12±0.19	nd	nd	nd	nd	nd	nd	0.17±0.02
Ester and acid														
Ethyl acetate	2.2±0.65	nd	nd	nd	nd	nd	2.24±0.35	1.75±0.27	nd	0.68±0.08	2.09±0.88	nd	0.23±0.02	0.66±0.32
Sulphur compounds														
Disulfide, dimethyl	0.19±0.06	nd	0.39±0.03	nd	nd	nd	nd	0.19±0.04	1.39±0.08	0.19±0.03	nd	nd	nd	nd
Methanethiol	0.11±0.04	nd	0.43±0.05	nd	nd	nd	0.31±0.04	0.11±0.02	nd	0.2±0.03	0.76±0.2	0.52±0.09	0.09±0.01	nd
Nitrogen compounds														
Pyrazine, methyl-	nd	nd	1.44±0.15	nd	nd	nd	nd	nd	nd	6.78±0.01	nd	nd	nd	nd
Pyrazine, ethyl-	nd	nd	1.19±0.05	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pyrazine, 2-ethyl-5-methyl-	nd	nd	2.61±0.11	nd	nd	0.31±0.11	nd	nd	nd	0.51±0.05	nd	nd	nd	nd
Pyrazine, 2-ethyl-6-methyl-	nd	nd	0.92±0.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pyrazine, 3-ethyl-2,5-dimethyl-	nd	nd	2.59±0.52	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pyridine	nd	nd	nd	nd	nd	nd	nd	nd	0.22±0.03	0.15±0.04	nd	nd	nd	nd
Guanidine	nd	nd	nd	nd	nd	0.96±0.09	nd	nd	nd	0.69±0.11	6.26±2.65	nd	nd	nd
Dimethylamine	0.19±0.07	0.15±0.06	0.28±0.03	1.6±0.34	4.63±0.14	0.49±0.11	0.69±0.09	0.33±0.01	0.37±0.05	0.27±0.04	1.04±0.35	0.83±0.15	0.19±0.01	0.51±0.06
Pyrrole	0.32±0.1	0.22±0.09	5.79±0.82	2.93±0.17	2.41±0.34	0.93±0.15	nd	nd	nd	0.16±0.03	nd	nd	nd	nd
1-Pentanamine	0.19±0.05	0.14±0.05	nd	0.79±0.19	nd	0.37±0.04	0.44±0.06	nd	nd	nd	nd	nd	nd	nd
Pyrazine, 3,5-diethyl-2-methyl-	nd	nd	0.54±0.09	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pyrazine, 2,5-dimethyl-	nd	nd	3.34±0.38	nd	nd	nd	nd	nd	nd	0.68±0.08	nd	nd	nd	nd
Pyrimidine, 4,6-dimethyl-	nd	nd	1.41±0.1	nd	nd	nd	nd	nd	nd	0.18±0.02	nd	nd	nd	nd

The results shown are the average of triplicate measurements and are expressed as mean±standard deviation, nd: not detected.

[a]RPA (%)=100×peak area of each volatile compound/total area counts (TAC)

*R: raw; RF: roasted flour; RS: roasted seeds; PCSE: pre-cooked seeds; PCFD: pre-cooked-freeze-dried; PCSD: pre-cooked-spray-dried .

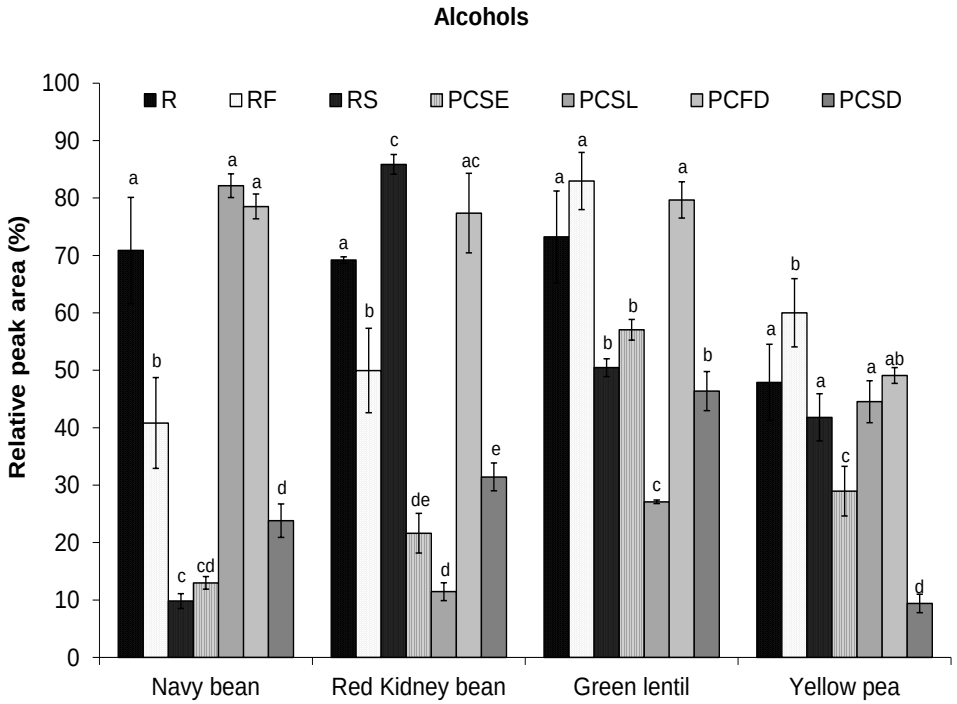


Fig. 4.2 Effect of thermal processing on the total relative peak area (RPA) of volatile alcohols present in navy bean, red kidney bean, yellow pea, and green lentil.
Results are the average value of three replications. For each type of pulse, mean values bearing different letters are significantly different (P<0.05) as per Tukey's multiple comparison test. (R: raw; RF: roasted flour; RS: roasted seed; PCSE: pre-cooked seeds; PCSL: pre-cooked slurry; PCFD: pre-cooked-freeze-dried; PCSD: pre-cooked-spray-dried).

4.3.3 Effect of Processing on Aldehydes

As shown in Tables 4.1 and 4.2, hexanal was the principal aldehyde found in navy bean, red kidney bean, green lentil, and yellow pea. The presence of hexanal has previously been reported in beans, soybeans, and peas (Oomah et al. 2007; Azarnia et al. 2011a; Del Rosario et al. 1984; Van Ruth et al. 1995). In the raw seeds, linoleic acid is oxidized to hydroperoxides in the presence of oxygen; *n*-hexanal may be formed by the cleavage of 13-hydroperoxylinoleic acid by lyases (Galliard et al. 1976). This process often occurs in pulse seeds that are disrupted during processing, and the hexanals formed contribute to the greeny and grassy flavor of legumes. Thermal processing either increased or decreased the RPA of hexanal (Tables 4.1 and 4.2).

Other aliphatic saturated aldehydes found in raw pulses (Tables 4.1 and 4.2) include 3-methyl butanal (a choking, powerful, arid, pungent, apple-like odor), 2-

methyl butanal (a powerful, choking odor with a peculiar cocoa and coffee-like flavor), heptanal (a very strong, fatty, harsh, pungent odor), nonanal (a strong, fatty odor developing orange and rose notes), octanal (a fatty, citrus, honey odor) and dodecanal (a characteristic fatty odor reminiscent of violets on dilution). The amino acid phenylalanine is likely the precursor of most of the aromatic aldehydes of the pulses, including benzaldehyde (Murray et al. 1976), as shown in Table 4.1 and 4.2, which has a characteristic odor and aromatic taste similar to bitter almond (Burdock 2002). The presence of 2-methyl butanal, 3-methyl butanal, heptanal, and benzaldehyde has been reported in a variety of bean cultivars (Barra et al. 2007; Van Ruth et al. 1995; Del Rosario et al. 1984).

The RPA of total aldehydes in the different type of pulse is presented in Fig. 4.3. The R samples of yellow pea had significantly lower RPA values ($P<0.05$), whereas higher values were found for red kidney bean and green lentils. The differences observed for R samples may be due to differences in the levels of fatty acids and linoleate (Del Rosario et al. 1984; Oomah et al. 2007; Choudhury and Rahman 1973). In addition, the pre-cooking process (i.e., PCSE, PESL, PCFD, and PCSD) generally led to either statistically equal or significantly increased ($P<0.05$) RPA values for total aldehydes in relation to the raw samples. The PCSD samples all exhibited significantly increased ($P<0.05$) RPA values for aldehydes compared to R samples. The roasted samples (including RF and RS) generally had statistically similar amounts of total aldehydes, except that a significantly decreased value was observed for the RS of red kidney bean compared with the R samples.

In general, the heat stabilities of lipoxygenase and alcohol oxidoreductase determine the levels of RPA of total aldehydes and alcohols. Lumen et al. (1978) reported that the lipoxygenase activity of green bean seeds was quite stable, persisting for up to 40 min during heating at 80 ℃. In contrast, alcohol oxidoreductase was very unstable; it remained stable for up to 8 min and then was completely inactivated. Thus, the changes observed in the RPA values after thermal processing may be due to differences in the heat stabilities of the two enzymes, which may have led to different impacts on the concentration of

alcohols and aldehydes upon heating. Additionally, the formation of these compounds due to non-enzymatic reaction may also be responsible for the changes observed.

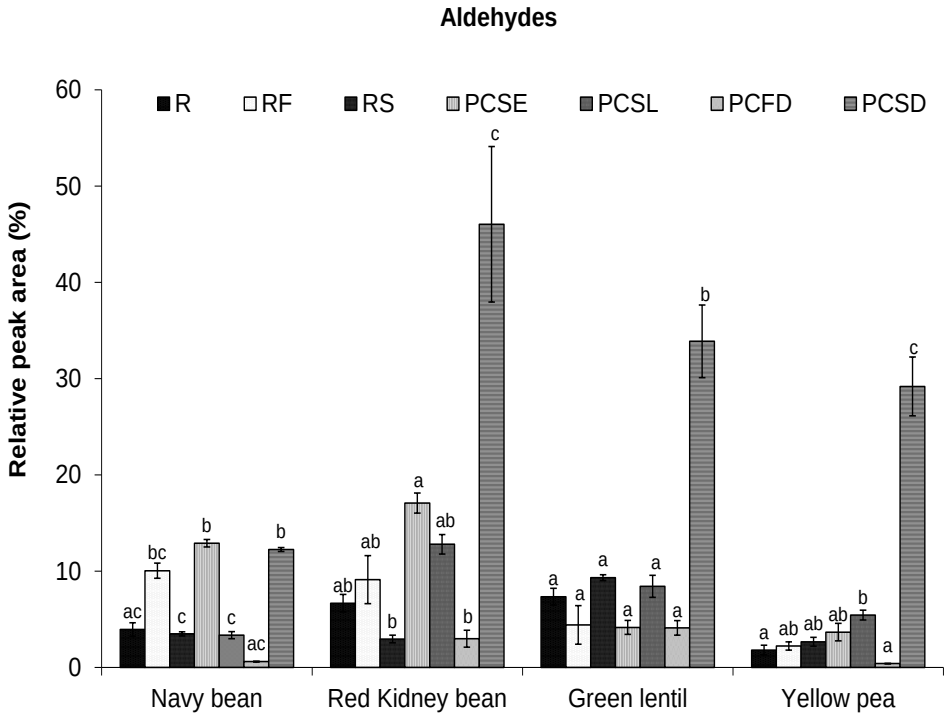


Fig. 4.3 Effect of thermal processing on the total relative peak area (RPA) of volatile aldehydes present in navy bean, red kidney bean, yellow pea, and green lentil.
Results are the average value of three replications. For each type of pulse, mean values bearing different letters are significantly different (P<0.05) as per Tukey's multiple comparison test. (R: raw; RF: roasted flour; RS: roasted seed; PCSE: pre-cooked seeds; PCSL: pre-cooked slurry; PCFD: pre-cooked-freeze-dried; PCSD: pre-cooked-spray-dried).

4.3.4 Effect of Processing on Ketones

Ketones are carbonyl compounds that are formed by lipoxygenase activity from the breakdown of unsaturated fatty acid hydroperoxides. As shown in Tables 4.1 and 4.2, 2-butanone, which has a sweet apricot-like odor, was found in the R samples of navy bean, red kidney bean, lentils, and peas. 2-Pentanone, which has a mild green, fuel-oil odor, was found in the R samples of yellow pea and RS green lentil. Heating produced small amounts of other ketones in the pulses, including 2-heptanone, which has a fruity, spicy, cinnamon, banana, slightly spicy odor (Burdock 2002). These compounds have distinctive characteristics that can affect the flavor of pulses and their flavor potency varies with their carbon chain length. The presence of 2-pentanone and 2-butanone has been identified in peas,

dry beans, and lentils (Azarnia et al. 2011b; Oomah et al. 2007; Lovegren et al. 1979).

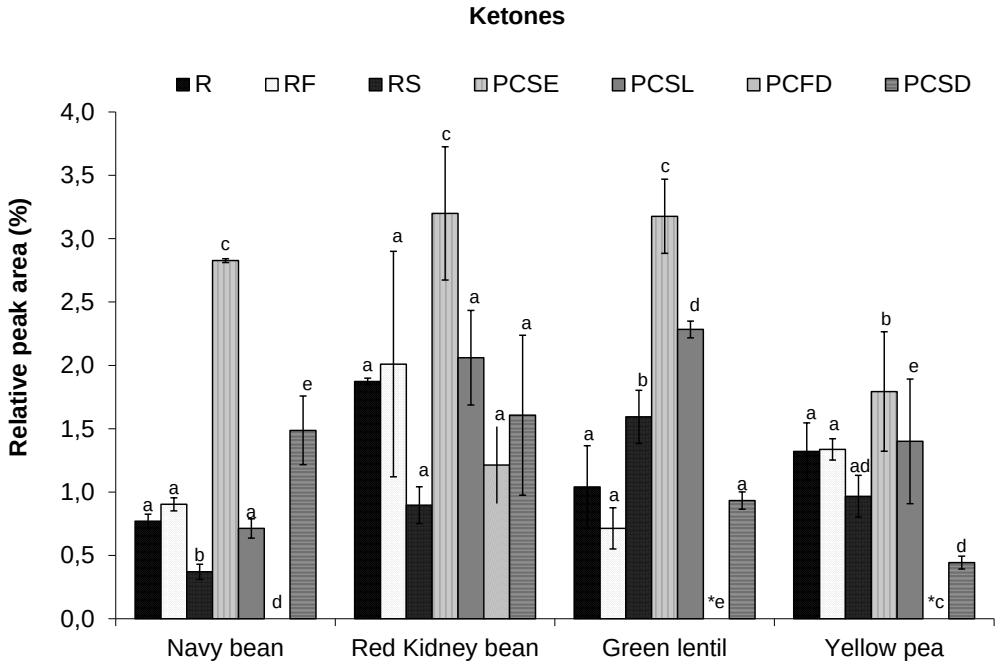


Fig. 4.4 Effect of thermal processing on the total relative peak area (RPA) of volatile ketones present in navy bean, red kidney bean, yellow pea, and green lentil.
Results are the average value of three replications. * Not detected. For each type of pulse, mean values bearing different letters are significantly different (P<0.05) as per Tukey's multiple comparison test. (R: raw; RF: roasted flour; RS: roasted seed; PCSE: pre-cooked seeds; PCSL: pre-cooked slurry; PCFD: pre-cooked-freeze-dried; PCSD: pre-cooked-spray-dried).

The PCSE of navy bean, red kidney bean, lentils, and yellow peas all had significantly increased (P<0.05) total RPA values for ketones compared with the R samples (Fig. 4.4). The PCSL had either statistically equal (for navy bean and red kidney beans) or significantly increased (for green lentil and yellow peas) total RPA values for ketones. Ketones were not detected in the PCFD samples of navy bean, green lentil or yellow pea. The PCSD samples exhibited significantly increased (P<0.05) total RPA values for ketones in navy beans, whereas significantly decreased values were observed for yellow peas relative to the R samples. The RS of navy beans showed significantly decreased RPA values for ketones, whereas the RS of green lentils exhibited significantly increased values (Fig. 4.4).

4.3.5 Effect of Processing on Aromatic Compounds

2-Methyl-furan was detected in the R samples of navy bean, red kidney bean, green lentils, and yellow peas (Table 4.1 and 4.2). Other substituted furans, e.g., 2-ethyl-furan, which has a powerful, sweet, burnt odor (Burdock 2002), appeared after heating (Tables 4.1 and 4.2). Aromatic compounds (cyclic compounds containing a certain number of double bonds) are present in a wide variety of foods in small quantities. In pulse seeds, they originate from the oxidation of unsaturated fatty acids (Oomah et al. 2007). Maga & Katz (1979) reported the appearance of 2-ethyl furan upon heating, and indicated that a series of alkylated analogues, in particular 2-substituted alkylfurans such as 2-methyl- and 2-ethyl furans, often accompanied the parent furans associated with thermal treatment of complex Maillard reaction precursors or lipids. Azarnia et al. (2011b) found furan-2-methyl and toluene to be the most abundant aromatic compounds in yellow peas. Other aromatics, including *o*-xylene and *p*-xylene, which are lipid-derived compounds, were also detected in this study (Table 4.1 and 4.2); they have also been found in beans, split peas, and lentils (Lovegren et al. 1979; Del Rosario et al. 1984). Additionally, styrene, which has a characteristic sweet, balsamic, almost floral odor, was detected; it has been reported in several field pea cultivars, split pea, beans, and lentils (Azarnia et al. 2011b; Lovegren et al. 1979).

PCSE and PCSL had generally increased RPA values for total aromatics; the values were significant ($P<0.05$), except for the PCSL of navy bean and yellow pea (Fig. 4.5). In contrast, the PCSD of red kidney bean and yellow pea showed significantly decreased RPA values for aromatics. The RF and RS of navy beans showed significantly increased ($P<0.05$) values, whereas the RS of red kidney beans showed significantly decreased RPA values for aromatic compounds (Fig. 4.5). The varying levels of oxidation of unsaturated fatty acid as a result of the different thermal treatments may explain the differences observed in the formation of aromatic compounds in the pulse samples studied.

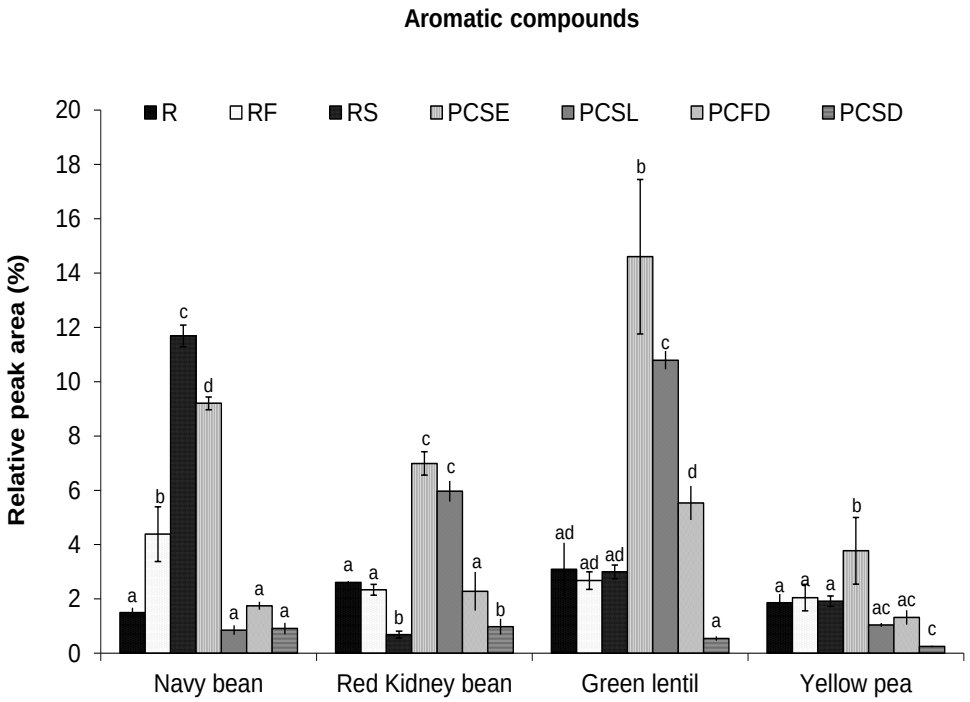


Fig. 4.5 Effect of thermal processing on the total relative peak area (RPA) of volatile aromatic compounds present in navy bean, red kidney bean, yellow pea, and green lentil.
Results are the average value of three replications. For each type of pulse, mean values bearing different letters are significantly different (P<0.05) as per Tukey's multiple comparison test. (R: raw; RF: roasted flour; RS: roasted seed; PCSE: pre-cooked seeds; PCSL: pre-cooked slurry; PCFD: pre-cooked-freeze-dried; PCSD: pre-cooked-spray-dried).

4.3.6 Effect of Processing on Alkane Compounds

The alkane compounds present in pulses originate mainly from the oxidative decomposition of lipids (Perkins 1988). They generally have weak odors and do not contribute much to flavors in foods; however, they may modify the volatility and flavor-imparting properties of other volatile compounds (Stevenson & Chen 1996).

Whereas 2-methyl decane was the most abundant alkane in navy bean and red kidney bean, 2,4-dimethyl hexane and 4-methyl decane gave the highest RPA values for alkanes in green lentils and yellow peas (Table 4.1 and 4.2). Chlorinated constituents (i.e., trichloromethane) were found in navy bean, red kidney bean, green lentil and yellow peas, as shown in Tables 4.1 and 4.2; they may be produced following minimal exposure of pulse plants to chlorinated organic compound. Their presence can be eliminated by making changes to cultural practices (Lovegren et al. 1979).

The RS and RF of the different pulses generally exhibited significantly decreased or statistically similar RPA values for alkanes in comparison with R samples (Fig. 4.6). Spray drying (PCSD) significantly decreased (P<0.05) the RPA of alkanes for red kidney bean, green lentil and yellow pea, whereas navy bean showed the opposite trend. The PCSE of red kidney bean also exhibited significantly decreased RPA values for alkane compounds (Fig. 4.6).

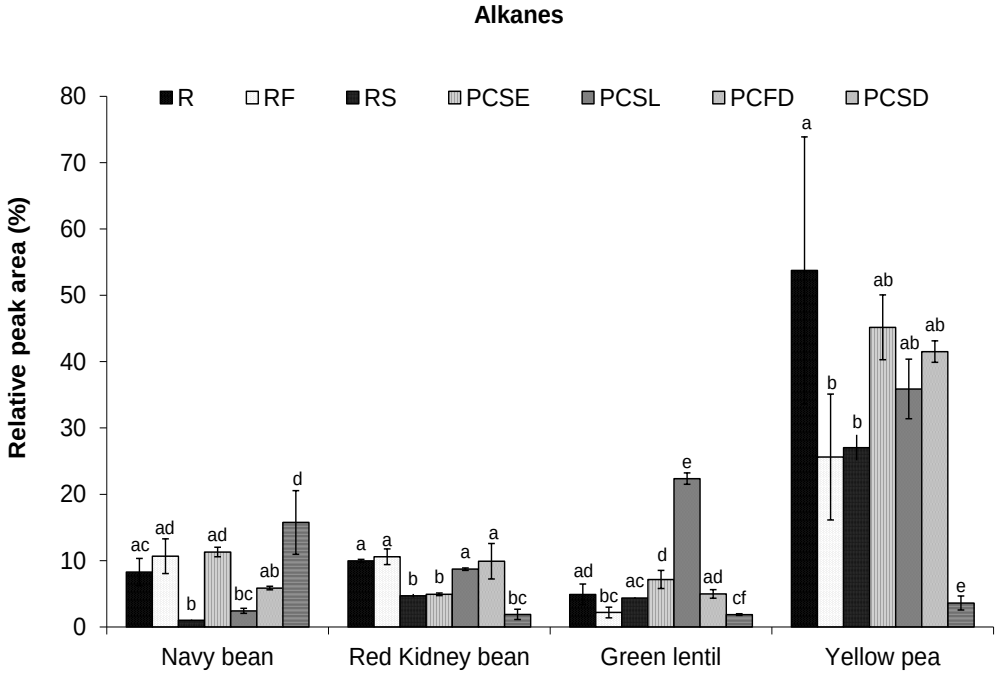


Fig. 4.6 Effect of thermal processing on the total relative peak area (RPA) of volatile alkane compounds present in navy bean, red kidney bean, yellow pea, and green lentil.
Results are the average value of three replications. For each type of pulse, mean values bearing different letters are significantly different (P<0.05) as per Tukey's multiple comparison test. (R: raw; RF: roasted flour; RS: roasted seed; PCSE: pre-cooked seeds; PCSL: pre-cooked slurry; PCFD: pre-cooked-freeze-dried; PCSD: pre-cooked-spray-dried).

4.3.7 Effect of Processing on Terpenoids

Terpenes, including 3-carene, camphene, and D-limonene, were found in raw and thermally treated navy bean, red kidney bean, green lentil, and yellow pea (Tables 4.1 and 4.2). The presence of monoterpenes in plants may result from endogenous isoprenoid biosynthesis or from carotenoid degradation by either LOX or hydroperoxides (Jakobsen et al. 1998). D-limonene, characterized as having a pleasant, lemon-like odor, is considered the most abundant and widespread terpene; it appeared in the heated samples of navy bean, red kidney

bean, and yellow pea (see Tables 4.1 and 4.2). Limonene and 3-carene have also been detected in dry and cooked beans, as well as in blanched green peas (Jakobsen et al. 1998; Oomah et al. 2007; Barra et al. 2007).

The comparison of the volatiles in raw and roasted pulse samples (RS and RF) showed a general increasing trend in the RPA of terpene for all varieties subjected to roasting (Fig. 4.7). A significant increase (P<0.05) in the RPA of terpene was observed for PCSL of red kidney beans. The PCFD and PCSD of green lentils showed significantly decreased (P<0.05) RPA for terpene (Fig. 4.7).

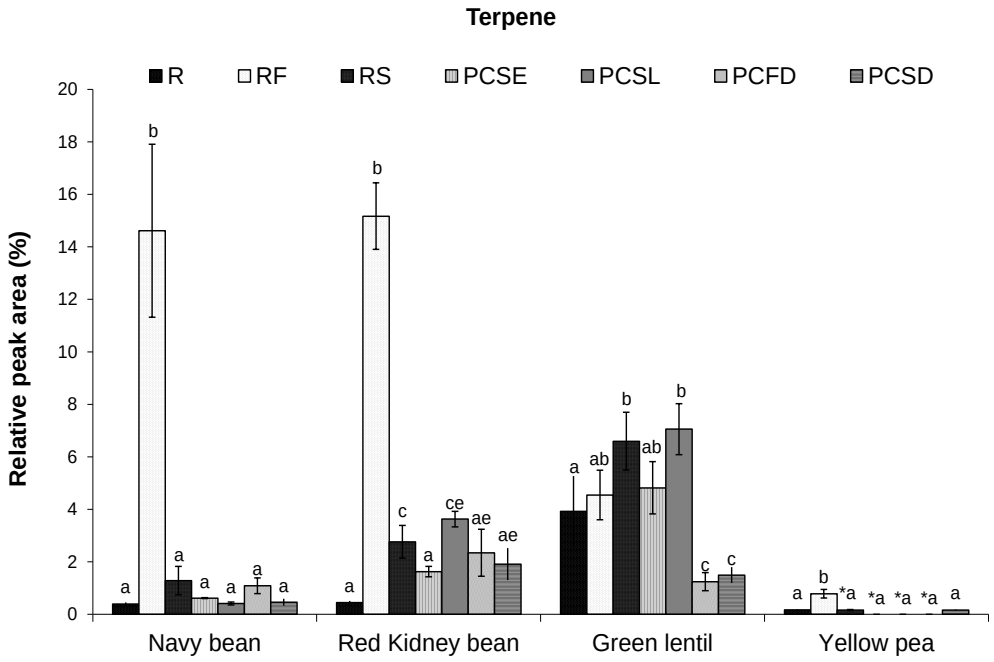


Fig. 4.7 Effect of thermal processing on the total relative peak area (RPA) of volatile terpenes present in navy bean, red kidney bean, yellow pea, and green lentil.
Results are the average value of three replications. * Not detected. For each type of pulse, mean values bearing different letters are significantly different (P<0.05) as per Tukey's multiple comparison test. (R: raw; RF: roasted flour; RS: roasted seed; PCSE: pre-cooked seeds; PCSL: pre-cooked slurry; PCFD: pre-cooked-freeze-dried; PCSD: pre-cooked-spray-dried).

4.3.8 Effect of Processing on Sulphur Compounds

Volatile sulphur compounds occur naturally in foods and can form during heat processing and storage. Sulphur-containing compounds are generally very flavor-active due to their low flavor thresholds and characteristic odors. Dimethyl disulfide, which has a diffuse, intense onion odor, was the predominant sulphur compound found in the pulses (Table 4.1 and 4.2); it is believed to result from the

decomposition of methanethiol. The presence of dimethyl disulfide has been reported in raw green peas and in cooked French beans (Azarnia et al. 2011b; Self et al. 1963). Methanethiol, which has an objectionable odor of decomposing cabbage or garlic, appeared in both the raw and thermally treated samples of navy bean, red kidney bean, green lentil and yellow pea (Table 4.1 and 4.2). The presence of small quantities of methanethiol was reported in the low boiling volatiles from cooked beans in a study using capillary column gas chromatography (Self et al. 1963).

As can be seen in Fig. 4.8, the RPA values for total sulphur compounds were either significantly increased or remained stable after the roasting process (RS and RF), except for the RF of green lentils. The PCSE of navy bean, red kidney bean and yellow peas showed significantly increased RPA values for total sulphur compounds. The PCFD and PCSD yellow pea, on the other hand, exhibited significantly decreased (P<0.05) total sulphur compounds compared with R samples.

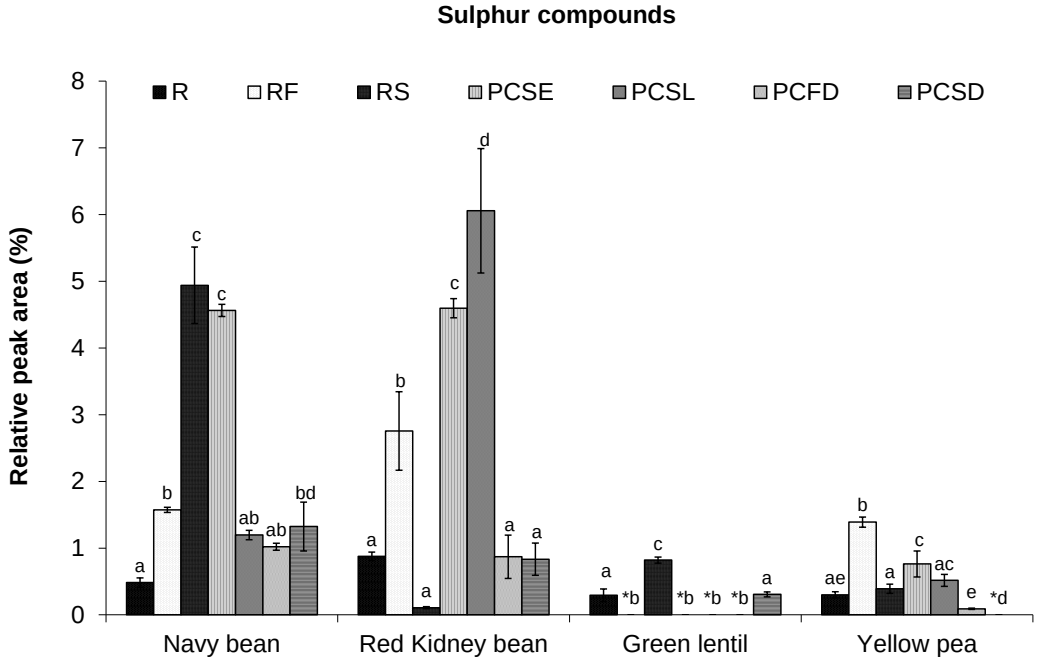


Fig. 4.8 Effect of thermal processing on the total relative peak area (RPA) of volatile sulphur compounds present in navy bean, red kidney bean, yellow pea, and green lentil.
Results are the average value of three replications. * Not detected. For each type of pulse, mean values bearing different letters are significantly different (P<0.05) as per Tukey's multiple comparison test. (R: raw; RF: roasted flour; RS: roasted seed; PCSE: pre-cooked seeds; PCSL: pre-cooked slurry; PCFD: pre-cooked-freeze-dried; PCSD: pre-cooked-spray-dried).

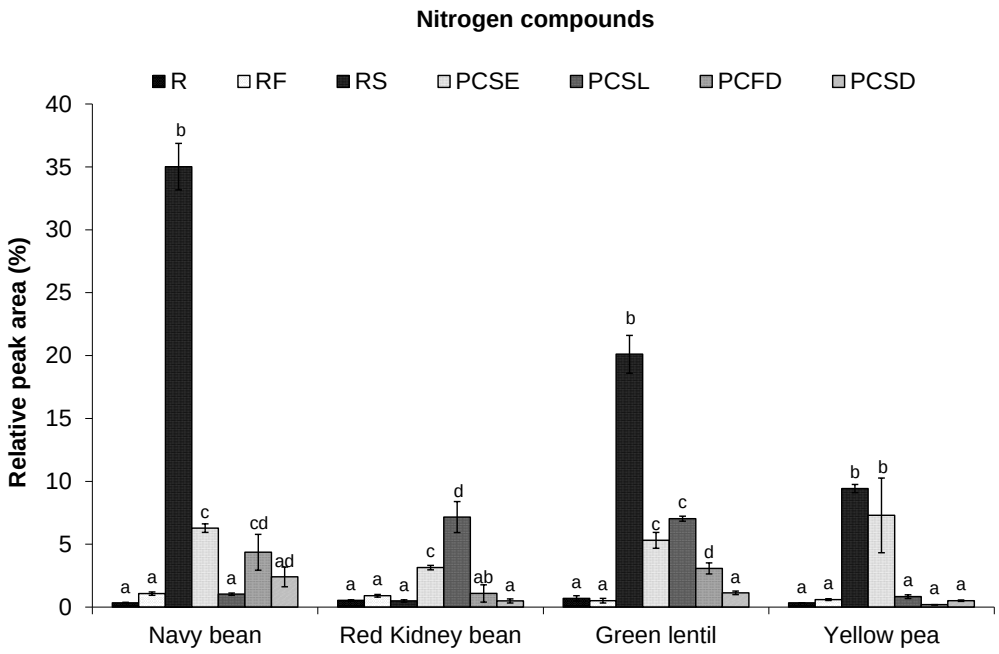


Fig. 4.9 Effect of thermal processing on the total relative peak area (RPA) of volatile nitrogen compounds present in navy bean, red kidney bean, yellow pea, and green lentil.
Results are the average value of three replications. For each type of pulse, mean values bearing different letters are significantly different (P<0.05) as per Tukey's multiple comparison test. (R: raw; RF: roasted flour; RS: roasted seed; PCSE: pre-cooked seeds; PCSL: pre-cooked slurry; PCFD: pre-cooked-freeze-dried; PCSD: pre-cooked-spray-dried).

4.3.9 Effect of Processing on Nitrogen Compounds

As shown in Tables 4.1 and 4.2, most of the nitrogenous flavor compounds (alkylated pyrazines, and pyrrole) were not detected in the raw samples; instead they were generally formed or increased after heating (cooking and roasting) most likely due to the Maillard reaction. Pyrazine compounds are produced by the reaction between amino acids and reducing sugars; they may also be observed upon dry-thermal degradation of proteins (Kato et al. 1981). However, in most cases, protein isolates do not produce pyrazines on wet heat treatment (i.e., under high moisture content treatment) (Qvist & Von Sydow 1974; Kato et al. 1981). A diverse group of pyrazines were found at low concentrations in the headspace of the thermally treated samples (Table 4.1 and 4.2). The highest levels of nitrogen compounds were found in the RS samples (Fig. 4.9). In particular, RS of navy bean, green lentil and yellow pea exhibited significantly increased (P<0.05) amounts of nitrogen compounds in comparison with the R samples (Fig. 4.9). The pre-cooking processes (PCSE, PCSL, PCFD, and PCSD) led to either

significantly increased or statistically equal RPA values for nitrogenous compounds. These compounds generally have a chocolate, roasted nut flavor and a sharp taste (Burdock 2002). This is consistent with the finding of Kato et al. (1981) that alkylated pyrazines were formed or increased markedly during roasting. Koehler et al. (1971) reported that, among the alkylated pyrazines, the mono-ethyl-mono-methyl-pyrazines had the lowest odor detection threshold level. The formation of 2-ethyl-5-methyl-pyrazine, 2-ethyl-6-methyl-pyrazine, 2-methyl-6-propyl-pyrazine and 2-methyl-5-propyl-pyrazine during roasting is of interest as they are desirable food flavors (Buttery et al. 1971; Wang et al. 1969) that could mask the beany flavor of pulses.

4.4 Conclusions

Basic knowledge of the volatile profiles of pulses such as red kidney bean, navy bean, lentils and peas, as well as the flavor changes that occur following different types of thermal processing, could ensure better quality control of raw materials and help product developers meet flavor-delivery challenges during product development. The results presented clearly show changes in the volatile flavor profiles of the treated pulse flours with some flavors disappearing and others appearing following heat treatment. Some of these changes are of interest in product development. For example, the novel volatile compounds such as pyrazines and alkylated pyrazines produced during the roasting and cooking processes may play an important role in masking the beany flavor associated with the presence of aldehydes, alcohols and sulfur compounds in raw pulses. The significant reduction in the total volatile compounds of the pre-cooked pulses, as well as the changes in volatile contents of different chemical families upon thermal processing, may also be of interest to relevant industries targeting specific pulse-based food product development. Further research using gas chromatography/sniffing port analysis, as well as quantitative descriptive analysis (QDA) and hedonic sensory evaluation, will be useful to determine which volatiles are significant contributors to the aroma of different pulse foods.

Connecting Statement to Chapter 5

In Chapter 3 and Chapter 4, the results obtained suggested that thermally treated pulse flours have good potential for use as value-added food ingredients in different food applications with improved nutritional quality, and, in some instances, superior functionality, as well as an altered flavour profile. Aside from the nutritional value of pulses, appealing appearance, and desirable texture attributes as well as sensory and rheological characteristics are of great importance for the development of high-quality food products. In this chapter, raw and thermally treated lentil flours were used to supplement salad dressings, and the colour, physical stability, and rheological and scanning electron microstructural properties, as well as sensory characteristics after production and during 28 days of storage, were studied and compared. The dressings with supplementation were also compared with a non-supplemented control sample. The effects of lentil flours on the colour, physical stability, and rheological properties were evaluated. It was hypothesized that close relationships might exist between the rheological properties, the microstructural characteristics, and the sensory attributes. This chapter addresses the third objective discussed in the "objective of study" section of Chapter 1. The results of this study are presented as follows:

Ma, Z., Boye, J. I., Fortin J., Simpson, B. K., Prasher, S. O., , Rheological, physical stability, microstructural and sensory properties of salad dressings supplemented with raw and thermally treated lentil flours. *Journal of Food Engineering*, (under revision).

Ma, Z., Boye, J. I., Simpson, B. K., Prasher, S. O., Fortin J., "Influence of processing on the rheological, physical stability, microstructural and sensory properties of lentil flour-supplemented salad dressings." *12th Annual Meeting of the Institute of Food Technologists (IFT)*, Las Vegas, Nevada, USA, June 25 to 28, 2012 (Poster Presentation).

Chapter 5. Rheological, Physical Stability, Microstructural and Sensory Properties of Salad Dressings Supplemented with Raw and Thermally Treated Lentil Flours

Abstract

Pulse flours have great potential for use as ingredients in the food industry because of their good functional properties and health benefits. In this study, raw lentil flour and lentil flour and seeds subjected to different thermal processing treatments (i.e., ground roasted seeds; roasted flours; pre-cooked, ground and freeze-dried seeds; pre-cooked, ground and spray-dried seeds) were used to supplement salad dressing emulsions. The effect of lentil addition on color, physical stability, rheological, microstructural and sensory properties was evaluated. The control sample (i.e., without pulse supplementation) had the lowest values for consistency coefficient (m), apparent viscosity (η_{ap}) and plateau modulus (G_N^0), which indicated lower viscoelastic properties, and further suggested that addition of pulse flours had a thickening effect. Dressings supplemented with pre-cooked freeze- and spray-dried lentil flours had the highest rheological properties. Overall, the results showed that thermally-processed pulse flours may be suitable as value-added ingredients in salad dressing applications.

5.1 Introduction

The nutritive and economic importance of dressings have continued to grow over the last two decades (Sikora et al. 2008). Salad dressings are widely consumed in North America and are frequently used by the food industry to enhance the attractiveness and tastiness of food products. Emulsifiers such as egg yolk are often used in salad dressings, as egg yolk lowers interfacial tension and forms an interfacial layer that prevents droplets from aggregating. Different hydrocolloids, including xanthan, guar, carrageenan, locust bean gum, gum Arabic, pectin, and propylene glycol alginate, are also frequently used to confer long-term stability to dressings (De Cássia da Fonseca et al. 2009; Mattes 1998; Paraskevopoulou et al. 2007; Jamison et al. 1978). These gums are usually highly

hydrated and are composed of extended molecules or aggregates which, depending on their molecular weight, degree of branching, conformation, and flexibility (McClements 2005b), also have the ability to increase viscosity.

A salad dressing is a non-Newtonian, pseudoplastic fluid with viscoelastic properties and yield stress. The significance of the rheological properties of dressings has to do with their close relationship with quality, sensory attributes, shelf life, and microstructure. Different rheological tests have been carried out to study the internal structural organization and interactions of components within emulsion systems. A steady-state flow test, for example, provides information about the strength of the colloidal interactions between droplets within the emulsion (Tadros, 1994). During the test, plots of shear stress (τ) versus shear rate (γ) can be obtained when samples are subjected to dramatic structural destruction due to both irreversible and reversible processes. Small-amplitude oscillatory experiments, carried out within the linear viscoelastic region, minimize destruction in the sample as little or no permanent structural breakdown occurs during the measurements. This approach allows the study of the viscoelasticity (i.e., corresponding storage and loss moduli) of complex systems which can be related to structural characteristics of the emulsion (Muńtoz & Sherman 1990). Creep and recovery tests performed by monitoring changes in a material's dimensions (strain or compliance) with time when a constant stress is applied and removed (Dolz et al. 2008; McClements 2005c) are also of interest. This type of test provides insight on the internal structure of a dressing emulsion and the structural variations associated with induced changes in composition.

The concept of supplementing salad dressings with pulse flour, such as lentil, is novel. Lentil addition may contribute to the rheological and sensory properties of salad dressing emulsions, owing to the increased numbers of rigid particles and the potential interactions between the pulse flour and other ingredients in the salad dressing. As pulses are rich in protein, dietary fiber, and various vitamins and minerals, supplementation with pulse flour may further enhance the nutritional value of salad dressings.

Our previous studies indicated that thermally treated pulse flours may have very good potential for use as value-added food ingredient because of their good nutritional value (i.e., significantly reduced trypsin inhibitor activity) and greater functionality in terms of fat-binding, water-holding and gelling capacity (Ma et al. 2011). So far, no research has been done to characterize salad dressings supplemented with various thermally processed pulse flours. The objective of this study, therefore, was to add lentils subjected to different processing treatments to salad dressings and investigate the effect on color, physical stability as well as the rheological, microstructural and sensory properties of the supplemented dressings.

5.2 Materials and Methods

5.2.1 Materials

Green lentil seeds (Laird variety) were provided by Pulse Growers in Saskatchewan (Saskatoon, Saskatchewan, Canada). Spray-dried egg yolk powder was obtained from Canadian Inovatech Inc. (Winnipeg, Manitoba, Canada). Xanthan gum was kindly provided by Tic Gums (Belcamp, Md., USA). Other ingredients used in the salad dressing preparation were purchased from a local supermarket. All other chemical reagents were of analytical grade.

5.2.2 Milling and Thermal Processing of Lentil Seeds

Green lentil seeds were repeatedly ground three times using a knife grinder (model 3600, Urschel Laboratories Inc., Valparaiso, Indiana, USA) to achieve a fine flour (i.e., "raw flour"). To obtain "roasted flour," the "raw flour" was thinly and evenly spread on an aluminum dish, and roasted for 1 min in a 100 ℃ oven (Double Model OD 302, Fisher & Paykel Appliances Ltd., Huntington Beach, CA, USA). To obtain flour from roasted seeds ("roasted seeds"), one kilogram of lentil seeds was evenly spread on an aluminum pan and roasted for 20 min at 120 ℃ in a rotating oven (Model MT-4-8, Picard Bakers LP, Victoriaville, Quebec, Canada). After roasting, the pan was placed in the freezer for 15 min and the seeds were ground using the same method as for raw flour. For the pre-cooked samples, lentil seeds were first rinsed with demineralized water and then cooked in water (35 kg seeds/110 L water) at 95 ℃ for 30 min. The pre-cooked samples were then either spray dried with an Atomizer spray dryer (model HT 10-530,

Niro Atomizer Ltd., Copenhagen, Denmark), or freeze dried with a VirTis freeze dryer (model 50-SRC-5, VirTis Co., Inc., Gardiner, NY, USA). Both samples were then ground three times using a knife grinder (Stephan Mikrocut Type MC15, A. Stephan u. Söhne GmbH & Co., Manelor 1 Germany). These samples are referred to as "pre-cooked spray-dried lentils" and "pre-cooked freeze-dried lentils", respectively. All samples were passed through a USA Standard Testing Sieve with 425 micrometer openings (No. 40 US Series Alternate Sieve Designation). Sieved flours were collected and stored in airtight plastic containers at 4 ℃ until further analysis.

5.2.3 Salad Dressing Sample Preparation

Lentil-supplemented salad dressings were made from the prepared lentil flours using the following ingredients [expressed as percentage (w/w)]: lentil flour (7%), canola oil (35%), egg yolk (5%), vinegar (containing 5% (w/v) acetic acid) (7.0%), lemon juice (5%), salt (1.0%), sugar (3.5%), and xanthan gum (0.25%). The method of preparation used was as follows: first, xanthan gum and sugar were dispersed in water and stored overnight to ensure complete hydration. All other ingredients except the oil were then added and mixed using a magnetic stirrer until a homogeneous mixture was obtained. Potassium sorbate (0.02 wt%) was then added as an antimicrobial agent. Lastly, the oil was added and emulsification was achieved using an Ultra-Turrax homogenizer (Model T25, Janke & Kunkel, Ika Labortechnik, Staufen, Germany) equipped with a S25–18G dispersing tool operated at 13,800 rpm for 3 min. A control salad dressing sample containing no lentil flour was also prepared. All salad dressings were stored in tightly capped bottles at 4 ℃ and analyzed during a 28 day period.

5.2.4 Rheological Measurements

Rheological measurements were taken with an AR 1000 rheometer (TA Instrument, New Castle, DE, USA) equipped with a plate/cone system. Steady-state flow, dynamic oscillatory, and creep and recovery tests were conducted using a stainless steel parallel plate (4 cm diameter). The gap was set at 1 mm. One tablespoon of sample was placed in the centre of the circular plate, and excess sample was removed from the edges of the plate. The plate was covered

with an annular ring/disc to prevent moisture loss during measurement. The linear viscoelastic (LVE) range was determined by performing amplitude sweeps at 1 Hz frequency over a strain range from 0.01% to 1000%. The steady-state flow test was performed at increasing shear rates (0.02−300 s^{-1}), and experimental flow curves were fitted to the power law model (Eq. (1)) and the Herschel-Bulkley model (Eq. (2)):

$$\eta = m\gamma^{(n-1)}, \qquad \text{(Eq. 1)}$$

$$\sigma = \sigma_0 + m\gamma^n, \qquad \text{(Eq. 2)}$$

where n is the flow behavior index (dimensionless), η is the shear viscosity (Pa.s), m is the consistency coefficient (Pa.s^n), γ is shear rate (s^{-1}), σ is the shear stress (Pa), and σ_0 is the yield stress (Pa). Each of these parameters was calculated.

The apparent viscosity (Pa.s) at a shear rate of 46.16 s^{-1} was also calculated in accordance with the power law model: the shear rate used was selected to correspond to the perceived mouthfeel or thickness of normal fluids (Baines and Morris, 1988).

The dynamic oscillatory test was performed over an angular frequency range of 0.1 to 100 rad/s. Storage modulus (G′, Pa) and loss modulus (G″, Pa) versus angular frequency were measured for all the samples with controlled strain of 0.1%. The plateau modulus (G^0_N, Pa) was obtained by the approximate procedure suggested by Wu (2003), which involves finding G′ in the plot of G′ versus frequency where the value of tan δ (which equals G″/G′) is a minimum, as shown in the following equation:

$$G_N^0 = [G']_{\tan\delta \to \min imum} \qquad \text{(Eq. 3)}$$

Creep and recovery tests were carried out to evaluate the elasticity of the salad dressings. The samples were pre-sheared at a shear strain of 300 s^{-1} for 2 min, 0.5 Pa stress was applied to the samples for 600 s, then the stress was totally removed and the strain was recorded as a function of time for 600s. Creep and recovery curves are plotted as compliance (J), which is the ratio of the strain to the applied stress during the monitored period. The recoverable strain or extent of

strain recovery (*Q(t)%*) was calculated according to the method of Zhang et al. (2008), with higher *Q(t)%* values indicating higher elasticity.

5.2.5 Color Measurement

The color of the salad dressing samples was measured with the *L**, *a**, *b** tristimulus system using a Minolta CM-503c spectrophotometer (Minolta Co. Ltd., Osaka, Japan). A fixed amount of salad dressing was poured into the measuring cup, which was then surrounded with a black paper strip. In this color system, *L** value is a measure of lightness to darkness (0=black and 100=white); *a** is a measure of redness (+ve) to greenness (−ve), with a higher positive *a** value indicating more redness; and *b** is a measure of yellowness (+ve) to blueness (−ve), with a higher positive *b** value indicating more yellowness. The data were also characterized in terms of chroma (C) and color difference (ΔE) to highlight the differences between the samples using the following equations:

$$C = (a^2 + b^2)^{1/2} \qquad \text{(Eq. 4)}$$

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2} \qquad \text{(Eq. 5)}$$

where ΔL, Δa, Δb are the color changes during storage when compared with the color of the freshly made dressing measured on the day it was prepared.

5.2.6 Physical Stability

The stability of the salad dressing emulsions was determined by light scattering measurements taken with a vertical scan analyzer (Quick Scan; Beckman Coulter, Fullerton, California, USA). The acquisition options were set at 0−60 mm. By repeating the sample scan at different time intervals during storage, the backscattering (BS) profile of every 40 μm as a function of sample height (50 mm) and time was acquired. Experiments were done in triplicate and the results were averaged.

5.2.7 Sensory Evaluation

Sensory evaluation was performed on salad dressings supplemented with raw lentil flour, roasted seeds, roasted flour, and pre-cooked-spray-dried samples. Prior to sensory testing, total micro-organism plate count was determined for the salad dressings according to the European Standard ISO 4833:2003 method to ensure the safety of the dressings for panel members. The pre-cooked-freeze-dried

sample was not included in the analysis as it was determined to be similar to the pre-cooked-spray-dried lentils with respect to its overall sensory attributes during preliminary studies. A quantitative descriptive analysis (QDA) was used for the sensory test, and the following attributes were determined and rated on a categorical scale ranging from 0 (attribute not detected) to 7 (attribute very strong): overall intensity, legume flavor, vinegar (aroma), acidity (taste), particle size and firmness. The test was carried out by a trained sensory panel consisting of 10 employees from the Food Research and Development Centre in Saint-Hyacinthe (Quebec) with three years of experience in QDA testing. The panel was trained in the modified Spectrum^{TM} method of descriptive analysis (Meilgaard et al. 2007). The panel members received 3 h training sessions on descriptive term familiarization, intensity scale use and performance measurements, allowing each panelist and the panel as a whole to improve discrimination and reproducibility performance as evaluated by ANOVA (Duncan's test).

A complete random block design was used and, in each session, four samples were presented and formal testing was performed in triplicate. The sensory evaluation was conducted 24 h after the dressing was produced, and during that time the samples were stored in sealed glass jars at 4 ℃. The supplemented salad dressings were portioned out (25 mL) and presented randomly in each session. The dressings were randomly labeled with a 3-digit number. The evaluation test took place in a panel room with individual booths lit with a red light. Prior to evaluating each sample, the panelists were given unsalted biscuits and water. The sample effect and the panelist effect were tested using the ANOVA statistical test.

5.2.8 Scanning Electron Microscopy

The salad dressing samples were encapsulated in 2% agar, cut into 2−3 mm cubes, and immersed in 2.5% glutaraldehyde for 2 h, then transferred into 0.1 *M* sodium cacodylate buffer solution (pH 7.1) and stored at 4 ℃. For each dressing, 2 samples were encapsulated. After the samples were washed with buffer, they were dehydrated in a graded ethanol series ranging from 30% to 100%, and critical point dried with CO_2 in a critical point dryer (SPI, USA). Dry sections

were fractured and fragments were mounted on aluminum stubs and coated with gold (10 nm). Observations were made under a scanning electron microscope (S-3000N model, Hitachi, Tokyo, Japan) at 5 kV.

5.2.9 Statistical Analysis

All analyses were conducted in triplicate. The values given in tables and figures are the means of three determinations. The statistical significance of difference was evaluated by one-way analysis of variance (ANOVA) using the PRISM software, version 3.02 (GraphPad Software, Inc., San Diego, CA, USA). Significant differences between means were determined by Tukey's multiple comparison tests at the 5% significance level. Sensory evaluation results were analyzed with the Fizz software (Version 2.4, Biosystems, Couternon, France).

5.3 Results and Discussion

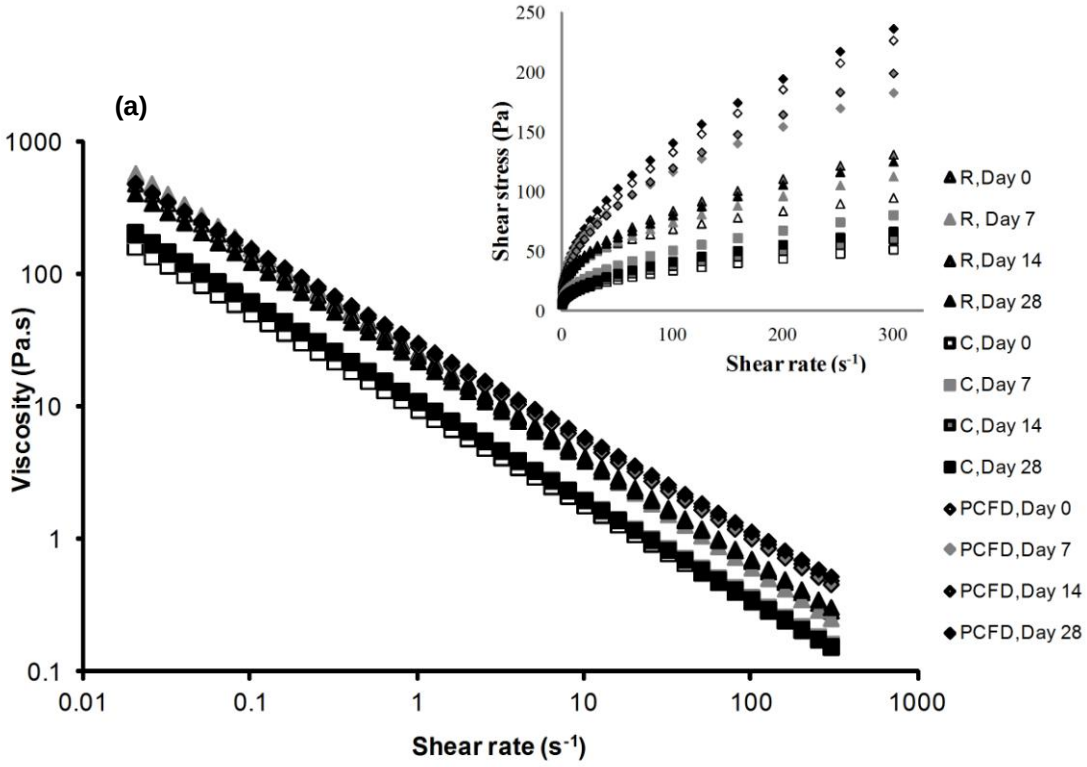
5.3.1 Rheological Measurements

5.3.1.1 Steady state flow curves

The steady-state flow curves for the different salad dressings are shown in Fig. 5.1 (a, b). The power law model, which is frequently used to characterize shear-thinning fluids, and the Herschel-Bulkley model (inserted figures in Fig. 5.1), which is mostly used to describe the flow behavior of fluids with a yield stress (σ_0), were both fitted to the experimental data. Flow behavior index (n), consistency coefficient (m), apparent viscosity (η_{ap}), and yield stress (σ_0) values obtained on different storage days (day 0, 7, 14 and 28) are presented in Tables 5.1 and 5.2. Although the control and all the lentil-supplemented dressings gave different values for the rheological parameters (Table 5.1, 5.2), all the samples showed pseudoplastic, non-Newtonian behavior (n<1) with yield stress.

On day 0 the control sample had the (P<0.05) lowest consistency coefficient (m) and apparent viscosity (η_{ap}) indicating its lower viscosity (Tables 5.1 and 5.2). Salad dressings supplemented with raw flour had a lower flow behavior index (n) than the control and the other supplemented samples. This effect was significant (P<0.05) for all the dressings except for those supplemented with roasted seeds, which suggests that dressings supplemented with raw flour had the most pronounced shear-thinning behavior. Thermal processing of the lentils (especially

pre-boiling) decreased the non-Newtonian behavior of the pulse-supplemented dressings. It suggested that the thermal processing, in particular pre-boiling process increased the stability of the dressing to shearing process, which could be attributed to the possibly increased chances of interaction in the emulsion system following protein denaturation. Yield stress (σ_0), defined as the minimum shear stress required to initiate flow, significantly increased (P<0.05) with lentil supplementation (Table 5.1). This observation may have important implications for the food industry, as yield stress is a key quality control parameter for technological processing, including mixing, pumping, transport and storage. It is also a very important parameter related to consumer acceptance (Juszczak et al. 2003). Salad dressings supplemented with pre-cooked-freeze/spray-dried lentils had higher consistency coefficient (m) and apparent viscosity (η_{ap}) values than the control and other supplemented dressings, which is indicative of an increase in the viscosity of these emulsion systems.



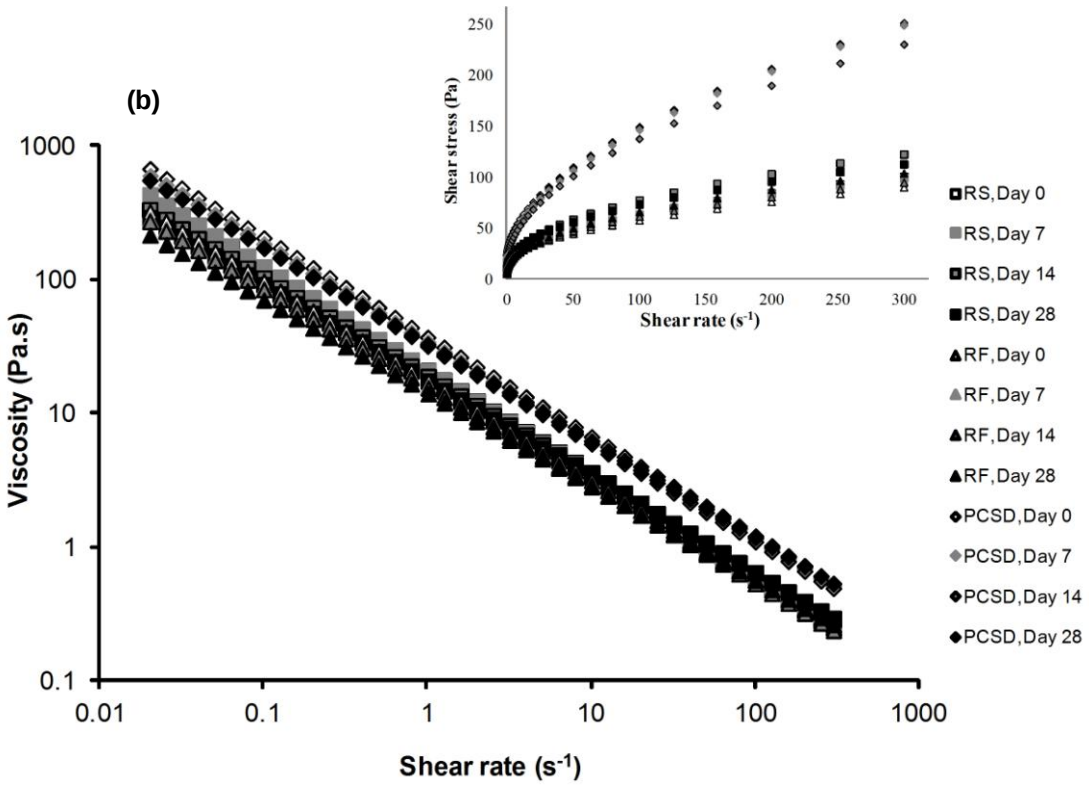


Fig. 5.1 Steady-state flow curves for (a) salad dressings supplemented with 7% (w/v) raw (R) lentil flour, pre-cooked freeze-dried (PCFD) lentil flour and control (C); (b) salad dressings supplemented with 7% (w/v) roasted lentil seeds (RS), roasted flour (RF), and pre-cooked spray-dried (PCSD) lentil flour on storage days 0, 7, 14 and 28 at 4 ℃.

Values for *n* increased with storage for all samples which indicated a decrease in pseudoplasticity, except the control (Table 5.1). This increasing trend was statistically significant ($P<0.05$) for dressings supplemented with raw lentil flour, roasted lentil seeds, and roasted lentil flour. In contrast, the consistency coefficient (*m*) for all samples, except for the control sample, decreased with storage indicating a decrease in viscous nature. The decrease in pseudoplasticity suggests fewer interactions and entanglements in the emulsion system, which agrees with the decreased *m* value observed during storage. The yield stress obtained by fitting the Herschel-Bulkley model also decreased during storage for all samples, except for the control. The effect was statistically significant for dressings supplemented with roasted flour and roasted seeds, and for the pre-cooked-freeze-dried samples on day 28 compared with the freshly prepared samples (day 0). The changes observed, therefore, suggest that rearrangements occurring during storage resulted in a weakening of the network structure.

5.3.1.2. Dynamic oscillatory tests

All the salad dressings were found to be more elastic than viscous over the studied frequency range, as indicated by their greater G′ than G″ values across the frequency interval studied Fig. 5.2 (a, b). For all samples, a gradual increase in both G′ and G″ was observed with increasing frequency. All the supplemented salad dressings and the control were weak gels (i.e., tan δ values larger than 0.1), which is typical of dressing and mayonnaise emulsions. Salad dressings supplemented with pre-cooked-freeze/spray-dried lentils (Fig. 5.2a and 5.2b) had higher G′ values compared to the other supplemented dressings and the control, indicative of greater elasticity. The control sample had the lowest G′ and G″ value. Salad dressings supplemented with roasted lentil flour exhibited the highest G″ value (Fig. 5.2 c, d).

Furthermore as shown in Table 5.2, compared to the supplemented salad dressings, the control sample had a lower G_N^0 value (P<0.05). Salad dressings supplemented with pre-cooked-freeze/spray-dried lentils had the highest G_N^0 values (P<0.05), suggesting a stronger emulsion network in the dressings supplemented with pre-cooked freeze/spray-dried lentils.

5.3.1.3. Creep and recovery tests

The control sample had the highest J values for both the creep and recovery tests, whereas the dressings supplemented with pre-cooked-freeze/spray-dried lentils had the lowest values (Fig. 5.3a, b). For the same applied stress (0.5 Pa), changes in the dimensions of the dressings with time (strain) were relatively large for the control and relatively small for the dressings supplemented with the pre-cooked lentils. This is consistent with the finding discussed earlier, specifically that in the case of dressing supplemented with pre-cooked lentils, a stronger network structure (higher values of m and G_N^0) would lead to less deformation, and for the control which had the weakest viscoelastic properties, a large change in its dimensions would be observed owing to the weaker emulsion structure.

Table 5.1 The power law model flow behavior index (*n*), the power law model consistency coefficient (*m*) and Herschel-Bulkley's yield stress (σ_0) for the control and lentil-supplemented salad dressings.

Salad dressings	*n* value (dimensionless)*				*m* value (Pa s^n)*				σ_0 (Pa)			
	Day 0	Day 7	Day 14	Day 28	Day 0	Day 7	Day 14	Day 28	Day 0	Day 7	Day 14	Day 28
Raw flour	0.2119^{acA} ±0.004	0.1947^{c} ±0.032	0.2331^{ab} ±0.015	0.2508^{b} ±0.005	26.27^{aAC} ±0.15	25.33^{a} ±2.51	24.68^{a} ±0.77	22.32^{a} ±1.93	19.33^{aAC} ±2.35	16.24^{a} ±2.85	14.13^{a} ±2.55	11.99^{a} ±3.15
Roasted seed	0.2348^{acAC} ±0.005	0.2196^{a} ±0.017	0.2655^{bc} ±0.008	0.2812^{b} ±0.004	21.10^{aA} ±1.06	20.97^{a} ±0.96	19.02^{a} ±0.34	17.65^{a} ±0.96	14.12^{abAC} ±1.57	15.11^{a} ±4.73	6.76^{bc} ±1.05	5.27^{c} ±0.65
Roasted flour	0.2537^{aB} ±0.009	0.2636^{a} ±0.011	0.2666^{a} ±0.007	0.3009^{b} ±0.009	17.61^{aA} ±0.79	15.93^{a} ±1.02	15.64^{a} ±0.29	14.18^{a} ±1.14	11.98^{aC} ±2.81	7.44^{ab} ±2.93	5.64^{ab} ±1.54	3.70^{b} ±0.81
Pre-cooked-spray-dried	0.2567^{aBC} ±0.015	0.2635^{a} ±0.002	0.2679^{a} ±0.007	0.2768^{a} ±0.001	37.23^{aC} ±2.55	34.22^{a} ±3.86	31.97^{a} ±0.77	33.01^{a} ±0.24	21.30^{aAC} ±2.70	16.54^{ab} ±1.41	14.55^{ab} ±0.35	13.37^{b} ±0.52
Pre-cooked-freeze dried	0.2810^{aB} ±0.005	0.2722^{a} ±0.007	0.2755^{a} ±0.008	0.2865^{a} ±0.008	30.16^{aC} ±7.68	29.38^{a} ±3.70	28.89^{a} ±4.81	28.03^{a} ±3.33	15.19^{aA} ±3.86	12.60^{a} ±3.91	12.88^{a} ±2.37	13.47^{a} ±1.93
Control	0.2759^{aB} ±0.002	0.2607^{a} ±0.007	0.2519^{a} ±0.002	0.2489^{a} ±0.007	10.10^{aB} ±0.24	11.17^{a} ±0.94	11.07^{a} ±0.65	11.29^{a} ±0.17	4.75^{aB} ±1.80	5.50^{a} ±1.14	5.78^{a} ±0.63	5.67^{a} ±0.86

Table 5.2 Plateau modulus, apparent viscosity and extent of recovery of control and lentil-supplemented salad dressings.

Salad dressings	Apparent viscosity η_{ap} (Pa)[a]				G_N^0 value (Pa)				*Q(t)* (%)			
	Day 0	Day 7	Day 14	Day 28	Day 0	Day 7	Day 14	Day 28	Day 0	Day 7	Day 14	Day 28
Raw flour	1.28^{aAD} ±0.01	1.16^{a} ±0.12	1.30^{a} ±0.04	1.26^{a} ±0.11	141.63^{aA} ± 8.39	103.83a ±9.54	130.27^{a} ±7.31	149.23^{a} ±4.68	63.84^{aA} ±11.29	68.58^{a} ±8.69	66.34^{a} ±5.99	66.14^{a} ±2.68
Roasted seed	1.12^{aAB} ±0.09	1.07^{a} ±0.06	1.14^{a} ±0.01	1.12^{a} ±0.04	117.08^{aA} ±9.10	111.11^{a} ±7.92	127.52^{a} ±8.71	120.93^{a} ±12.07	51.13^{aA} ±7.84	65.12^{a} ±1.04	53.73^{a} ±8.92	65.36^{a} ±1.01
Roasted flour	1.01^{aAB} ±0.07	0.95^{a} ±0.03	0.94^{a} ±0.02	0.97^{a} ±0.09	106.51^{aA} ±7.89	84.67^{a} ±4.91	78.11^{a} ±1.98	83.99^{a} ±4.45	52.71^{aA} ±6.27	58.11^{a} ±0.88	58.52^{a} ±4.18	56.07^{a} ±2.15
Pre-cooked-spray-dried	2.16^{aC} ±0.11	2.04^{a} ±0.24	1.93^{a} ±0.08	2.06^{a} ±0.01	326.9^{aC} ± 5.59	307.85^{a} ±8.84	247.75^{ab} ±9.15	202.57^{b} ±9.81	139.70^{aB} ±6.35	125.08^{a} ±5.29	131.47^{b} ±6.92	114.01^{b} ±10.68
Pre-cooked-freeze dried	1.73^{aCD} ±0.32	1.81^{a} ±0.22	1.80^{a} ±0.33	2.01^{a} ±0.47	310.87^{aC} ±8.19	242.53^{a} ±9.85	104.95^{b} ±7.68	329.35^{a} ±10.25	133.23^{abB} ±9.69	116.91^{a} ±6.91	276.47^{b} ±8.90	193.28^{ab} ±6.70
Control	0.63^{aB} ±0.02	0.66^{a} ±0.06	0.64^{a} ±0.03	0.63^{a} ±0.01	27.99^{aB} ±6.74	42.23^{a} ±5.46	31.02^{a} ±5.42	35.27^{a} ±9.19	46.69^{aA} ±1.39	48.69^{a} ±3.21	48.35^{a} ±2.99	49.42^{a} ±2.88

* For the salad dressing samples on day 0, mean values with different capital letters (A, B, and C) for a given parameter within the same column are significantly different (p<0.05) based on Tukey's multiple comparison test. For a given parameter, different lower case letters within the same row are significantly different (p<0.05) based on Tukey's multiple comparison test. * [a] Apparent viscosity was calculated according to the power law model at a shear rate of 46.16s^{-1}

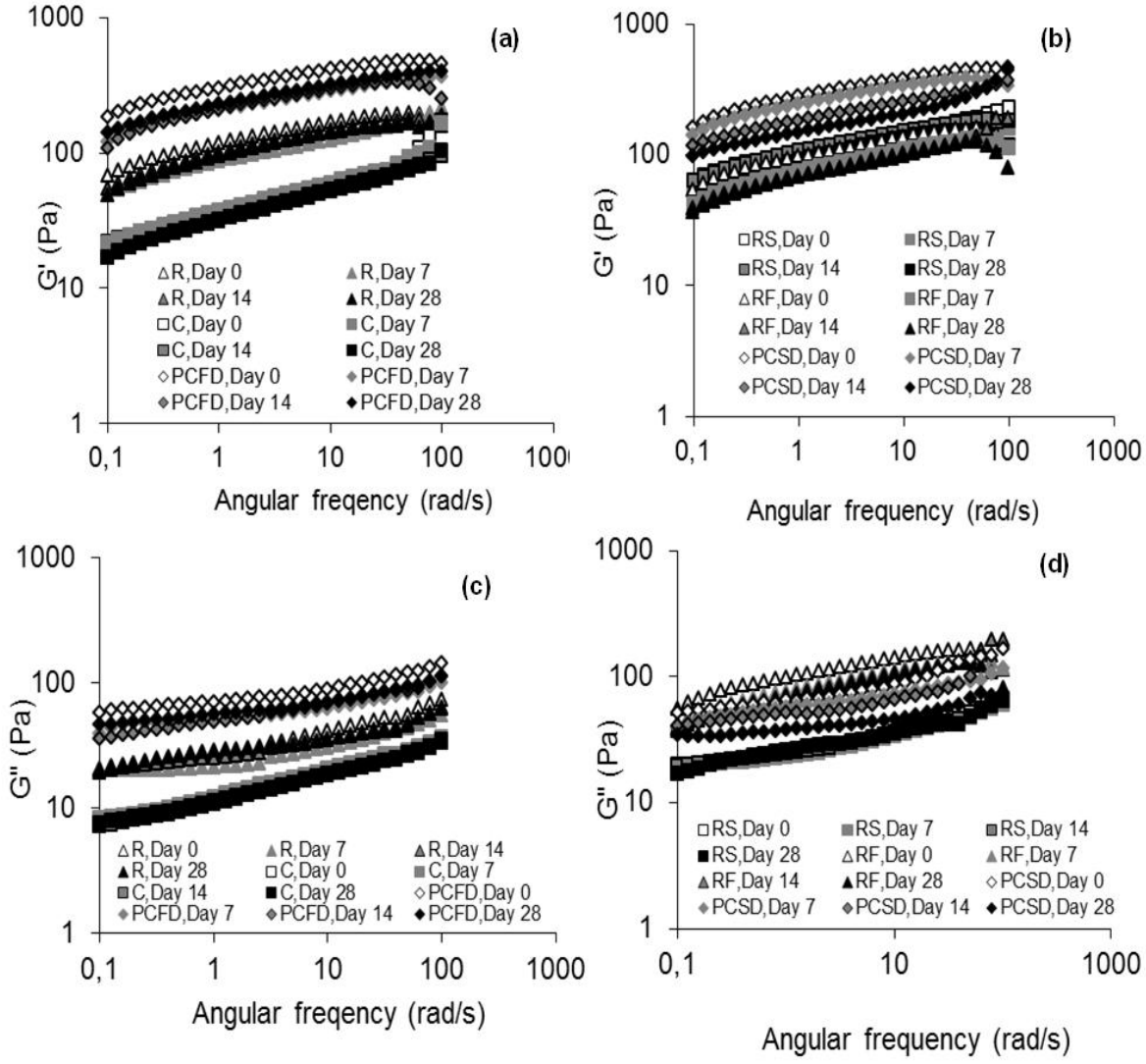
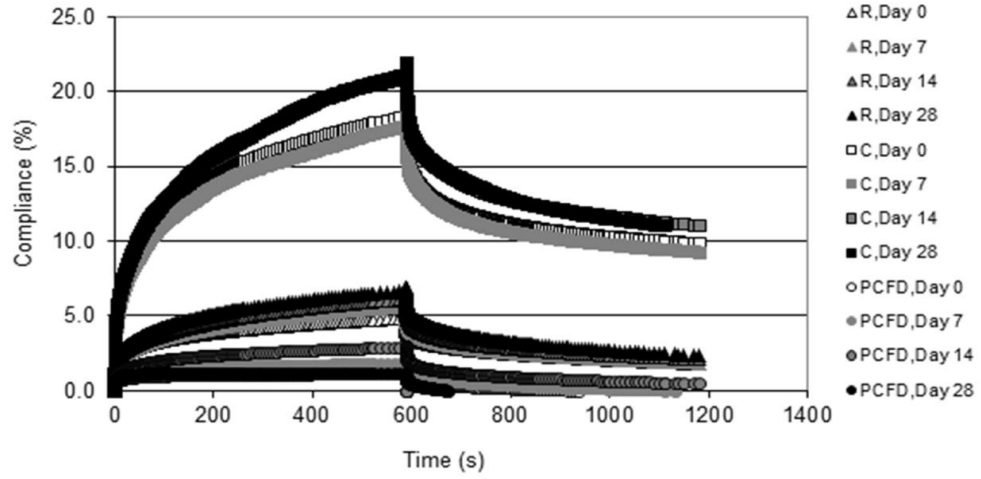


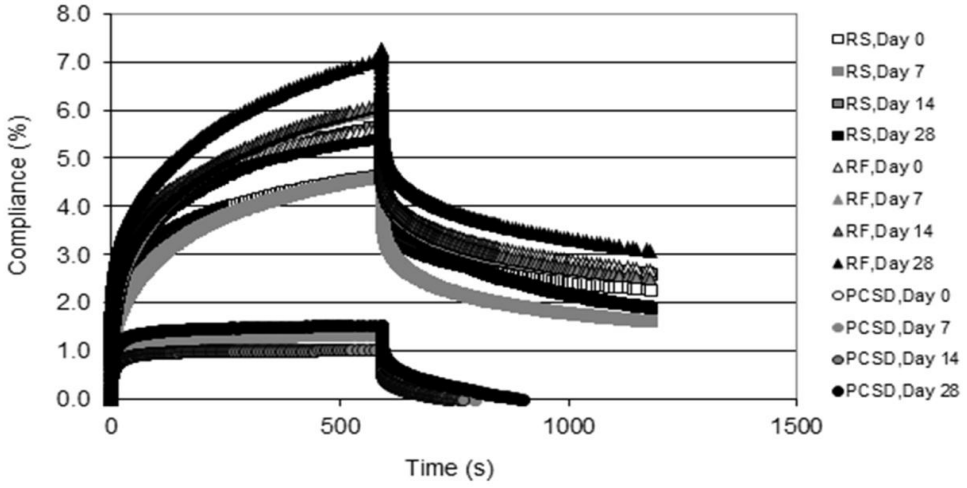
Fig. 5.2 (a) Storage modulus (G′) as a function of angular frequency during dynamic oscillatory tests of (a) salad dressings supplemented with 7% (w/v) raw (R) lentil flour, pre-cooked freeze-dried (PCFD) lentil flour and control (C) on storage days 0, 7, 14 and 28; (b) salad dressings supplemented with 7% (w/v) roasted lentil seeds (RS), roasted lentil flour (RF), and pre-cooked spray-dried (PCSD) lentil flour on storage days 0, 7, 14 and 28; (c) loss modulus (G″) of salad dressings supplemented with 7% (w/v) raw (R) lentil flour, pre-cooked freeze dried (PCFD) lentil flour and control (C); (d) loss modulus (G′) of salad dressings supplemented with 7% (w/v) roasted lentil seeds (RS), roasted lentil flours (RF), and pre-cooked spray dried (PCSD) lentil flour on storage days 0, 7, 14 and 28.

As shown in Table 5.2, the dressings supplemented with pre-cooked-freeze/spray-dried lentils exhibited the highest (P<0.05) recoverable strain ($Q(t)\%$) indicative of greater elasticity. As these samples also showed the highest consistency coefficient (m), apparent viscosity (η_{ap}), storage modulus (G′), and plateau modulus (G_N^0) values, the

results collectively indicate that this type of dressing had the highest viscoelasticity and compact structure.



(a)



(b)

Fig. 5.3 Creep and recovery curves of (a) salad dressings supplemented with 7% (w/v) raw (R) lentil flour, pre-cooked freeze-dried (PCFD) lentil flour and control (C); (b) salad dressings supplemented with 7% (w/v) roasted lentil seeds (RS), roasted lentil flour (RF), and pre-cooked spray-dried (PCSD) lentil flour on storage days 0, 7, 14 and 28 at 4 ℃.

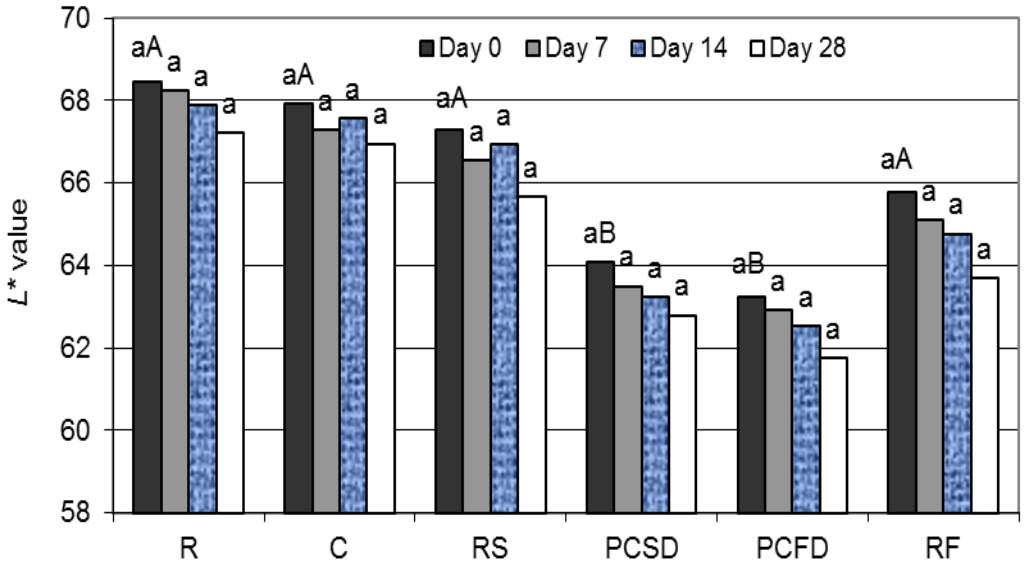
5.3.2 Color Measurement

The variations in lightness (L^*), redness (a^*) and yellowness (b^*) for all samples during the 28 days of storage are shown in Fig. 5.4a, b, and c, respectively. Dressings supplemented with pre-cooked freeze-dried and spray-dried lentils had lower lightness values ($P<0.05$) (L^* values) than the other dressings. Although not statistically significant,

the general decreasing trend in L^* values during storage for all samples, is suggestive of a possible increase in droplet size over time (Chantrapornchai et al. 1998). Significantly higher a^* values were obtained for dressings supplemented with pre-cooked-freeze/spray-dried lentils (indicative of less greenness) (Fig. 5.4b). Additionally, a significant increase was observed in a^* values for all supplemented samples after 28 days compared with the values on day 0, indicative of a decrease in greenness over time. Compared to the supplemented dressings, the control sample had significantly (P<0.05) lower b^* values, which suggests that addition of lentils increased the yellowness hue of the dressings.

Changes in ΔE of all samples increased significantly during the 28 days of storage (Fig. 5.4d). This observation is in good agreement with those of Laca et al. (2010). Chroma (C) is a parameter which gives a better description of the spatial position of the measured color. The variation in C during storage was slight (Fig. 5.4e), indicating that the color intensity of all dressings remained stable over time. The control sample had a significantly lower C value (P<0.05) than that of all the supplemented dressings which indicates that supplementation with lentils increased the total color intensity of the dressings.

(a)



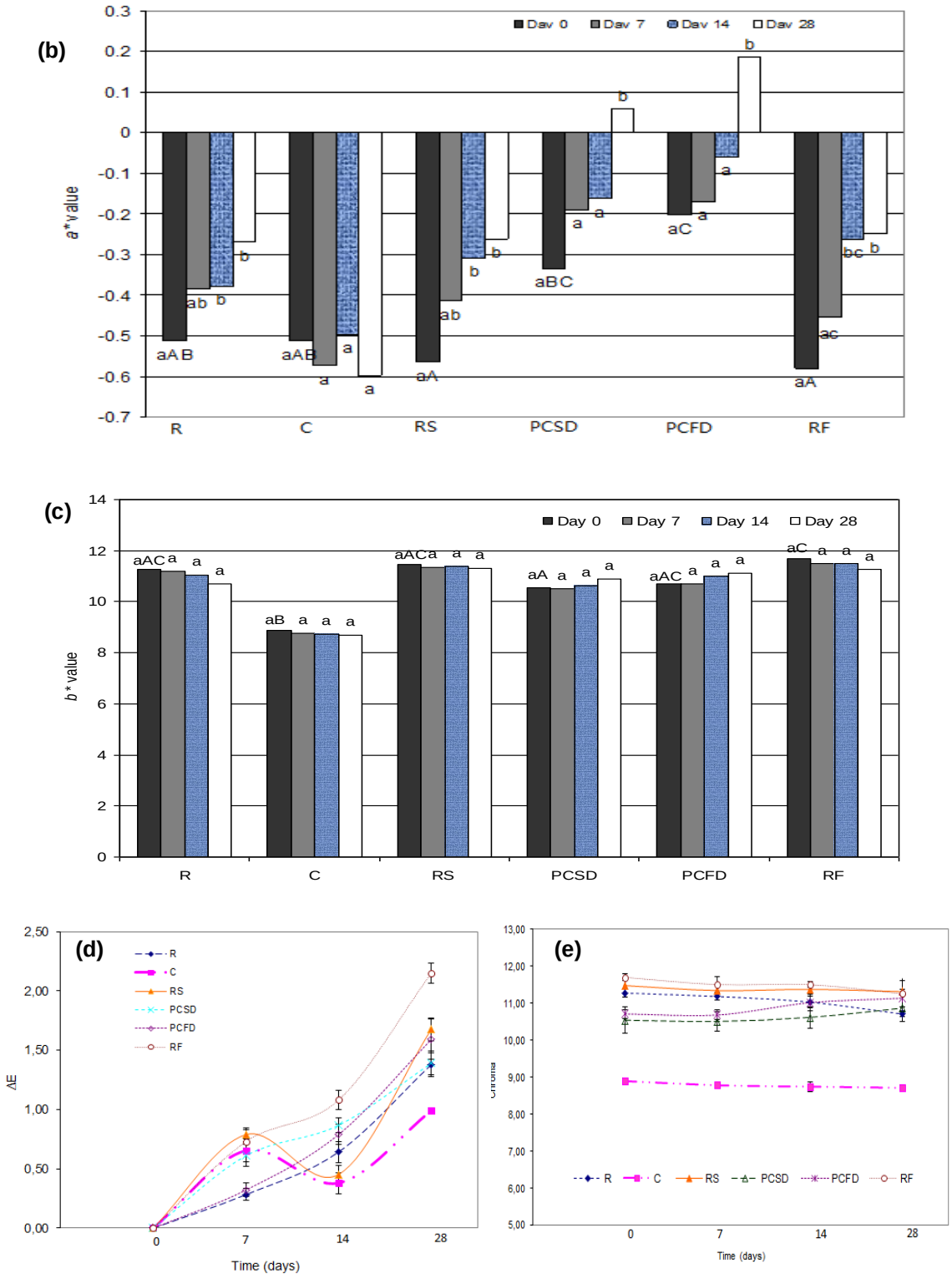


Fig. 5.4 Variation in (a) *L**, (b) *a**, (c) *b** (d) Δ*E* and (e) Chroma of salad dressings supplemented with 7% (w/v) raw (R) lentil flour, roasted lentil flour (RF), roasted lentil seeds (ground) (RS), pre-cooked spray-dried (PCSD), pre-cooked freeze-dried (PCFD) lentil flour, and control (C) on storage days 0, 7, 14 and 28 at 4 ℃.

5.3.3 Physical Stability

The initial mean values of percent backscattering (*BS*%) along the entire tube (BS_{av0}, i.e., from *BS* profile at t=0) for the control, the dressings supplemented with raw flour, roasted flour, roasted seeds, pre-cooked-freeze-dried lentils, and pre-cooked-spray-dried lentils were 82.80%, 82.52%, 81.13%, 81.22%, 79.33%, and 79.11%, respectively (figures not shown).

Physical stability is an important property of emulsion products. Stability of emulsions is usually attained by preventing droplet coalescence, flocculation, creaming and sedimentation. The stability of salad dressings is influenced by, amongst other things, their interfacial composition, emulsion droplet size, and/or continuous phase rheology (Zhang et al. 2008). All the samples had a similar *BS*% profile and no significant differences were observed. In general, all the salad dressings were quite stable with slightly negative or positive ΔBS values recorded along the entire tube, except for dressings with roasted seeds, which showed a decreasing trend in the mean *BS*% values at the bottom, middle and top zones of the tube over 28 days (figures not shown).

5.3.4 Sensory Evaluation

Statistical differences were observed in the intensity scores for most of the attributes studied, except for legume flavor and vinegar. The sensory profiling graph for all samples, generated by plotting the average values for each scale, is shown in Fig. 5.5. Each attribute had an intensity score ranging from slight to moderate, which is in agreement with other work reported by Meilgaard et al. (2007) for national brand dressings. The highest score for overall flavor was obtained for salad dressings supplemented with roasted flour, which was statistically different (P<0.05) from the scores for dressings supplemented with roasted seeds and pre-cooked-spray-dried lentils, but similar to the values obtained for dressings supplemented with raw flour. The legume flavor was perceived to be similar among the four lentil-supplemented dressing samples, and ranged from mild to moderate. No statistical difference was found for intensity of vinegar among the samples, which was rated as lower than mild. The roasted flour-supplemented dressings had the highest rating (P<0.05) for acidity, which

was similar to the observation for overall flavor, indicating that acidity had a large influence on the overall flavor of the dressing products.

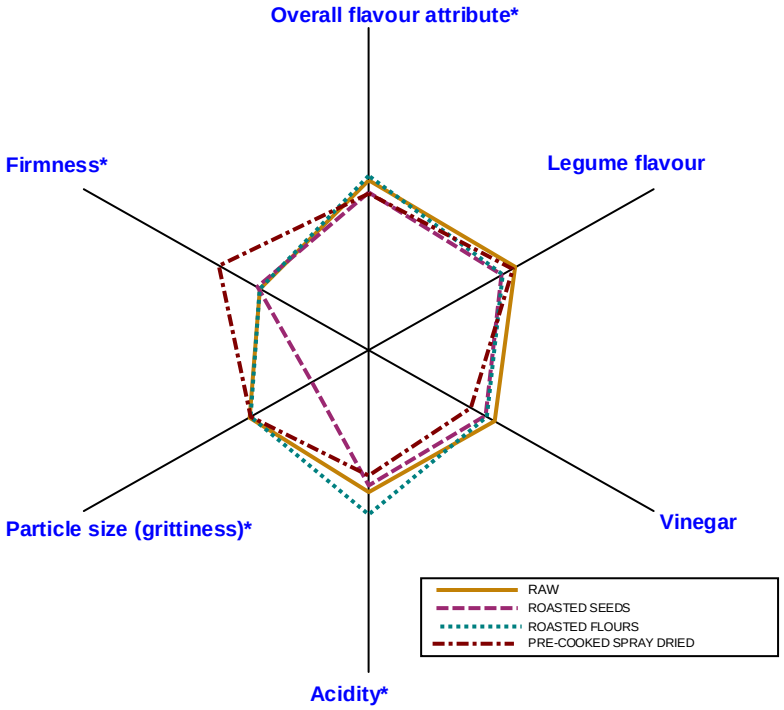
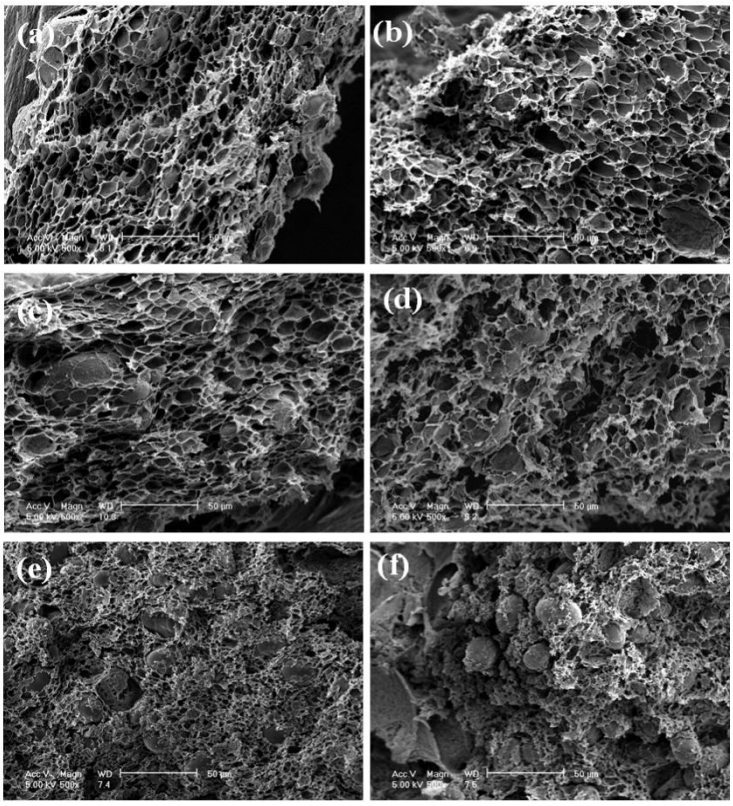


Fig. 5.5 Sensory profiling graph of salad dressings supplemented with lentils of raw (R) flour, roasted seeds (RS), roasted flours (RF), and pre-cooked spray dried (PCSD) samples.
*indicates an attribute that significantly discriminates at least one of the samples. The attributes are positioned as the spokes of a wheel around a center (zero, or not detected) point, with the spokes representing attribute intensity scale and with higher values (more intense) radiating outward.

In terms of particle size (grittiness), salad dressing supplemented with roasted seeds received a significantly lower score (more grittiness) (p<0.0001); this type of dressing was more powdery than the others. The dressing supplemented with pre-cooked-spray-dried lentils obtained a significantly higher score for firmness. This was consistent with the results of the rheological tests, in which the dressings supplemented with pre-cooked-spray-dried lentils exhibited higher values for consistency coefficient (m), apparent viscosity (η_{ap}), storage modulus (G′), plateau modulus (G_N^0), and recoverable strain ($Q(t)\%$) (Table 5.1, 5.2, Fig. 5.2b).

5.3.5 Scanning Electron Microscopy

The scanning electron microscope (SEM) images for selected dressings on different storage days are presented in Fig. 5.6 a-l. A highly packed three-dimensional network was observed for all samples. Void spaces with varying shapes in the SEM images represent the aqueous phase of the emulsion (this results from the dehydration step prior to imaging). In general, the void spaces in the control sample (Fig. 5.6a-d) were generally larger than those in the supplemented dressings, indicating that lentil flour (regardless of the type of processing) played an important role in increasing crosslinking in the emulsion system. Starch granules from the lentil flours, embedded in the network structure, could be seen in dressings supplemented with raw flour, roasted flour, and roasted seeds. These three samples exhibited very similar micrographs to the one shown for the dressing supplemented with raw lentil flours in Fig. 5.6 (e-h). No major differences were observed between the micrographs of the dressings supplemented with pre-cooked spray-dried and pre-cooked freeze-dried lentils, thus, only the micrographs of the latter are shown (Fig. 5.6 (i-l)).



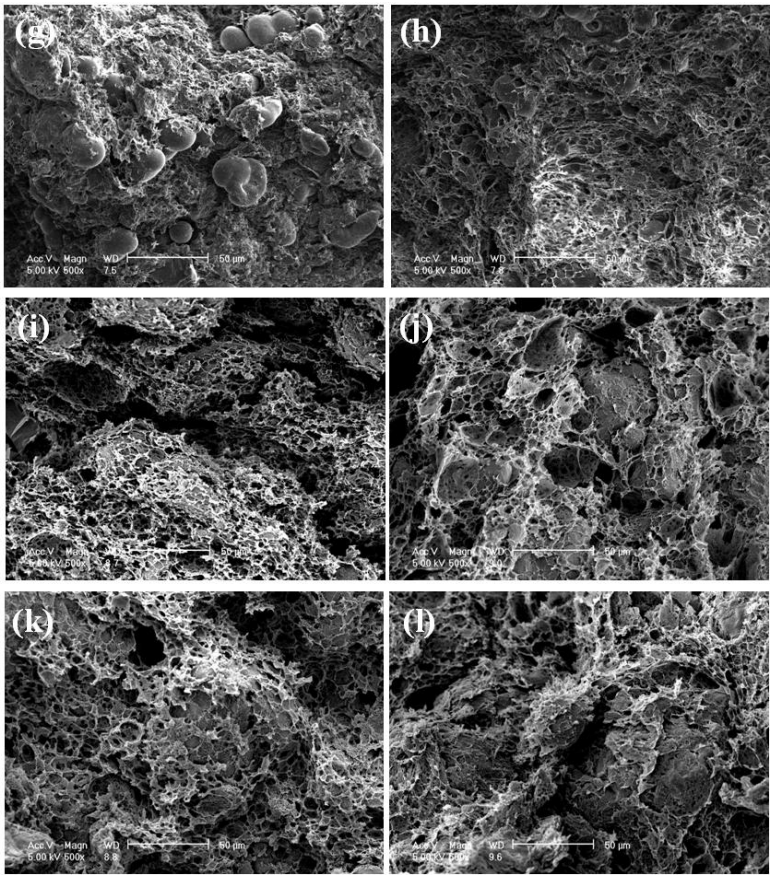


Fig. 5.6 Scanning electron micrograph of (a-d) control (without lentil flour supplementation) on days 0, 7, 14, and 28, respectively; (e-h) salad dressings supplemented with raw lentil flours on days 0, 7, 14, and 28, respectively; (i-l) salad dressings supplemented with pre-cooked freeze-dried lentils on days 0, 7, 14, and 28, respectively.

Dressings supplemented with pre-cooked lentils, however, had very different microstructure compared to the other samples. No starch granules were observed in these micrographs, which indicates that pre-gelatinization of starch and crosslinking with protein likely occurred during the pre-boiling process as reported previously (Ma et al., 2011). The predominant viscoelastic properties observed for the dressings made with pre-cooked spray/freeze-dried lentils may, thus, be attributable to the formation of a more compact network resulting from the interactions between the lentil flour components (e.g., pre-gelatinized starch) and other ingredients in the emulsion system which together bind to neighboring oil droplets. Franco, et al. (1998) have also reported that heating of protein solutions prior to addition of an oil phase increases crosslinking among the

protein molecules. In contrast to the pre-cooked samples, the raw flour, roasted flour/seeds did not undergo gelatinization, hence, their less compact structure as observed in the SEM images.

5.4. Conclusions

This study demonstrates the high potential for using lentil flours as an ingredient in salad dressings. In particular, lentil flours subjected to different thermal treatments hold good promise for the production of value-added salad dressings which could benefit from the inherent nutritional value of lentils. Furthermore, the results showed a thickening effect associated with lentil flour supplementation. Pre-boiling of lentil flour significantly increased this thickening effect. Lentil supplementation significantly increased the yellowness hue and the total color intensity of the salad dressings and all of the dressings maintained acceptable consistency and stability over 28 days of storage. In addition, the quantitative descriptive sensory analysis scores showed promising results. Overall, the data presented is of interest as it provides useful information for potential commercial and industrial application of pulses, especially thermal treated pulse flours, as techno-functional ingredients in salad dressings.

Connecting Statement to Chapter 6

In Chapter 5, the results obtained showed that lentil flour supplementation significantly (P<0.05) increased the rheological parameters, including the consistency coefficient, apparent viscosity, and plateau modulus, which suggested that the addition of pulse flour had a thickening effect. In addition, the pre-boiling process significantly (P<0.05) increased this thickening effect, indicating that the thermally processed pulse flours may have good potential for use as value-added ingredients in salad dressing products. The work described in this chapter involves the evaluation of the quantitative and interactive effects of factors (i.e., pulse flour concentration, 3.5%–10.5%; egg yolk concentration, 3%–7%; and oil concentration, 20%–50%) on the physical properties of salad dressings supplemented with raw flours prepared from dehulled green lentil, green lentil with hulls, dehulled Desi chickpea, and dehulled yellow pea. Response surface methodology (RSM) was chosen in this study as a statistical technique for modelling the responses of interest. The physical stability, scanning electron microscope observations, and consumer sensory tests were examined for selected dressing samples. This chapter addresses the fourth objective discussed in the "objective of study" section of Chapter 1. The results of this study are presented as follows:

Ma, Z., Boye, J. I., Swallow K., Montpetit, D., Malcolmson L., Simpson, B. K., Prasher, S. O., Techno-functional characterization of pulse flour supplemented salad dressing emulsions. *Journal of Food Engineering*, (submitted).

Chapter 6. Techno-Functional Characterization of Pulse Flour Supplemented Salad Dressing Emulsions

Abstract

A three-factor face-centered central composite design (CCD) was used to determine the effect of pulse flour concentration (3.5%, 7%, 10.5% w/w), egg yolk concentration (3%, 5%, 7% w/w) and oil concentration (20%, 35%, 50% w/w) on the rheological and color characteristics of salad dressings supplemented with pulse flours. The effect of pulse flours on consistency coefficient *m*, plateau modulus G_N^0, recoverable strain *Q(t)* and color values varied depending on the concentration used within the experimental range studied. Scanning electron microscopy showed that dressings with lower oil and egg yolk contents had a less densely packed network compared with dressings with higher oil and egg yolk contents. Sensory results were most promising for salad dressings supplemented with the whole green lentil, yellow pea with low flour content, and chickpea with high oil content. This study should be useful for designing novel types of salad dressings to meet market requirements as well as helping to increase pulse consumption.

6.1 Introduction

Pulses, the edible seeds of legumes, including dry beans, peas, chickpeas and lentils, are emerging ingredients in North America. Due to their unique nutritional and functional properties, pulses are increasingly gaining recognition as potential ingredients that can boost the health attributes of different food products. Several studies have focused on developing new products, such as low-fat meatballs (Serdaroglu et al. 2005), extruded snacks (Lazou & Krokida, 2009) and macaroni (Rasmay et al. 2001), by using pulse flours and pulse fractions (protein isolates, starch and fiber fractions) as ingredients.

Salad dressing is an important category of semi-solid foods, containing egg yolk, thickening agent, salt, sugar, vinegar and flavoring materials. Incorporation of pulse flours into salad dressing products is a novel idea that offers the opportunity to expand the use of pulse ingredients, while enhancing the nutritional value of foods. There are many reports in the literature on salad dressing

emulsions prepared with different protein sources and various emulsifiers, such as whey protein (De Cássia da Fonseca et al. 2009), lupin seed protein isolate (Papalamprou et al. 2006), soybean protein isolate (Diftis et al. 2005), binary blends of egg yolk and different types of amphiphilic molecules, including pea protein, sodium caseinate, Tween 20 and sucrose distearate (Riscardo et al. 2003). So far, however none of these studies have looked at the development of salad dressing products using pulse flours.

The rheological behavior of an emulsion is a critical characteristic that must be studied as it is closely related to the sensory attributes, quality, and processing properties of a food product. Most importantly, it provides fundamental insights into the structural organization and interactions of the components within the emulsion (McClements 2005c). Rheological behavior is basically controlled by the nature of the interactions between emulsifiers, stabilizer and oil droplets, as well as by the phase behavior between the continuous phase and the emulsifiers. In addition, taste, nutrition and overall appearance are important features that consumers look for in a product and which must be studied in the development of products with new ingredients.

In this study the impact of pulse flour, egg yolk and oil concentrations on the rheological behavior and color characteristics of pulse supplemented salad dressings was studied using response surface methodology (RSM). Additionally, the effect of storage on rheological, color characteristics, microstructure and physical stability was investigated. Sensory evaluation was further conducted on five selected dressings to determine their acceptability.

6.2 Materials and Methods

6.2.1 Materials

Spray-dried egg yolk powder was obtained from Canadian Inovatech Inc. (Winnipeg, Manitoba, Canada) and xanthan gum was kindly provided by Tic Gums (Belcamp, MD., USA). Green lentil (with or without hulls) flours were obtained from the Canadian International Grains Institute (Winnipeg, MB, Canada). Dehulled Desi chickpea and dehulled yellow pea flours (commercial products) were provided by Diefenbaker Seed Processors Ltd. (Elbow, SK,

Canada) and Parrheim Foods Inc. (Saskatoon, SK, Canada), respectively. Other ingredients used in the salad dressing preparations were purchased from local supermarkets. All chemicals used were of analytical grade.

6.2.2 Sample Preparation

The pulse supplemented salad dressings were prepared using different concentrations of the following three major components: pulse flour (3.5−10.5%, w/w), canola oil (20−50%, w/w), and egg yolk (3−7%, w/w) (Table 6.1). The other ingredients, expressed as a percentage (w/w), were as follows: vinegar (5% (w/v) acetic acid) 7.0%; lemon juice 2.5%; salt 1.0%; sugar 3.5%; and xanthan gum 0.25%.

The dressings were prepared as follows: first, the xanthan gum and sugar were dispersed in water and stored overnight to ensure complete hydration. All other ingredients, except oil, were added and mixed homogeneously and 0.02 wt% potassium sorbate added as an antimicrobial agent. Oil was added next, and emulsification was achieved using an Ultra-Turrax homogenizer (Model T25-S1, Janke & Kunkel, Ika-Labortechnik, Staufen, Germany) with the S25-18G dispersing tool at 13,500 rpm for 3 min. The emulsions were pasteurized at 71.7 ℃ for 20 s and then stored in tightly capped bottles for 28 days at 4 ℃ until further analysis.

6.2.3 Effect of Storage

The effect of storage on rheological properties, color, physical stability, and microstructure was evaluated on selected salad dressings as follows: formulation in run 3 and run 17 (Table 6.1) for salad dressing supplemented with dehulled green lentil (DGL) flour; run 8 and 16 for dressings supplemented with green lentil with-hulled (GLWH) flour; run 5 and 8 for dressings supplemented with dehulled Desi chickpea (DDC) flour; and run 2 and 16 for salad dressings supplemented with dehulled yellow pea (DYP) flour. These dressings were selected based on their techno-functional and sensory properties which showed them to have greater potential and marketability. This evaluation was done on days 0, 14, and 28 of storage. All studies were done in triplicate.

6.2.4 Rheological Measurements

Rheological measurements were performed with an AR 1000 rheometer (TA Instrument, New Castle, DE, USA) equipped with a plate/cone system. Steady state flow test, dynamic oscillatory, and creep and recovery tests were conducted using a stainless steel parallel plate (4 cm diameter). The gap setting was 1 mm. One tablespoon of sample was placed in the centre of the circular plate, and excess sample was removed from the edges of the plate. The linear viscoelastic (LVE) range was determined by performing amplitude sweeps at 1 Hz frequency over a strain range from 0.01% to 1000%. Samples were covered with an angular ring/disc during analysis to prevent hydration.

Steady state flow test was performed at increasing shear rates (0.02−300 s^{-1}), and experimental flow curves (log (τ) *vs*. log (γ)) were fitted to the power law model (Eq. 1):

$$\eta = m\gamma^{(n-1)} \qquad \text{(Eq. 1)}$$

where n is the flow behavior index (dimensionless), with $n<1$ for a shear-thinning fluid and $n=1$ for a Newtonian fluid, η is the shear viscosity (Pa.s), m is the consistency coefficient (represents the inherent viscosity, $Pa.s^n$), τ is the shear stress (Pa), and γ is the shear rate (s^{-1}).

Dynamic oscillation test was performed at 0.1% strain with an angular frequency ranging from 0.1 to 100 rad/s. Storage modulus (G′, Pa) and loss modulus (G″, Pa) *vs.* angular frequency (rad/s) were measured for all samples. The plateau modulus, G_N^0 (Pa), defined as the extrapolation of the entanglement contribution to G′ at high frequencies (Baurngaertel et al. 1992), was estimated by using an approximation procedure. This corresponds to the value of G′ at a minimum in tan δ (G″/G′) evident in the plot of G′ versus frequency (Wu, 1989):

$$G_N^0 = [G^{'}]_{\tan\delta \to \min imum} \qquad \text{(Eq. 2)}$$

This parameter can be considered a measure of the intensity of the entangled network that develops between the adsorbed and non-adsorbed protein molecules (Franco et al. 1997). It is related to the formation of a structural network in o/w emulsions due to an extensive flocculation process (Moros et al. 2002).

A creep and recovery test was carried out by applying 0.5 Pa stress to the samples for 600 s, after which the stress was removed and the strain was recorded as a function of time (600 s). The sample was pre-sheared at a shear strain of 300 s^{-1} for 2 min before each test. Strain *vs.* time in seconds was recorded during the test. The recoverable strain (*Q(t)*), a quantity used to estimate the extent of strain recovery and the elasticity of the material (Zhang et al. 2008), was calculated from the recovery zone (Eq. 3).:

$$Q(t) = \Delta(\chi_{c(t)}) = \frac{\chi_{(600)} - \chi_{(1200)}}{\chi_{(600)}} \quad \text{(Eq. 3)}$$

where $\chi_{(600)}$ and $\chi_{(1200)}$ are the strains at the equilibrium of the creep and recovery zones, respectively.

6.2.5 Color Measurements

The color of the salad dressings was measured with the *L**, *a**, *b** tristimulus system using a Minolta CM-503c spectrophotometer (Minolta Co. Ltd., Osaka, Japan). In this color system, the *L** is a measure of lightness or darkness (0=black and 100=white), and *a** and *b** are the color opponent dimensions, in which *a** is a measure of redness (+ve) to greenness (−ve), and *b** is a measure of yellowness (+ve) to blueness (−ve). A fixed amount of salad dressing was poured into the measuring cup, which was then surrounded with a black paper strip prior to measurement.

6.2.6 Physical Stability

The stability of the selected pulse supplemented salad dressings was determined by light scattering measurements using a vertical scan analyzer (QuickScan; Beckman Coulter, Fullerton, CA, USA). The principle and features of this method are described elsewhere (Pan et al. 2002). The acquisition options were set at 0−60 mm. By repeating the scan of a sample at different time intervals, the backscattering (BS) profile of every 40 μm was acquired as a function of sample height (50 mm) and time.

6.2.7 Scanning Electron Microscopy

The salad dressings selected for the storage studies were encapsulated in 2% agar, cut into 2−3 mm cubes, and immersed in 2.5% glutaraldehyde for 2 h.

Subsequently, they were transferred to 0.1 M sodium cacodylate buffer solution (pH 7.1) and stored at 4 ℃. Three samples of each dressing were encapsulated. Samples were dehydrated in a graded ethanol series with concentrations ranging from 30% to 100%; and they were critical point dried with CO_2 in a critical point dryer (SPI, USA). Dry sections were fractured and fragments were mounted on aluminum stubs and coated with gold (10 nm). Observations were made under a scanning electron microscope (model S-3000N, Hitachi, Tokyo, Japan) at 5 kV.

6.2.8 Sensory Evaluation

A consumer acceptability test with 80 untrained panelists (43 male and 37 female) aged from 18 to 65 years was conducted at the Consumer Product Testing Centre in Alberta (Canada). Five salad dressings were prepared using different varieties and concentrations of pulse flours. Prior to the sensory evaluation session, dressings (96 g) were mixed with lettuce (180 g). A 30-g portion of salad was placed on a 15-cm white foam plate identified with a 3-digit random number code. It was served at room temperature with water and unsalted crackers to cleanse the palate between samples. Salad dressings were evaluated on the basis of aroma, appearance on lettuce, color, flavor, texture and overall acceptability using a 9-point hedonic scale (9 = like extremely and 1 = dislike extremely). In addition, flavor intensity and consistency were scored using a 5-point just-about-right (JAR) scale (i.e., from "much too weak" to "much too intense" for flavor intensity, and from "much too runny" to "much too thick" for consistency).

6.2.9 Statistical Analysis

A face-centered central composite design (CCD) with three independent variables (pulse flour, x_1; egg yolk, x_2; oil concentration, x_3) at three coded levels (-1, 0, +1) and five replicates at the center point was programmed as shown in Table 6.1. A total of 19×4 experiments were conducted for the four salad dressings supplemented with the following types of flour: dehulled green lentil (DGL); green lentil with hulls (GLWH); dehulled Desi chickpea (DDC); and dehulled yellow pea (DYP) (i.e., a complete design consisted of 19 experimental runs for each pulse supplemented salad dressing). A commercial statistical package, Design-Expert, version 7 (Stat-Ease Inc., Minneapolis, MN, USA), was

used for designing, response surface plotting and optimization. Response surface methodology (RSM) was used to estimate the effect of the three independent variables on the response variables, specifically, consistency coefficient *m* (Y_1), plateau modulus G_N^0 (Y_2), recoverable strain *Q(t)* (Y_3), *L** value (Y_4), *a** value (Y_5), and *b** value (Y_6). Mathematical models (Eq. 4) of the relationship between a dependent response (*Y*) and the independent variables (*x*) were generated. The basic model equation (Eq. 4) used to fit the data was:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii}{}^2 x^2{}_{ii} + \sum \sum \beta_{ij} x_i x_j + \varepsilon \quad \text{(Eq. 4)}$$

where *Y* is the predicted response, β_0 is the constant, β_i , β_{ii} , β_{ij} are the regression coefficients, and x_i, x_j are the levels of the independent variables (Myers et al. 2009). The significant terms in the model were evaluated by one-way analysis of variance (ANOVA). The adequacy of the models was checked by removing the non-significant terms ($p > 0.05$) using a step-wise "backward" multiple reduction algorithm. The R^2 and adjusted R^2 values, together with the Adeq precision (i.e., signal-to-noise ratio that measures the ratio of the range of variation in the predicted response to an estimate of the standard error of the predictions), were computed. The models were expressed as three-dimensional surface plots to help in visualizing the interaction effect of main ingredients on the responses studied. The plots were generated by keeping one independent variable constant at the center point and varying the other two variables within the experimental range.

The individual optimizations were carried out within the range of experimental conditions to determine the optimal levels of the three independent variables leading to the desired properties. Four types of commercial dressings were tested; the resulting averaged rheological parameters were used as target values for the optimization procedure. Maximum lightness (*L** value) was selected as the optimum condition.

PRISM software, version 3.02 (GraphPad Software, Inc., San Diego, CA, USA), was used for statistical analysis for storage effects by conducting Tukey's multiple comparison tests at the 5% significance level. The SAS (v. 9.1.3, SAS

Institute Inc., Cary, NC) software (Tukey's test) was used for statistical analysis of the sensory results.

6.3 Results and Discussion

6.3.1 Model Fitting

Fig. 6.1 shows the visual appearance of the GLWH flour supplemented salad dressings prepared for the 19 runs (Table 6.1). Regression models for samples with adjusted determination coefficients (R^2) of at least 0.80 or greater (Joglekar & May 1987) as presented in Table 6.2, are discussed below. These selected responses had statistically significant predicted models ($p < 0.05$) with non-significant lack of fit ($p > 0.05$) (i.e., the R^2 and adjusted R^2 values were fairly high, ensuring a satisfactory fit of the response surface models to the actual data).

Table 6.1 Face-centered central composite experimental design with coded and actual experimental data for formulating the pulse supplemented salad dressings.

Run no.	Pulse flour con. (%, w/w)	Egg yolk con. (%, w/w)	Oil con. (%, w/w)
1	10.5 (+1)	3 (-1)	20 (-1)
2	7 (0)	5 (0)	35 (0)
3	3.5 (-1)	3 (-1)	20 (-1)
4	3.5 (-1)	7 (+1)	20 (-1)
5	10.5 (+1)	7 (+1)	20 (-1)
6	10.5 (+1)	7 (+1)	50 (+1)
7	7 (0)	7 (+1)	35 (0)
8	3.5 (-1)	3 (-1)	50 (+1)
9	7 (0)	5 (0)	35 (0)
10	7 (0)	5 (0)	20 (-1)
11	7 (0)	5 (0)	50 (+1)
12	7 (0)	5 (0)	35 (0)
13	7 (0)	5 (0)	35 (0)
14	10.5 (+1)	3 (-1)	50 (+1)
15	7 (0)	5 (0)	35 (0)
16	7 (0)	3 (-1)	35 (0)
17	3.5 (-1)	5 (0)	35 (0)
18	10.5 (+1)	5 (0)	35 (0)
19	3.5 (-1)	7 (+1)	50 (+1)

Code 0 is for center point of the parameter range studied; 1 for factorial points.

6.3.2 Flow Behavior

6.3.2.1 Salad dressing supplemented with dehulled green lentil (DGL) flour

For DGL flour supplemented salad dressing, a second-order polynomial model was fitted to the *m* value, and analysis of variance was performed (Table 6.2). All the linear terms of the variables had significant effects ($p < 0.001$).

Similarly, all the quadratic terms, except egg yolk (x_2), and the interaction of DGL flour (x_1) with oil (x_3) content, had significant effects ($p < 0.05$) (Table 6.2). The significant quadratic and interaction effects of the independent variables on *m* values showed that the variations in viscous nature can be described as a nonlinear function of the main components studied. The multiple regression model is given by the following equation (Eq. 5)

$$Y_1 = 33.35 - 11.02x_1 + 4.69x_2 - 2.93x_3 + 0.798x_1^2 + 0.055x_3^2 + 0.19x_1x_3 \quad \text{(Eq. 5)}$$

All the response graphics showed similar trends with *m* value increasing with DGL flour (x_1), egg yolk (x_2), and oil content (x_3) (Figs. 6.2a, b, c). In Fig. 6.2a, b, the effect of oil content was more pronounced than that of DGL flour and egg yolk. Similarly, the effect of DGL flour was greater than that of egg yolk (Fig. 6.2c). The maximum *m* value was obtained at the highest concentrations of either one of the two independent variables studied. This suggests that the combination of high levels of the main components (i.e., DGL flour, oil, and egg yolk) resulted in a salad dressing with the most pronounced viscous characteristics.

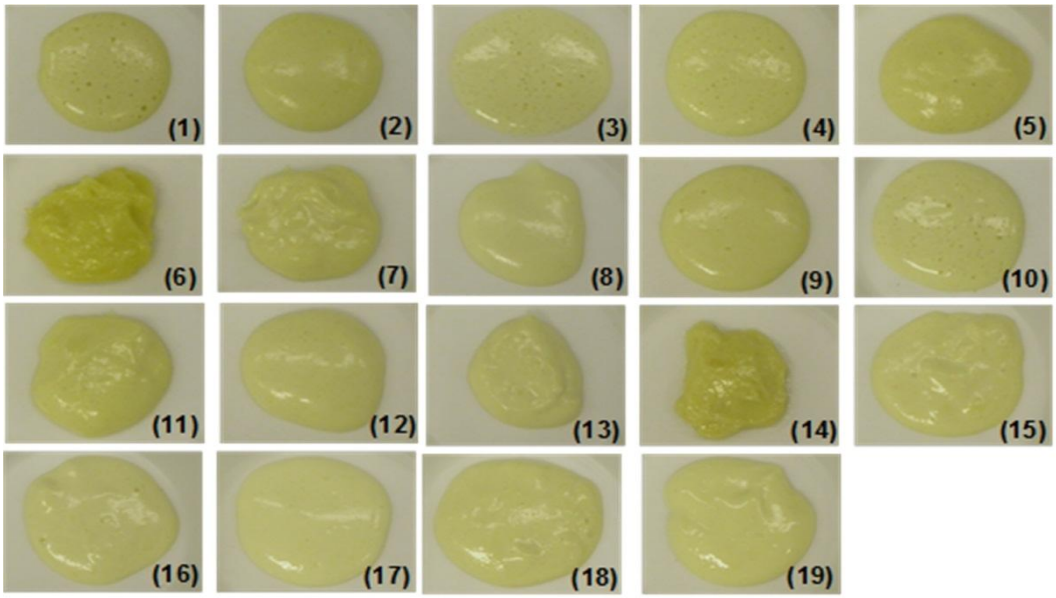


Fig. 6.1 Visual appearance of salad dressing samples supplemented with green lentils with hulls from Run 1 to Run 19 based on the face-centered central composite design. The specific formulation of the different compositions for each run is shown in Table 6.1

During homogenization the low density lipoproteins from egg yolk adsorbs to the oil droplets and interlinks with other lipoproteins from neighboring droplets

(Kiosseoglou & Sherman 1983). It can, thus, be assumed that the higher the egg yolk content, the more compact the network structure, which may explain the more pronounced viscous structure observed. It is also reasonable to expect that the total number of rigid particles will increase with the addition of increased levels of DGL flours, which could enhance the resistance to flow and result in increased *m* values as observed. Moreover, the oil content, expressed as the dispersed phase volume fraction ϕ, is one of the factors with the greatest influence on emulsion viscosity, as suggested by the equations defined by McClements & Demetriades (1998) for various emulsion types. The oil effect findings in this study are in good agreement with those reported by Mirhosseini et al. (2009), Dluzewska et al. (2006) and Moros et al. (2002) who showed that viscosity increases with an increase in the concentration of the dispersed phase.

6.3.2.2 Salad dressing supplemented with green lentil with hulls (GLWH) flour

A significant ($p < 0.0001$) linear model was fitted to the consistency coefficient (*m*) of salad dressings supplemented with GLWH flour (Eq. 6),

$$Y = -50.78 - 1.51x_1 + 8.54x_2 + 1.54x_3 + 0.16x_1x_3 - 0.25x_2x_3 \quad \text{(Eq. 6)}$$

As shown in Table 6.2, all the linear terms of the variables had significant effects; the interactions of GLWH flour (x_1) with oil (x_3), and egg yolk (x_2) with oil (x_3) also had significant effects on *m* values. Thus, a salad dressing with higher GLWH flour (x_1), egg yolk (x_2) and oil (x_3) contents was predicted to be the most viscous (Fig. 6.2d, e, f). The independent variables with the most significant ($p < 0.05$) effect on *m* value were oil, followed by GLWH flour and egg yolk. This is in agreement with the findings for DGL supplemented salad dressings, as discussed above. In addition, it was observed that the effect of GLWH flour on *m* values was more pronounced at higher oil contents; similarly the effect of oil on *m* value was more pronounced at higher GLWH flour contents (Fig. 6.2d).

The main difference between the DGL and GLWH supplemented dressings was that the change in *m* value as a function of the three independent variables was linear for dressings with GLWH but nonlinear for DGL supplemented dressings. This might be due to differences in the fiber content of the dressings

(i.e., due to the presence of lentil hulls in the GLWH sample), which may have altered the rheological properties.

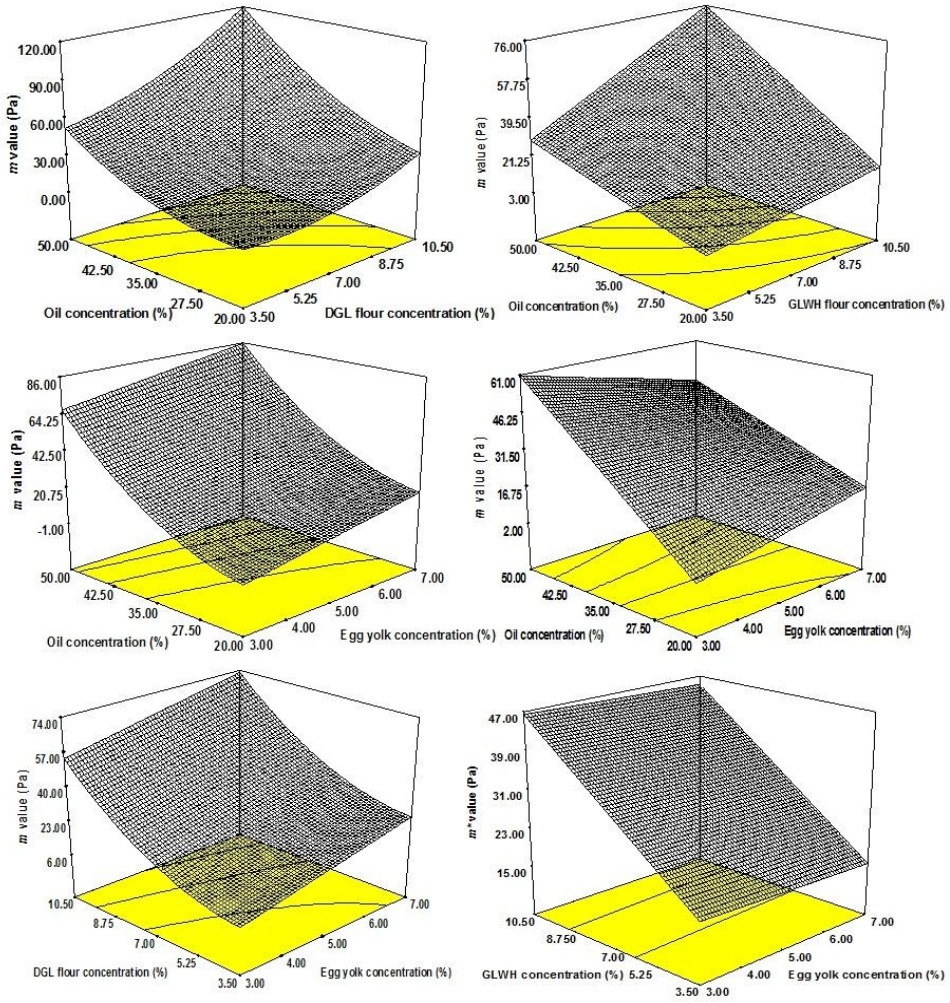


Fig. 6.2 (a, b, c): Response surface for the effect of dehulled green lentil (DGL) flour (x_1), egg yolk (x_2) and oil content (x_3) on the consistency coefficient (m); **(e, f, g):** Response surface for the effect of green lentil with hulls (GLWH) flour (x_1), egg yolk (x_2) and oil content (x_3) on the consistency coefficient (m). The graphs show the interaction effect of two independent variables by holding the other two variables at their central points, specifically 35 wt% for oil, 5 wt% for egg yolk, and 7 wt% for DGL flour.

The optimization studies showed that concentrations of 7.09%, 6.80% and 32.18% (w/w) of GLWH flour (x_1), egg yolk (x_2) and oil (x_3), respectively, gave an optimum m value using the m value for the commercial dressing (m=28.095 Pa) as target value. The salad dressing that had GLWH flour = 10.50%, egg yolk = 3.0% and oil = 50.0% (w/w) gave the maximum m value (83.97 Pa). The minimum value of m (1.79 Pa) was achieved using 4.25% GLWH flour, 3.97% egg yolk and 20.38% oil (w/w).

Table 6.2 Analysis of variance for the fit of experimental data to the response surface model for the pulse supplemented salad dressing samples (with relatively high R^2 values)

Source	*m* value (Pa.s^n) for DGL				*a** value for GLWH				*b** value for GLWH				*m* (Pa.s^n) value for GLWH				G^0_N (Pa) value for GLWH				*Q(t)* value for DDC			
	Coefficier	Sum of squares	DF	*p* value	Coefficient	Sum of squares	DF	*p* value	Coefficient	Sum of squares	DF	*p* value	Coefficient	Sum of squares	DF	*p* value	Coefficient	Sum of squares	DF	*p* value	Coefficient	Sum of squares	DF	*p* value
Model	33.35	20590.03	6	<0.0001	-1.54	0.36	4	<0.0001	6.8	29.02	4	<0.0001	-50.78	7778.25	5	<0.0001	-903.06	617599.50	6	<0.0001	-1.10	23.50	7	<0.0001
Linear																								
P	-11.02	5659.58	1	<0.0001	0.105	0.13	1	0.0003	0.19	21.29	1	<0.0001	-1.51	2184.19	1	<0.0001	177.71	65985.89	1	0.03	-1.19	0.56	1	0.033
E	4.69	877.94	1	0.0008					0.41	6.81	1	<0.0001	8.54	4.25	1	0.8	-7.22	26783.19	1	0.00	0.63	0.11	1	0.31
O	-2.93	11415.53	1	<0.0001	0.059	16	1	0.084	-0.048	0.016	1	0.74	1.54	4536.05	1	<0.0001	17.80	65959.90	1	0.03	0.27	0.44	1	0.055
Quadratic																								
*P*P*	0.798	301.23	1	0.023													-11.37	91908.57	1	0.01	0.08	4.22	1	<0.0001
*E*E*																								
*O*O*	0.055	484.06	1	0.0062	-6.80E-04	0.11	1	0.0008																
Interaction																								
*P*E*																	12.7215	63439.75	1	0.03	0.10	4.20	1	<0.0001
*P*O*	0.19	795.78	1	0.0011	-2.06E-03	45	1	0.0016	6.43E-03	0.91		0.022	0.16	591.68	1	0.0096	-1.68	62473.52	1	0.03	-0.014	4.04	1	<0.0001
*E*O*													-0.25	462.08	1	0.0189					-0.037	9.93	1	<0.0001
		394.96	8				10			1.51	1				9			102305.30	8			0.97	7	
Lack of fit				0.37NS		16								764.073		0.0737NS				0.32 NS				0.0313NS
Pure error		133.20	4			0.0061	4			0.41				71.39	4			30337.35	4			0.07	4	
R^2		0.97				0.81				0.94				0.90				0.82				0.96		
Adj R^2		0.96				0.75				0.92				0.87				0.73				0.93		
Adeq precision		33.25				11.16				25.57				19.39				10.44				20.37		

*Coefficients are based on actual data. *P*, *E*, and *O* are pulse flour concentration, egg yolk concentration, and oil concentration respectively. DGL, GLWH, DDC represent the salad dressing samples supplemented with flours of dehulled green lentil, green lentil with hulls, dehulled Desi chickpea, respectively. **N**S = non-significant. [a] The consistency coefficient (*m*) and the flow behavior index (*n*) were obtained from raw data fitted to a power-law model $\eta = m\gamma^{(n-1)}$ after performing a steady state flow test, where η is the shear viscosity, γ is the shear rate, *n* is the flow behavior index, *n*<1 for a shear-thinning fluid, *n*=1 for a Newtonian fluid, *n*>1 for shear-thickening fluid, *m* is the consistency coefficient which is an indicator of the viscous nature of emulsion samples; [b] G^o_N is the plateau modulus obtained based on a dynamic oscillation test; [c] *Q(t)* refers to the extent of recovery of the dressing samples; it is calculated as the percent difference of the strains measured at the equilibrium of the creep and recovery zones, respectively.

6.3.2.3 Salad dressing supplemented with dehulled yellow pea (DYP) flour

The consistency coefficient (*m*) of salad dressings supplemented with the DYP flour with R^2 of 0.78 was fitted using Eq. (7),

$$Y = 165.76 - 5.148x_1 + 19.88x_2 - 8.26x_3 + 0.56x_2x_3 + 0.095x_3^2 \quad \text{(Eq. 7)}$$

The viscous nature of the DYP flour supplemented salad dressings increased with an increase in DYP flour (x_1), egg yolk (x_2) and oil (x_3) contents. The level of importance of the three independent variables on *m* was similar to the order for the DGL/GLWH supplemented dressings. Synergistic effects resulting in the highest consistency coefficient (*m*) values occurred between DYP flour (x_1) and oil (x_3), and egg yolk (x_2) with oil (x_3). The only difference observed was the effect of oil content, which was less pronounced between 20% and 35% than between 35% and 50%, where the consistency coefficient increased nonlinearly with oil content.

An optimum *m* value (using the value of the commercial dressing as target) was obtained with a combination of 7.01%, 3.95% and 34.19% (w/w), DYP flour (x_1), egg yolk (x_2) and oil (x_3), respectively. The salad dressing containing DYP flour = 10.41%, egg yolk = 6.99% and oil = 49.98% (w/w) was estimated to have maximum *m* value (101.70 Pa), whereas a minimum *m* value (7.83Pa) was estimated for salad dressing containing 3.62% DYP flour, 4.78% egg yolk and 28.66% oil (w/w).

6.3.3 Linear Viscoelasticity

6.3.3.1 Salad dressing supplemented with green lentil with hulls (GLWH) flour

A significant second-order model ($p < 0.0001$) for G_N^0 with relatively high R^2 value (0.8232) was fitted for the GLWH flour supplemented salad dressing and is given by the following equation:

$$Y = -903.06 + 177.71x_1 - 7.22x_2 + 17.20x_3 + 12.72x_1x_3 - 1.68x_1x_3 - 11.37x_1^2 \quad \text{(Eq. 8)}$$

All the linear terms, the quadratic terms for pulse flour (x_1) and the interactions of GLWH flour (x_1) with egg yolk (x_2), and GLWH flour (x_1) with oil (x_3) had significant effects on G_N^0. As shown in Fig. 6.3, G_N^0 increased linearly with an increase in both oil and egg yolk. This is in good agreement with the finding of Moros et al. (2002), Raymundo et al. (2002) and Gallegos et al. (1992) who found that the viscoelasticity (G_N^0 values) of commercial mayonnaise or oil in water (o/w) emulsions increases with

increasing oil, protein, or xanthan gum content. Furthermore as shown in Fig. 6.3b, egg yolk had a greater effect on the G_N^0 value than did oil. In addition, a synergistic effect was observed between egg yolk (x_2) and oil (x_3). The maximum value of G_N^0 was observed at the highest combined concentrations of egg yolk and oil (Fig. 6.3b). Furthermore, the effect of egg yolk concentration on G_N^0 was more pronounced at a higher GLWH flour content (Fig. 6.3a), and the effect of oil concentration was more significant at a lower GLWH flour content (Fig.6.3c). In general, the results indicate that an enhancement of shear sensitivity in the viscoelastic network (i.e., lower G_N^0 values) (Gallegos, et al. 1992; Raymundo et al. 2002) occurred at low oil and egg yolk concentrations. The enhancement of the G_N^0 value with increased egg yolk and oil contents may be attributable to an enhanced emulsion network. The effect of GLWH flour was, however, quite different from that of the other two variables (Fig. 6.3a and 6.3c); G_N^0 first increased nonlinearly with an increase in GLWH flour concentration within the experimental range of 3.5−7.0% (w/w); after the center point, it started to decrease especially at lower egg yolk and higher oil content. In addition, the G_N^0 value tended to remain stable with an increase in GLWH flour content after reaching the center point at higher egg yolk and lower oil concentrations (Fig. 6.3a and 6.3c). The optimization procedure predicted that a salad dressing formulation containing 4.5% (w/w) GLWH flour (x_1), 3.41% (w/w) egg yolk (x_2) and 27.0% (w/w) oil (x_3) would provide the optimum plateau modulus (i.e., using a target value for the commercial mayonnaise, G_N^0 =98.35 Pa). Similarly, the highest G_N^0 would be achieved (639.28 Pa) with salad dressing containing GLWH flour = 8.04% (w/w), egg yolk =7.0% (w/w) and oil = 50.0% (w/w).

6.3.4 Creep and Recovery Behavior

6.3.4.1 Salad dressing supplemented with dehulled Desi chickpea (DDC) flour

Eq. 9 shows the significant second-order mathematical model for the recoverable strain *Q(t)* for salad dressing supplemented with DDC.

$$Y = -1.098 - 1.19x_1 + 0.63x_2 + 0.27x_3 + 0.10x_1x_2 - 0.01x_1x_3 - 0.04x_2x_3 + 0.08x_1^2 \quad \text{(Eq. 9)}$$

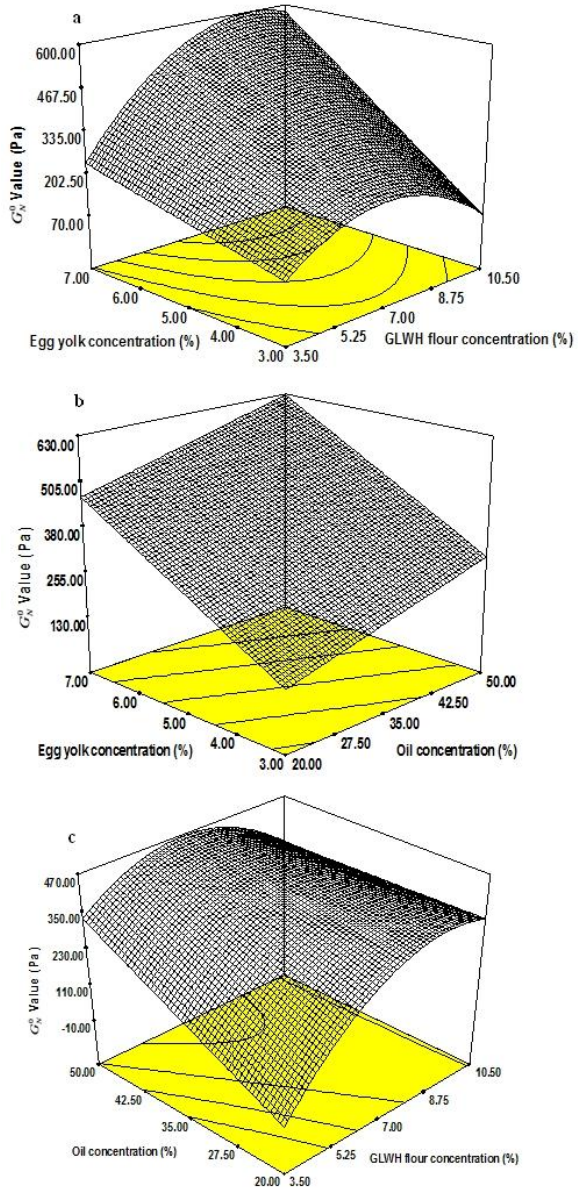


Fig. 6.3 Response surface for the effect of green lentil with hulls (GLWH) flour (x_1), egg yolk (x_2) and oil content (x_3) on the plateau modulus (G_N^0). The graphs show the interaction effect of two independent variables by holding the other two variables at their central points, specifically 35 wt% for oil, 5 wt% for egg yolk, and 7 wt% for whole green lentil flour.

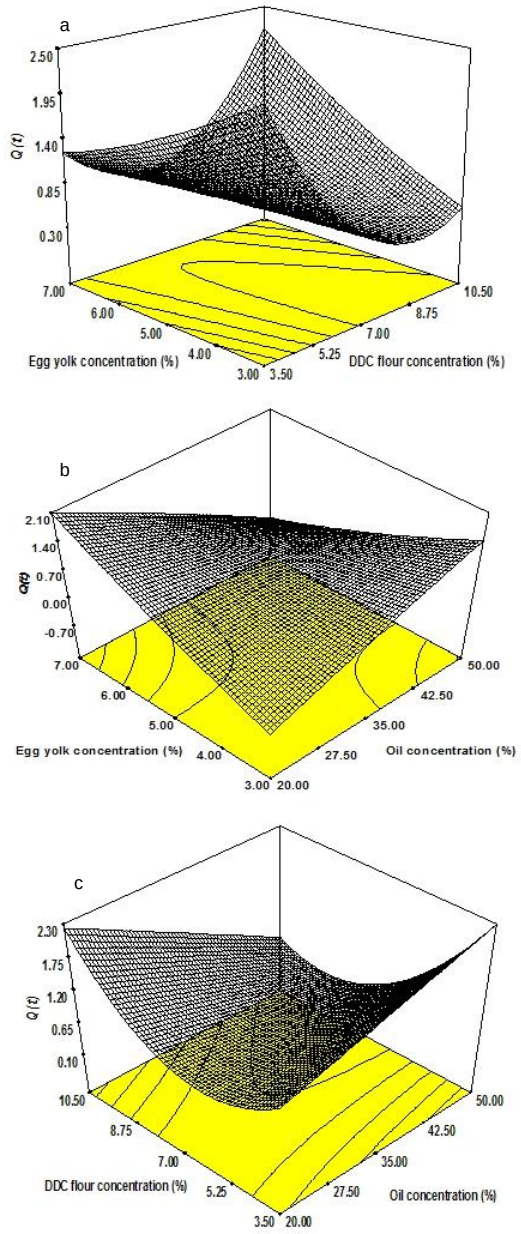


Fig. 6.4 Response surface for the effect of dehulled Desi chickpea (DDC) flour (x_1), egg yolk (x_2) and oil content (x_3) on $Q(t)$. The graphs show the interaction effect of two independent variables by holding the other two variables at their central points, specifically 35 wt% for oil, 5 wt% for egg yolk, and 7 wt% for dehulled Desi chickpea flour.

As shown by the three-dimensional response surface plots in Fig. 6.4 (b, c), *Q(t)* increased with an increase in oil content (x_3) at lower egg yolk (x_2) or DDC flour (x_1) concentrations. However, at higher egg yolk or DDC flour levels, *Q(t)* decreased with an increase in oil content (x_3) (Fig. 4b, c). Thus, higher egg yolk or DDC flour content at higher oil concentration appeared to constrain the formation of a compact elastic network. Dickinson et al. (1995) earlier reported that, optimum viscoelasticity is achieved at about saturation protein monolayer coverage. A similar trend was observed for the effect of egg yolk (x_2): *Q(t)* started to increase with an increase in egg yolk at higher DDC flour or lower oil concentrations (Fig. 6.4a, 6.4b); however, a decreasing trend was observed at lower DDC flour or higher oil contents.

The effect of DDC flour on *Q(t)* was in contrast with the trend observed in the G_N^0 of GLWH supplemented dressings. An initial decrease in *Q(t)* was observed as the concentration of DDC flour increased up to a critical concentration of 7% w/w; a further increase in DDC flour concentration produced a more elastic emulsion (i.e., higher *Q(t)* value) within the experimental range (Fig. 6.4a, c). In addition, the increasing trend is more pronounced at higher yolk and lower oil concentrations. The study predicted that a salad dressing containing 10.46% (w/w) DDC flour, 3.04% (w/w) egg yolk and 20.08% oil (w/w) would yield an optimum recoverable strain using a target value of 85.70% (i.e., value for the commercial dressing). Similarly, a dressing formulation containing 8.03% (w/w) DDC flour, 7.0% (w/w) egg yolk and 50.0% (w/w) oil would provide the highest *Q(t)* value.

6.3.5 Color Characteristics

Fig. 6.5 and 6.6 show the response surface plots of the three color coordinate values (*L*, a*, and b**) of salad dressings supplemented with GLWH as affected by the main components. The regression model generated for *L** ensuring a satisfactory fit of the model to the data is provided below:

$$Y = 53.22 + 0.58x_1 + 6.58x_2 + 0.11x_3 - 0.03x_1x_3 - 0.71x_2^{\,2} \qquad \text{(Eq. 10)}$$

In general, the *L** value decreased with an increase in GLWH flour (x_1) at higher oil content; and it decreased with an increase in oil (x_3) at higher GLWH flour content (Fig. 5a). An increase in both GLWH flour (x_1) and oil content (x_3) produced a linear decrease in the lightness of the dressings, in the experimental range considered. With regard to egg

yolk (x_2), an increase in egg yolk content yielded higher values for lightness; however, after a concentration of 5% (w/w) was reached at the center point, a further increase in egg yolk concentration yielded lower values for lightness (Fig. 5b, 5c). It is likely that the highest lightness point was related to the optimal level of lipoproteins absorbed at the o/w interface in the salad dressing emulsion (i.e., when the absorption reached saturation levels, the droplets tended to flocculate which restrained the formation of smaller oil droplets in the system). As indicated by Chantrapornchai et al. (1999), the "lightness" increased with decreasing droplet size, thus further decreased L^* values was observed at higher egg yolk content. The optimization results indicated that a combination of 3.5% (w/w) GLWH flour, 4.6% (w/w) egg yolk and 20.0% (w/w) oil would result in maximum lightness (70.39).

For a^* values, the increase in GLWH flour content (x_1) in the dressings slightly increased their reddish hue (Fig. 6.6), which might be due to the reddish component presented in GLWH flours. However this effect was not observed at higher oil contents. The a^* values increased with higher oil content (x_3) initially, but after the center point was reached at 35% w/w, a^* started to decrease (Fig. 6.6). Egg yolk content (x_2) did not affect the a^* values of the GLWH flour supplemented dressings. The regression model generated for a^* value of GLWH flour supplemented dressing is described in Eq. 11.

$$Y = -1.54 + 0.10x_1 + 0.06x_3 - 0.002x_1x_3 - 0.0007x_3^2 \quad \text{(Eq. 11)}$$

The reddish hue (a^* value) of the salad dressings supplemented with DDC flour was fitted as shown in Eq. 12 with an R^2 of 0.9391.

$$Y = -1.97 + 0.12x_1 + 0.13x_2 + 0.064x_3 - 0.0095x_1x_2 - 0.0019x_1x_3 - 0.002x_2x_3 - 0.0007x_3^2$$

(Eq. 12)

The effects of DDC flour and oil on the a^* value were similar to those found for GLWH supplemented samples. In addition, egg yolk was found to decrease the reddish color of the DDC supplemented salad dressings slightly.

The b^* coordinate value (yellowness) increased with increasing GLWH flour content (x_1) and egg yolk content (x_2) (Fig. 6.5d, 6.5e). A synergistic effect of x_1 and x_2 was found, indicating that salad dressings with higher egg yolk and GLWH flour contents had more yellowness (Fig. 6.5e). Oil had only a minor effect on the b^* value. The relationship between the independent variables and the b^* responses of GLWH

supplemented salad dressings was described with the following equation (Eq. 13), respectively.

$$Y = 6.82 + 0.19x_1 + 0.41x_2 - 0.05x_3 + 0.006x_1x_3 \quad \text{(Eq. 13)}$$

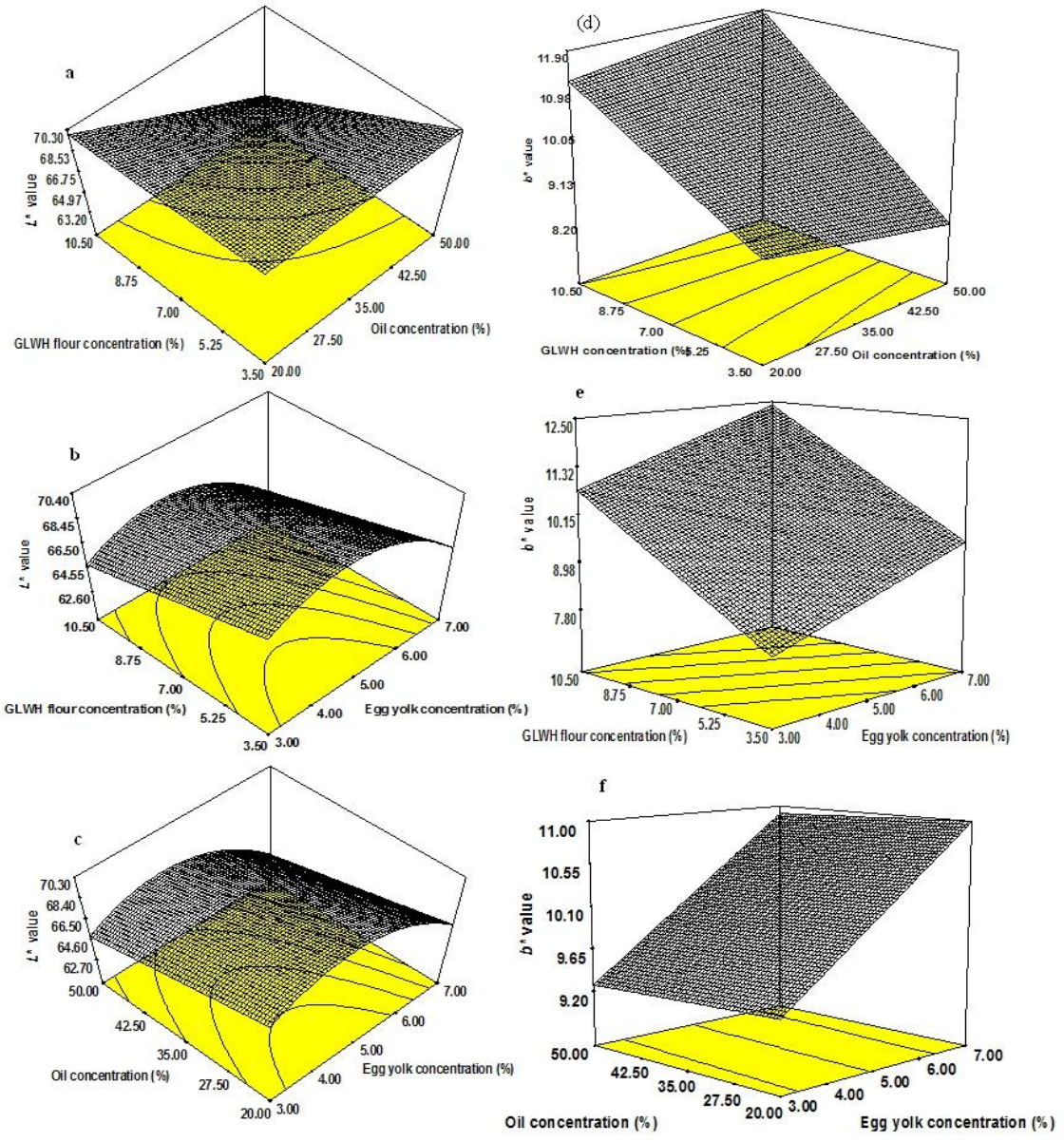


Fig. 6.5 (a, b, c): Response surface for the effect of green lentil with hulls (GLWH) flour (x_1), egg yolk (x_2) and oil content (x_3) on lightness (L^*); **(d, e, f):** Response surface for the effect of green lentil with hull (GLWH) flour (x_1), egg yolk (x_2) and oil content (x_3) on b^* values. The graphs show the interaction effect of two independent variables by holding the other two variables at their central points, specifically 35 wt% for oil, 5 wt% for egg yolk, and 7 wt% for whole green lentil flour. Response surface for the effect of green lentil with hulls (GLWH) flour (x_1), egg yolk (x_2) and oil content (x_3) on lightness (L^*).

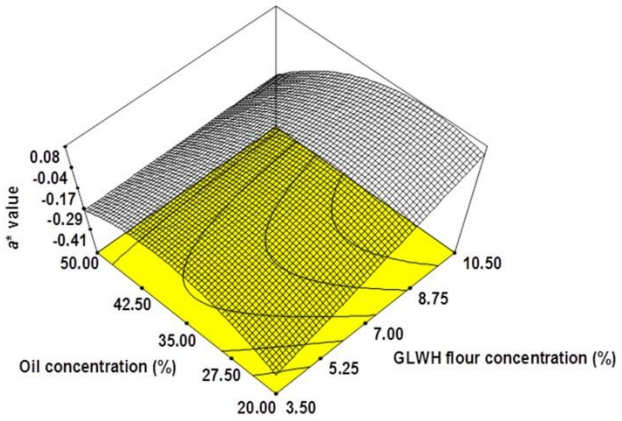


Fig. 6.6 Response surface for the effect of green lentil with hulls (GLWH) flour (x_1), egg yolk (x_2) and oil content (x_3) on the a^* values. The graphs show the interaction effect of two independent variables by holding the other two variables at their central points, specifically 35 wt% for oil, 5 wt% for egg yolk, and 7 wt% for whole green lentil flour.

6.3.6 Effect of Storage on Color and Rheology

The effect of storage on rheology, color, physical stability, and microstructure of the selected pulse supplemented salad dressings was investigated.

The L^* values remained stable for all the flour supplemented dressing samples. There was a slight variation in a^* and b^* values over 28 days relative to the initial color but it was not statistically significant (data not shown). The creep and recovery curves (Fig. 7a) were similar for all the samples. In general, the compliance, J, which is the ratio of the strain to the applied stress, showed a steady increase during the creep stage. After the stress was removed, J recovered part of its elastic component independent of time. Dressings prepared based on the run 16 formulation (7%, DYP; 3%, egg yolk; 35%, oil) on days 0, 14, and 28 generally had higher J values over the creep and recovery stages than the run 2 formulation (7%, DYP; 5%, egg yolk; 35%, oil). This indicated that the stronger network structure of dressings with higher egg yolk content underwent smaller deformation when subjected to the same stress than the dressings with lower levels of egg yolk. Fig. 7b shows the typical flow curves obtained for the selected salad dressings stored over a 28 day period; all the samples exhibited shear thinning behavior (pseudoplastic characteristic) as confirmed by the flow behavior index $n<1$ during the flow test where both irreversible and reversible process may happen which can result in dramatic structure destruction in the sample. Both the storage (G′) and loss moduli (G″) were frequency-dependent and increased with increasing frequency during the oscillation

test where little or no permanent structure breakdown is expected within the linear viscoelastic (LVE) region (Fig. 7c). G′ values were greater than G″ values across the tested frequency range, indicating that the elastic properties were more pronounced than the viscous properties. This type of behavior has been linked to extensive bridging flocculation in a series of protein-stabilized emulsions (Moros, et al., 2002).

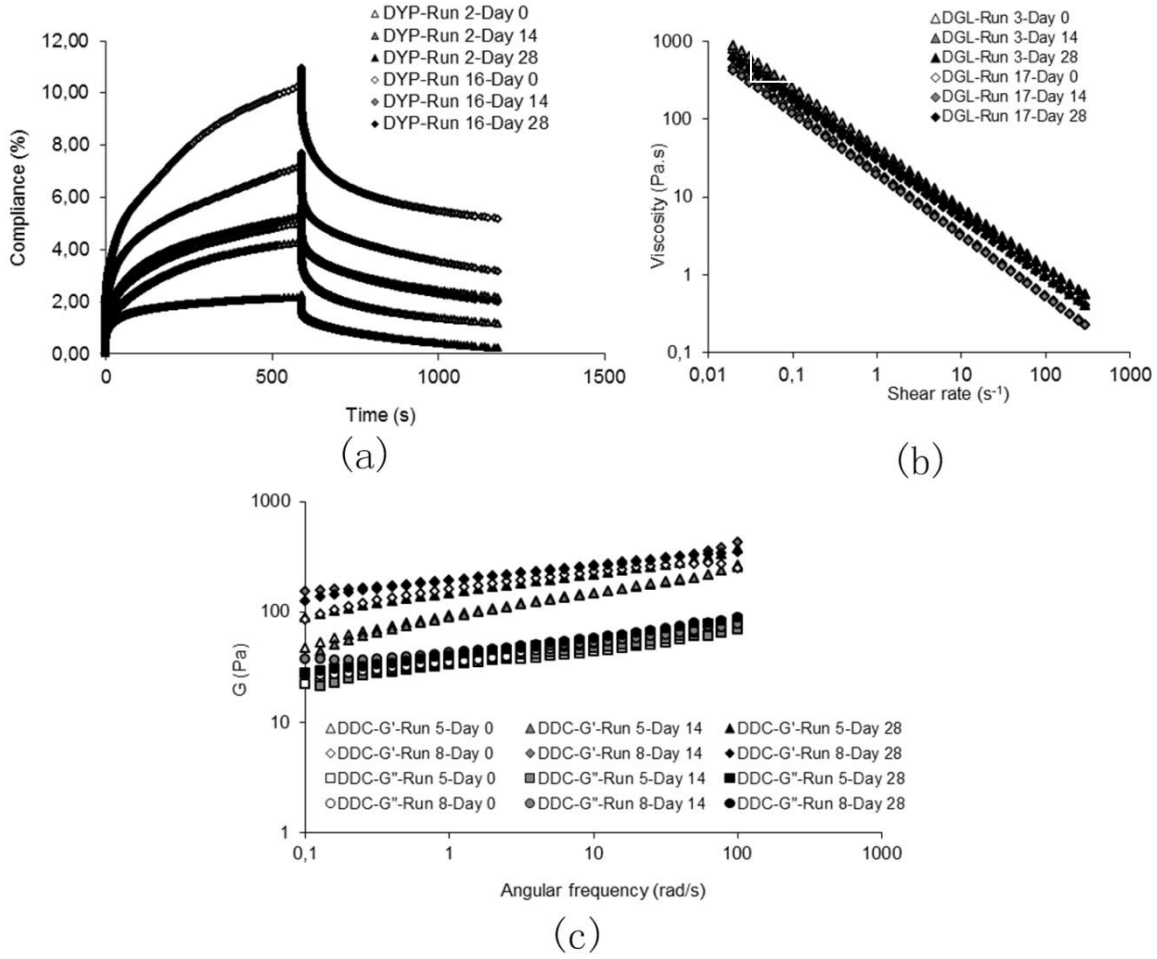


Fig. 6.7 (a) Creep and recovery curves of salad dressings supplemented with dehulled yellow pea (DYP) flours of run 2 and run 16 on storage days 0, 14, and 28 at 4 ℃; (b) steady state flow curves for salad dressings supplemented with dehulled green lentil (DGL) flours of run 3 and run 17 on storage days 0, 14, and 28 at 4 ℃; (c) Storage and loss moduli as a function of angular frequency during dynamic oscillation tests of salad dressings supplemented with dehulled Desi chickpea (DDC) flour of run 5 and run 8 on storage days 0, 14, and 28 at 4 ℃. The specific formulation of the different compositions for each run is shown in Table 6.1.

With respect to the other rheological parameters (data not shown), the variations in the consistency coefficient (*m*) and the flow behavior index (*n*) were small and not statistically different. The plateau modulus G_N^0 and extent of recovery *Q(t)* showed a

general decreasing trend over the 28 days of storage. This effect was significant ($p < 0.05$) in the case of *Q(t)* values for samples supplemented with DGL (run 17), samples supplemented with DYP (run 2), and samples supplemented with DYP (run 16), suggesting that the elasticity of these dressings decreased with storage.

6.3.7 Physical Stability

The stability of the stored salad dressings was determined by monitoring their backscattering (BS) profiles (figures not shown). The initial averaged BS value along the entire tube (i.e., $BSav_0$) ranged from 81.02% to 84.47. In general, the samples remained stable over the 28 days of storage indicating that the oil phase was well protected against creaming, coalescence and sedimentation. There was a slight negative ΔBS at the top zone, which varied among the salad dressings. This finding indicates that slight flocculation occurred at the top of the tube. A larger ΔBS was observed for samples supplemented with lower oil, egg yolk, and pulse flour contents.

6.3.8 Scanning Electron Micrographs

The microstructures of the salad dressing formulations selected for the storage study are presented in Fig. 6.8. The samples generally had a similar microstructure with a highly packed three-dimensional network similar to that reported by Laca et al. (2010). Void spaces between the network represent the aqueous continuous phase in the emulsion which was removed during the dehydration of the samples in preparation for the scanning electron microscopy study (Fig. 6.8). For some samples, starch granules could be seen embedded in the network. A larger number of the starch granules were observed in the SEM images of the salad dressing samples supplemented with larger amounts of pulse flour, such as run 5 in Fig. 6.8(g-i) which contained 10.5% DDC flour.

In comparing the micrographs of dressings based on run 3 (Fig. 6.8a-c) and run 8 formulations (Fig. 6.8d-f) (i.e., samples containing the same amount of egg yolk and pulse flour but differing in oil content), it was observed that the dressings with lower oil content (run 3 formulation in Fig. 6.8a-c) exhibited a less densely packed and relatively loose network, where the interspaced voids were larger in structure compared with the samples in Fig. 6.8d-f. This may explain why the reduction in fat content caused a dramatic decrease in the viscous nature (consistency coefficient *m*) of the salad dressings as discussed in section 3.2.

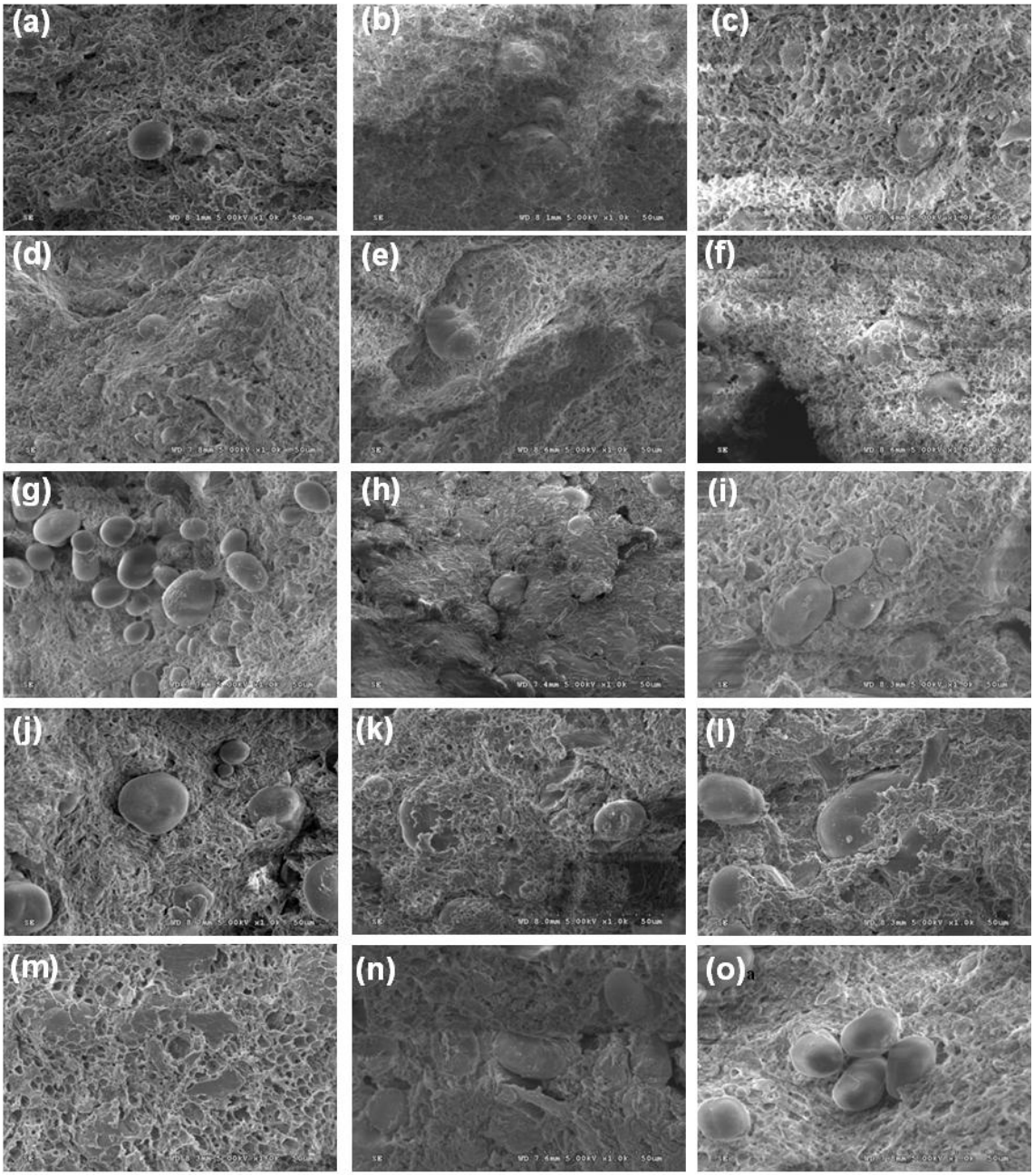


Fig. 6.8 Scanning electron micrograph of salad dressing samples supplemented with pulse flours. From (a) to (c): samples supplemented with dehulled green lentil (DGL) (Run 3) on days 0, 14, and 28; from (d) to (f): samples supplemented with green lentil with hull flour (GLWH) (Run 8) on days 0, 14, and 28; from (g) to (i): samples supplemented with dehulled Desi chickpea (DDC) flour (Run 5) on days 0, 14, and 28; from (j) to (l): samples supplemented with dehulled yellow pea (DYP) flour (Run 2) on days 0, 14, and 28; from (m) to (o): samples supplemented with dehulled yellow pea (DYP) (Run 16) on days 0, 14, and 28.

The findings are also consistent with those of Worrasinchai et al. (2006) for mayonnaise samples with a low oil content. Furthermore, the dressings with lower egg yolk content, such as the run 16 samples (3% w/w egg yolk) shown in Fig. 6.8 (m-o), had larger interspaced voids surrounded by more loosely aggregated droplets than the samples with higher egg yolk content (5% w/w), such as the run 2 samples shown in Fig. 6.8 (j-l).

6.3.9 Sensory Evaluation

Five of the promising salad dressings in terms of physical properties were selected for sensory evaluation. They included, salad dressings supplemented with whole green lentil flour (3.5% flour, 3% egg yolk, and 50% oil), yellow pea with high flour content (7% flour, 5% egg yolk, 35% oil), yellow pea with low flour (3.5% flour, 5% egg yolk, 35% oil), chickpea with high oil content (3.5% flour, 5% egg yolk, 35% oil), and chickpea with low oil content (3.5% flour, 5% egg yolk, 20% oil). A comparison of consumer acceptance scores for the five salad dressing formulations is presented in Table 6.3. The results indicate that the acceptability scores for the whole green lentil, yellow pea with low flour and chickpea with high oil dressings did not differ significantly ($p > 0.05$) for all of the attributes studied. The mean hedonic scores showed that the yellow pea with low flour content dressing was significantly ($p < 0.05$) preferred over yellow pea with high flour and chickpea with low oil content dressings for all attributes except appearance and aroma. Flavor or taste, which is more important than the other attributes for the acceptability of a product (Luckow & Delahunty, 2004), is of crucial importance for pulse supplemented products because of the beany and astringent flavor they usually have. As shown in Table 6.3, dressings supplemented with yellow pea with high flour content and chickpea with low oil content had significantly lower ($p < 0.05$) mean scores for flavor than the other dressings. Both of them also had overall acceptability scores below 5.0 (neither like nor dislike), indicating that they do not have market potential. A correlation analysis of flavor and overall acceptability (r=0.9) showed that significant improvements to the flavor of all treatments could increase overall acceptability scores. The dressing supplemented with yellow pea with low flour content was significantly ($p < 0.05$) preferred over the one with high yellow pea flour content for aroma acceptability, and it was also significantly preferred ($p < 0.05$) over the chickpea with high oil content dressing for appearance acceptability. Whole green lentil and chickpea with high oil

contents were significantly preferred over the yellow pea with high flour content dressing for aroma acceptability and significantly preferred over yellow pea with high flour content and chickpea with low oil content dressings for flavor acceptability. The general trend in acceptability testing appeared to be that the less pulse flour added, the higher the scores for all attributes studied; and the more oil added, the higher the acceptability for each attribute.

Table 6.3 Acceptability scores for various pulse supplemented salad dressing attributes

Acceptability Attribute[1]	**Pulse Supplemented Salad Dressing Treatment**					
	P value	Whole green lentil	Yellow pea with high flour content	Yellow pea with low flour content	Chickpea with low oil content	Chickpea with high oil content
Aroma	<0.01	5.8^{a}	5.2^{b}	6.0^{a}	5.8^{ab}	5.9 ^{a}
Appearance	0.04	6.2^{ab}	6.1^{ab}	6.5^{a}	6.1^{b}	6.2^{ab}
Color	0.02	6.2^{ab}	6.1^{b}	6.5^{a}	6.1^{b}	6.3^{ab}
Flavor	<0.01	5.6^{a}	4.6^{b}	5.6^{a}	4.7^{b}	5.2^{a}
Texture	0.02	5.9^{ab}	5.8^{b}	6.4^{a}	5.8^{b}	6.2^{ab}
Overall	<0.01	5.3^{a}	4.4^{c}	5.5^{a}	4.6^{bc}	5.1^{ab}

*The formulation of each sample in the table was based on the following: whole green lentils (3.5% flour, 3% egg yolk, and 50% oil), yellow pea with high flour content (7% flour, 5% egg yolk, 35% oil), yellow pea with low flour content (3.5% flour, 5% egg yolk, 35% oil), chickpea with high oil content (3.5% flour, 5% egg yolk, 35% oil), and chickpea with low oil content (3.5% flour, 5% egg yolk, 35% oil).
[1]For acceptability scales, a score of 1=dislike extremely and 9=like extremely
Mean values with different lower case letters a, b, and c in the same row for each pulse are significantly different (P<0.05).

In the present study, less than 35% of panelists found the flavor intensity of each treatment to be "just right," and the net scores indicated that all treatments were considered to be too bland (data not shown). This may be because the salad dressings were produced with no added flavor ingredients such as green/black pepper or onion powders, unlike the case for commercial dressings. Over 60% of the panelists indicated that the consistency of all the treatments was 'just right." Most panelists who did not find the consistency to be "just right" indicated that it was too thick, except in the case of the dressings based on chickpea with low oil formulation, where the net score showed that the treatment was "too runny" in the consistency tests using just-about-right (JAR) scales.

6.4 Conclusions

The use of pulse flours in salad dressing applications represents a new avenue of research. Utilization of pulse flours in food products takes advantages of both their nutritional value and techno-functional properties. The modeling of experimental data in this study allowed the generation of useful equations for predicting the color and rheological behavior of pulse flour supplemented salad dressings which should be useful for food industries and food scientists interested in using pulse ingredients in food formulation. In addition to the physicochemical properties, consumer acceptability scores, which is of major interest to food industries, suggested that the dressings supplemented with whole green lentil flour, yellow pea with low flour content, and chickpea with high oil contents have promising potential. Further studies to improve flavor and sensory acceptability (e.g., through the addition of flavor ingredients) could be explored to increase the overall acceptability of any novel products developed.

Connecting Statement to Chapter 7

In Chapter 6, the effects of main components on the physical properties of pulse flour-supplemented salad dressings were studied. The results showed that these factors markedly affected color and rheological behaviour. The scanning electron microscope results were consistent with the trend of the rheological parameters observed in the response surface plots. The consumer acceptability tests indicated that the dressings based on selected formulations were quite promising. The work described in this chapter involved using protein isolates prepared from lentil, pea, and chickpea to fortify salad dressings and studying the effects of the main components (i.e., pulse protein concentration, 3%–8%; egg yolk concentration, 0%–5%; and oil concentration, 20%–50%) on the rheological and textural properties, color, water activity, and droplet size using response surface methodology. The adequacy of the experimental models was validated under optimized conditions. This chapter addresses the fifth objective discussed in the "objective of study" section of Chapter 1. The results of this study are presented as follows:

Ma, Z., Boye, J. I., B. K., Prasher, S. O., Preparation of salad dressing emulsions using lentil, chickpea and pea protein isolates: a response surface methodology study. *Journal of Food Science*, (submitted).

Ma, Z., Boye, J. I., B. K., Prasher, S. O., "Use of Response Surface Methodology to Optimize Conditions for the Production of Pulse protein-supplemented Salad Dressing Using Protein Isolates from Lentil, Chickpea and Pea." *12th Annual Meeting of the Institute of Food Technologists (IFT)*, Las Vegas, Nevada, USA, June 25 to 28, 2012 (Poster Presentation).

Chapter 7. Preparation of Salad Dressing Emulsions Using Lentil, Chickpea and Pea Protein Isolates: A Response Surface Methodology Study

Abstract

In this study, pulse protein isolates were used as ingredients in the preparation of salad dressing. The effect of pulse protein, egg yolk, and oil contents on the physical properties (i.e., static and dynamic rheological behavior, texture, color, water activity, droplet size) of lentil-, pea- and chickpea-supplemented salad dressings was studied using a three-factor central composite design (CCD). Multiple regression equations were developed to describe the effects of the independent variables on several response variables. In general, an increase in oil and emulsifier (pulse protein or egg yolk) contents modified the rheological and textural properties and led to either a linear or a nonlinear increase in several parameters, including G_N^0, m, η_{ap}, σ_0, $Q_{(t)}\%$, and firmness. Response surface methodology was used to optimize the salad dressing formulations based on selected response variables, which were either maximized or minimized, or targeted using average values of parameters for several commercial salad dressings. The validation test confirmed the overall adequacy of the response surface models in predicting specific properties of the set formulations. The results showed that it is possible to produce pulse protein-supplemented salad dressings with physical properties similar to those of commercial dressings by using different levels of protein, egg yolk and oil; furthermore, dressings can be designed to meet market specifications.

7.1 Introduction

Salad dressing and mayonnaise are frequently prepared with egg yolk, which is a complex mixture of lipoproteins, phospholipids (lecithin) and cholesterol. In addition to having a high emulsifying capacity, egg yolk can impart desirable flavor, mouth-feel, and color to foods. However, health concerns related to dietary cholesterol intake have stimulated interest in developing emulsifiers from alternative protein sources. Several different animal and vegetable proteins, such as whey protein (Dickinson & Yamamoto 1996), sodium caseinate (Dickinson & Golding, 1997), soy protein (Diftis & Kiosseoglou,

2004), lupin protein (Raymundo et al. 2002), and wheat protein (Ghoush et al. 2008), have been used as alternatives or substitutes in oil-in-water emulsions.

Proteins can stabilize emulsions by forming an interfacial layer that prevents droplets from aggregating, by providing electrostatic and steric repulsion between droplets, and by reducing interfacial tension between the two phases during homogenization. Proteins from pulses may have good potential for use as value-added food ingredients in emulsion-type food applications such as salad dressing and mayonnaise for several reasons: (i) Pulses are excellent, inexpensive sources of proteins, with a protein content ranging from 18% to 32% (Boye et al. 2010c); (ii) Pulse proteins have potential health benefits as they are rich in most of the essential amino acids, especially lysine, they are cholesterol free, and they can serve as alternatives to some of the major allergenic proteins (Boye et al. 2010c); (iii) In previous studies, protein isolates from pulses were found to have excellent functional properties, including good solubility, emulsifying, fat/water binding, and gelling properties, making them potentially suitable for use in food emulsions (Boye et al. 2010a; Paredes et al. 1991); (iv) Several effective technologies (such as air classification, membrane separation, alkaline/isoelectric precipitation, acid/salt extraction, and ultrafiltration) have been developed to fractionate pulses and extract the proteins (Boye et al. 2010a; Boye et al. 2010c; Mondor et al. 2009). (v) Additionally, the use of pulse ingredients in different products can stimulate production of pulse crops and increase the consumption of these beneficial foods.

Salad dressings show viscoelastic characteristics and pseudoplastic behavior with yield stress. Several rheological equations, such as the power law and the Herschel-Bulkley and Carreau models, have been used to describe the stress response to deformation (flow behavior) in dressing-type emulsions. The rheological and textural properties of dressings are governed by several factors, including: the phase volume of the dispersed droplets; the rheology of the continuous phase; average particle size and distribution; the nature of the particle-particle interactions; and the internal viscosity (Barnes 1994; Tadros 2004). Variations in salad dressing formulations could thus have a great impact on the rheology, texture, and physical properties of salad dressings, including color, water activity, and particle size distribution.

Surprisingly, very little research has been conducted to (a) explore the use of pulse protein extracts in salad dressing preparations, (b) determine the influence of formulation composition on the physical properties of pulse protein-supplemented salad dressings, and (c) to optimize novel formulations using these ingredients. In addition, few studies have compared the behavior of proteins derived from different pulses, such as lentil, pea and chickpea. The present study was therefore undertaken to systematically investigate the effect of three types of pulse protein (green lentil, pea, chickpea) and their concentration (3−8%, w/w), egg yolk content (0−5%, w/w) and oil content (20−50%, w/w), on the rheology, texture, color, water activity, and particle size of salad dressings. A three-factor face-centered central composite design (CCD) and response surface methodology (RSM) was used in the study.

7.2 Materials and Methods

7.2.1 Materials

Green lentil, yellow pea, and Desi chickpea were provided by pulse growers in Saskatchewan. The specific varieties and suppliers are as follows: CDC Grandora Green Lentil (Simpson Seeds Inc., Saskatchewan, Canada); CDC Golden Pea (Wagon Wheel Farms of Churchbridge, Saskatchewan, Canada); Mylese Desi Chickpea (R Young Seeds Inc., Saskatchewan, Canada). Spray-dried egg yolk powder was obtained from Canadian Inovatech Inc. (Winnipeg, Manitoba, Canada). Xanthan gum was kindly provided by Tic Gums (Belcamp, MD, USA). All other ingredients used in the preparation of the salad dressings were purchased from a local supermarket. All chemicals used were of analytical grade.

7.2.2 Preparation of Pulse Protein Isolates

Protein isolates from green lentil, yellow pea, and Desi chickpea were prepared using alkaline extraction followed by isoelectric precipitation as described by Boye et al. (2010a), and are referred to as GLPI, YPPI, and DCPI, respectively. The protein content of the isolates was 79.1 ±0.3% for green lentil, 81.7 ±0.3% for yellow pea and 73.6 ±0.1% for Desi chickpea.

7.2.3 Preparation of Salad Dressing Samples

The salad dressings were prepared using different concentrations of pulse protein, oil, and egg yolk as shown in Table 7.1. Other ingredients used, expressed as a

percentage (w/w), were as follows: vinegar 7.0% (with 5% acetic acid), lemon juice 5.0%, salt 1.0%, sugar 3.5%, and xanthan gum 0.25%. To prepare the dressings, the xanthan gum and sugar were dispersed in water and stored overnight to ensure complete hydration. All other ingredients except oil were then added and mixed homogeneously using a blender. Canola oil was subsequently added and emulsification was achieved using an Ultra-Turrax homogenizer (Model T25, Janke & Kunkel, Ika-Labortechnik, Staufen, Germany) with a S25-18G dispersing tool at 13,800 rpm for 3 min. All measurements were made on the day the samples were prepared.

7.2.4 Rheological Measurements

Rheological measurements were performed using an AR 1000 rheometer (TA Instrument, New Castle, DE, USA) with a plate/cone system. Steady state flow tests, dynamic oscillatory tests, and creep and recovery tests were conducted using a stainless steel parallel plate (4 cm diameter). The gap setting was 1 mm. One tablespoon of sample was placed at the centre of the circular plate, and excess sample was removed from the edges of the plate. The linear viscoelastic (LVE) range was determined by performing amplitude sweeps at 1 Hz frequency over a strain range from 0.01% to 1000%. The steady state flow tests were performed by increasing shear rates from 0.02 to 300 s^{-1}. Experimental flow curves were fitted to the power law (Eq. 1) and Herschel-Bulkley (Eq. 2) models:

$$\eta = m\gamma^{(n-1)} \qquad \text{(Eq. 1)}$$

$$\sigma = \sigma_0 + m\gamma^n \qquad \text{(Eq. 2),}$$

where n is the flow behavior index (dimensionless), η is the shear viscosity (Pa.s), m is the consistency coefficient (Pa s^n), γ is the shear rate (s^{-1}), σ is the shear stress (Pa), and σ_0 is the yield stress (Pa). The apparent viscosity (η_{ap}) at a shear rate of 46.16 s^{-1} was calculated according to the power law model (Eq. 1). The specific shear rate was selected based on the perceived in-mouth thickness of normal fluids (Baines & Morris 1988).

Dynamic oscillatory tests were performed within the (linear viscoelastic) LVE range at 0.1% strain with angular frequency ranging from 0.1 to 100 rad/s. Storage modulus (G′, Pa) and loss modulus (G″, Pa) versus angular frequency (rad/s) were measured for all samples. The linear viscoelasticity is defined as the extrapolation of the

entanglement contribution to G′ at high frequencies and expressed as the plateau modulus (G_N^0), which was obtained by an approximation procedure using the following equation:

$G_N^0 = [G^{'}]_{\tan\delta \to \min imum}$, where tan δ =G″/G′.

A creep and recovery test was carried out by recording compliance (*J*) versus time, where *J* is the ratio of the strain to the applied stress. The samples were pre-sheared at a shear strain of 300 s^{-1} for 2 min; 0.5 Pa stress was applied to the samples for 600 s, then the stress was removed and the strain was recorded as a function of time (600 s). The recoverable strain or extent of recovery *Q(t)%* was calculated according to the method of Zhang et al. (2008).

$Q(t)\% = [\Delta(\chi_{c(t)})] \times 100$, in our study, $\Delta(\chi_{c(t)}) = \frac{\chi_{(600)} - \chi_{(1200)}}{\chi_{(600)}}$, where $\chi_{(600)}$ and $\chi_{(1200)}$ are the strains at the equilibrium of the creep and recovery zones, respectively.

7.2.5 Color and Water Activity (a_w) Measurements

The color of the salad dressing samples was measured with the *L**, *a**, *b** tristimulus system using a Minolta CM-503c spectrophotometer (Minolta Co. Ltd., Osaka, Japan). A fixed amount of salad dressing was poured into the measuring cup, which was then surrounded with a black paper strip. In this color system, *L** is a measure of lightness or darkness (0=black and 100=white), *a** is a measure of redness (+ve) to greenness (−ve), and *b** is a measure of yellowness (+ve) to blueness (−ve). The data were also characterized in terms of chroma (*C*) to highlight differences between the samples: $C = (a^2 + b^2)^{1/2}$.

Water activity, was measured using an AquaLab Water activity meter (CX-2 model) AquaLab CX-2 Water Activity System (Decagon Devices Inc., Pullman, WA, USA).

7.2.6 Texture Profile Analysis

Texture profile analysis was performed with a TA-XT2 texture analyzer (Stable Micro Systems, UK). Salad dressing samples were placed in cylindrical bottles (60 mm diameter × 10 mm height) and punctured with a cylindrical probe of 25 mm diameter × 35 mm height in a load cell of 5,000 g at a crosshead speed of 1.0 mm/s. Firmness, considered as the height of the force peak during the first compression cycle of force versus time, was calculated from the force versus time texturograms.

7.2.7 Droplet Size Distribution

The particle size distribution of the salad dressings was analyzed by laser light scattering using a Mastersizer 2000 MU particle size analyzer (Malvern Instruments Ltd, Worcestershire, UK) with a Hydro 2000 MU sample dispersion unit. Distilled water was used as a dispersant at a speed of 2,000 rpm. In keeping with the procedure described by Worrasinchai et al. (2006), the results were expressed as the Sauter mean diameter: $D(3,2) = \sum n_i d_i^3 / \sum n_i d_i^2$, where n_i is the number of droplets of diameter d_i.

7.2.8 Statistical Analysis

A face-centered central composite design (CCD) with three independent variables (pulse protein, x_1; egg yolk, x_2; oil concentration, x_3) at three coded levels (-1, 0, +1) and five replicates at the center point was programmed using the commercial statistical package Design-Expert, version 7 (Stat-Ease Inc., Minneapolis, MN, USA). This software was also used to generate the response surface and to optimize the variables. A complete design consisted of 19 experimental runs for each pulse protein-supplemented dressing (Table 7.1). The effect of the three independent variables on the responses was modeled and optimized using response surface methodology (RSM): the flow behavior index *n* (Y_1), consistency coefficient *m* (Y_2), yield stress σ_0 (Y_3), plateau modulus G_N^0 (Y_4), recoverable strain $Q_{(t)}$% (Y_5), apparent viscosity η_{ap} (Y_6), firmness (Y_7), *a** value (Y_9), *b** value (Y_9), chroma (Y_{10}), and Sauter mean diameter *D(3,2)* (Y_{11}). The relationship between a dependent response (Y) and the independent variables (x) was expressed by fitting the data to the following equation:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii}{}^2 x^2{}_{ii} + \sum \sum \beta_{ij} x_i x_j + \varepsilon \qquad \text{(Eq. 3)}$$

where Y is the predicted response, β_0 is the constant, β_i , β_{ii} , β_{ij} are the regression coefficients, and x_i, x_j are the levels of the independent variables (Myers et al. 2009). The significant terms in the model were evaluated by one-way analysis of variance (ANOVA) for each response. The adequacy of the models was checked by removing the non-significant terms ($P > 0.05$) using a step-wise "backward" multiple reduction algorithm. The response surface plots were generated by holding one independent variable constant at the center point and varying the other two variables within the experimental range.

The overall optimization was carried out within the range of experimental conditions. The optimal levels of the three independent variables leading to the desired properties were determined using values for a commercially purchased salad dressing as target. The properties of five types of commercial salad dressing were tested to obtain target values for the responses, in order to predict the optimal formulation during the optimization procedure.

Table 7.1 Face-centered central composite experimental design with coded and independent variables, along with experimental data for preparing the salad dressings supplemented with pulse protein isolates.

Run no.	Pulse Protein isolates con. (%, w/w)	Egg yolk con. (%, w/w)	Oil con. (%, w/w)
1	3 (-1)	5 (+1)	50 (+1)
2	5.5 (0)	0 (-1)	35 (0)
3	3 (-1)	0 (-1)	50 (+1)
4	8 (+1)	5 (+1)	20 (-1)
5	8 (+1)	2.5 (0)	35 (0)
6	5.5 (0)	2.5 (0)	35 (0)
7	8 (+1)	0 (-1)	50 (+1)
8	5.5 (0)	2.5 (0)	35 (0)
9	5.5 (0)	2.5 (0)	35 (0)
10	8 (+1)	5 (+1)	50 (+1)
11	5.5 (0)	2.5 (0)	35 (0)
12	5.5 (0)	2.5 (0)	20 (-1)
13	8 (+1)	0 (-1)	20 (-1)
14	5.5 (0)	5 (+1)	35 (0)
15	3 (-1)	0 (-1)	20 (-1)
16	5.5 (0)	2.5 (0)	35 (0)
17	3 (-1)	2.5 (0)	35 (0)
18	3 (-1)	5 (+1)	20 (-1)
19	5.5 (0)	2.5 (0)	50 (+1)

*Code 0 is for center point of the parameter range studied; 1 for factorial points.

7.3 Results and Discussion

7.3.1 Model Fitting

As shown in Table 7.1, a face-centered CCD with 19×3 experiments was conducted for dressings supplemented with green lentil protein isolates (GLPI), Desi chickpea protein isolates (DCPI), and yellow pea protein isolates (YPPI), respectively. The R^2 and adjusted R^2, along with the Adeq precision (i.e., signal-to-noise ratio that measures the ratio of the range of variation in the predicted response to an estimate of the standard error of the predictions), were computed. The values of these parameters for salad dressings supplemented with YPPI are shown in Tables 7.5 and 7.6. Joglekar & May (1987) suggested that R^2 should be at least 0.80 for a good fit of a model. A few of the responses could not be fitted satisfactorily to mathematical models owing to lack of fit or

poor fit with low adjusted determination coefficients (R^2). The discussion below focuses on the responses for which the predicted models were statistically significant ($P < 0.05$) with nonsignificant lack of fit ($P > 0.05$) and relatively high R^2 values.

7.3.2 Flow Behavior

7.3.2.1 Flow Behavior Index (*n* value)

The flow behavior index (n) values for all salad dressings obtained by fitting the data to the power law model (Eq. 1) were less than 1 and ranged from 0.16 to 0.49, indicative of pseudoplastic (shear-thinning) behavior, a result of the increasing breakdown in structure. A second-order polynomial model (Eq. 4) with relatively high R^2 value (0.894) which suggested a satisfactory fit of the mathematical model to the experimental data was fitted to the n values for YPPI-supplemented dressings (Table 7.5).

$$Y_1 = 0.6146 - 0.016x_1 - 0.09x_2 - 0.006x_3 + 0.001x_2x_3 + 0.0077{x_2}^2 \qquad \text{(Eq. 4)}$$

The n value decreased with increasing oil (x_3) and YPPI contents (x_1), as shown in Fig. 7.1B. For the effect of egg yolk (x_2), an initial decrease in n was recorded as the concentration of egg yolk increased up to a critical concentration of 2.5% (w/w); a further increase in egg yolk produced less pronounced shear-thinning behavior, as evidenced by the higher n value (Fig. 7.1A, 7.1C).

The effect of the main components on the pseudoplastic properties of DCPI-supplemented salad dressings was quite different from that for dressings prepared with YPPI. For these samples, the multiple regression equation with R^2 of 0.814 is shown in Eq. 5:

$$Y_1 = 1.0152 - 0.05x_1 - 0.06x_2 - 0.03x_3 + 0.001x_1x_3 + 0.002x_2x_3 + 0.00025{x_3}^2 \quad \text{(Eq. 5)}$$

As shown in Fig 7.1(D,E), n values decreased with increasing DCPI content (x_1). Values of n also decreased with decreasing egg yolk content (x_2) at lower oil (x_3) and higher DCPI (x_1) concentrations (Fig. 7.1D, 7.1F). The linear effect of x_2 and the interaction between x_2 and x_3, and between x_1 and x_3 was significant ($P < 0.01$). An increase in oil content (x_3) yielded lower n values until the center point (35%, w/w), beyond which a further increase in oil yielded slightly higher n values (Fig. 7.1E, 7.1F). Among the formulated samples, the dressings prepared in run 7 (8% protein, 0% egg yolk, and 50% oil) had the lowest n values, whereas the run 15 dressings (3% protein, 0% egg yolk, and 20% oil) had the highest n values for both the YPPI- and DCPI- supplemented

dressings. It appears that a more pronounced pseudoplasticity (i.e., decreased *n* values) occurred in the dressings with a more compact network formulated at higher YPPI and DCPI concentrations.

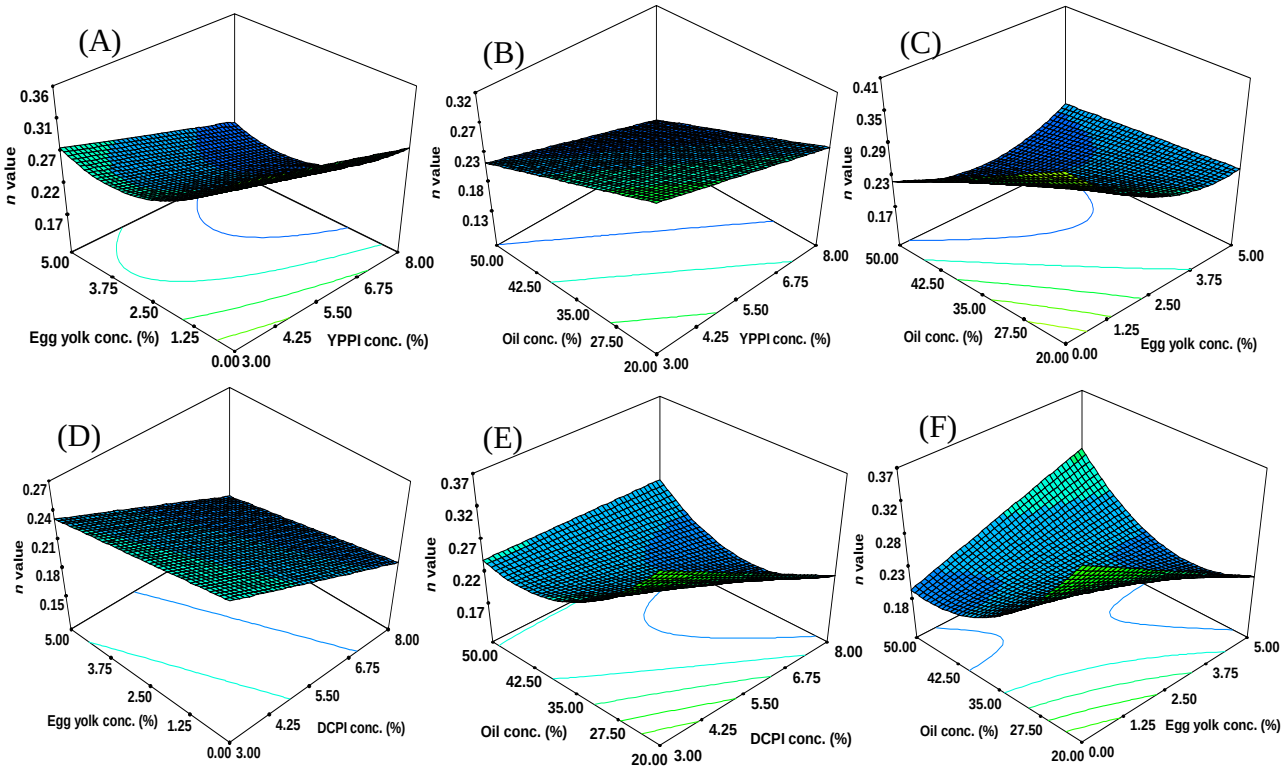


Fig. 7.1 (A-C). Response surface plots for *n* values for salad dressings supplemented with yellow pea protein isolates (YPPI). (D-F). Response surface plots for *n* values for salad dressings supplemented with Desi chickpea protein isolates (DCPI). The plots show the interaction effect of two independent variables by holding the other two variables at their central points.

7.2.2.2 Consistency Coefficient (*m* value)

A second-order polynomial response surface model (Eq. 6) was applied to the consistency coefficient (*m*) values for GLPI-supplemented salad dressings with a very high R^2 of 0.97, indicative of a satisfactory fit.

$$Y_2 = 11.03 - 1.29x_1 - 3.77x_2 - 1.18x_3 + 0.57x_1x_2 + 0.096x_1x_3 + 0.12x_2x_3 + 0.024{x_3}^2 \quad \text{(Eq. 6)}$$

Fig. 7.2(A-C) shows the increasing trend in *m* values for dressings prepared with high GLPI (x_1), egg yolk (x_2), and oil contents (x_3). Based on the sum of squares, oil content had a more pronounced effect than either egg yolk or GLPI on the viscous characteristics (*m* value) of the dressings. The synergistic effect of each set of two independent variables on *m* values can be seen in the response plots, with maximum *m*

values achieved with the highest concentrations in each two-variable set (Fig 7.2A-C). The total number of rigid particles in the dressing emulsions is expected to increase with the increase in egg yolk and GLPI levels, which would enhance resistance to flow (Hunter 1986) and therefore cause an increase in the viscous characteristics of the dressings. Additionally, as the emulsifier (egg yolk and pulse protein) content increased, a more compact network would be expected which would explain the increasing trend observed in *m* values (Fig. 7.2A). On the other hand, as oil content decreases, the mean distance between droplets can be expected to grow, which will result in a less compact network structure, and decreasing *m* values as observed in Fig. 7.2B and 7.2C. This finding is in agreement with the results of earlier studies (Gladwell et al. 1986; Ma & Barbosa-Cánovas 1995b) which showed that the viscous properties of oil in water emulsion and mayonnaise samples increased with oil concentration.

YPPI- and DCPI-supplemented salad dressings, showed an increasing trend in *m* values with an increase in pulse protein (x_1) and oil content (x_3) (Figs. 7.2D and 7.2E). The statistical models for the dependent variables with an R^2 of 0.9 and 0.83 for YPPI and DCPI- supplemented dressings are given by Eq. 7 and Eq. 8, respectively:

$$Y_2 = 132.44 - 13.63x_1 - 8.53x_3 + 0.70x_1x_3 + 0.105x_3^2 \qquad \text{(Eq. 7)}$$

$$Y_2 = 71.93 - 40.27x_1 + 0.0999x_3 + 0.3917x_1x_3 + 3.068x_1^2 \qquad \text{(Eq. 8)}$$

Unlike for GLPI-supplemented dressings, in the formulations for YPPI- and DCPI-supplemented dressings, egg yolk did not contribute to the statistical models generated. For dressings prepared with GLPI, the synergistic effect between egg yolk and pulse protein led to an enhanced *m* value. The emulsifying capacity of egg yolk is mainly the result of phospholipids, lipoproteins (LDL and HDL) and non-associated proteins (livetin and phosvitin), with LDL playing the most important role in emulsification (Kiosseoglou 2003). The composition of the emulsifiers around the oil droplets depends on the competitive adsorption and/or displacement of egg yolk and pulse proteins. The synergistic interaction in the case of GLPI-supplemented dressings indicated that GLPI interacted well with egg yolk, and both emulsifiers were important in structuring and stabilizing the emulsion. However, for YPPI and DCPI dressings, YPPI/DCPI seemed to be more competitive than egg yolk, and the pulse proteins may have preferentially adsorbed at the oil/water interface.

For all three pulse protein salad dressings, the lowest *m* values were those in the formulation used in run 15 (3% protein, 0% egg yolk, and 20% oil), whereas the run 10 formulation (8% protein, 5% egg yolk, and 50% oil) had the highest values.

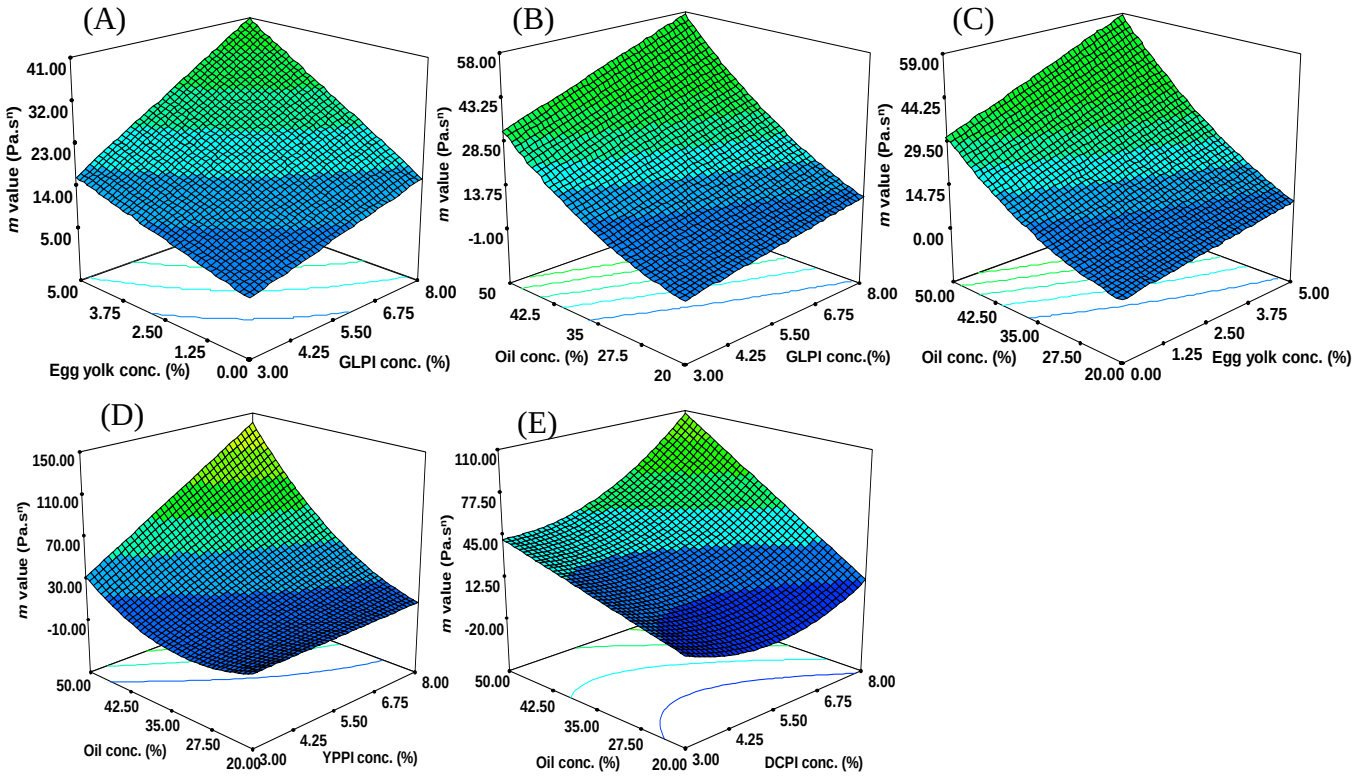


Fig. 7.2 (A-C). Response surface plots for *m* values for salad dressings supplemented with green lentil protein isolates (GLPI). (D). Response surface plots for *m* values for salad dressings supplemented with yellow pea protein isolates (YPPI). (E). Response surface plots for *m* values for salad dressings supplemented with Desi chickpea protein isolates (DCPI). The plots show the interaction effect of two independent variables by holding the other two at their central points.

7.3.2.3 Apparent Viscosity (η_{ap})

Significant second-order regression models fitted to η_{ap} with relatively high R^2 values of 0.82 and 0.93 were fitted for GLPI- and YPPI-supplemented dressings, respectively, and are given by

$$Y_3 = -1.88 + 0.18x_1 - 0.22x_2 + 0.04x_3 + 0.013x_2x_3 \qquad \text{(Eq. 9)}$$

$$Y_3 = 4.89 - 0.53x_1 + 0.17x_2 - 0.34x_3 + 0.028x_1x_3 + 0.004x_3^{\,2} \qquad \text{(Eq. 10)}$$

An increase in the concentrations of the three components yielded higher η_{ap} values. The response surface plots (not shown) for dressings prepared with GLPI and YPPI showed a similar trend to that observed for *m* values, except that the increase was linear.

Based on the sum of squares, the increase in η_{ap} was more pronounced with the addition of oil (x_3) than with an increase in GLPI/YPPI (x_1) and egg yolk (x_2). This result suggests that the perceived mouthfeel or thickness (i.e., η_{ap} at 46.16 s^{-1}) increased as expected with increasing egg yolk, GLPI and oil contents probably due to the formation of a more compact viscoelastic network. Dickie & Kokini (1983) found a good correlation between shear stress on the tongue and sensory thickness, and suggested that the tested η_{ap} be used as an indicator of texture in the mouth when formulating a new salad dressing product. By performing a quantitative descriptive analysis (QDA) on texture attributes, Wendin et al. (1997) also found that a higher fat content enhanced the thickness, fattiness and elasticity of mayonnaise samples. In addition, the obtained rheological values correlated very well with their sensory evaluations.

The regression model (Eq. 11) was fitted to η_{ap} values of salad dressings supplemented with DCPI with a R^2 of 0.933.

$$Y_3 = 4.42 - 0.3375x_1 - 0.305x_3 + 0.0191x_1x_3 + 0.0045x_3^2 \qquad \text{(Eq. 11)}$$

An increase in DCPI and oil contents resulted in higher η_{ap} values (not shown), which is consistent with the results observed for *m* values. Based on the sum of squares, the effect of oil was more pronounced than that of DCPI.

7.3.2.4 Yield Stress (σ_0)

Yield stress (σ_0), which is an important property of mayonnaise and dressing products, was obtained by fitting the flow curves to the Herschel-Bulkley model. A second-order polynomial model was fitted to σ_0 for YPPI-supplemented salad dressings (Eq. 12).

$$Y_4 = 33.62 - 14.90x_1 - 2.89x_2 - 0.42x_3 + 0.97x_1x_2 + 0.24x_1x_3 + 0.81x_1^2 \qquad \text{(Eq. 12)}$$

As observed for apparent viscosity (η_{ap}), increasing oil (x_3), egg yolk (x_2) and YPPI (x_1) contents resulted in higher σ_0 values (Fig. 7.3A-C). This is consistent with the findings of previous studies (Ma & Barbosa-Cánovas 1995; Peressini et al. 1998; Wendin et al. 1997) indicating that mayonnaise-type emulsions exhibited higher yield stress values as fat content increased. Based on the sum of squares, the importance of the independent variables can be ranked as follows: oil>YPPI>egg yolk (Fig. 7.3B, 7.3C, Table 7.5). Formulations used in run 13 (8% protein, 0% egg yolk, and 20% oil) and run

10 (8% protein, 5% egg yolk, and 50% oil) had the lowest and highest yield stress values, respectively, for the three pulse protein-based salad dressings. A similar trend in the response surface plot for σ_0 compared with that for η_{ap} was observed, suggesting that the minimum shear stress required to initiate flow increased owing to the comparatively rigid structure formed with higher x_1, x_2, and x_3 contents.

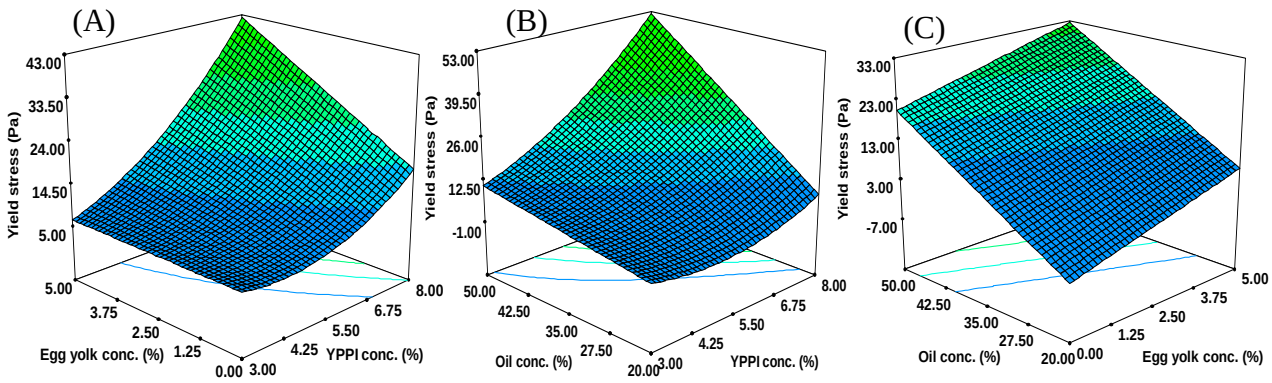


Fig. 7.3 (A-C). Response surface plots for yield stress for salad dressings supplemented with yellow pea protein isolates (YPPI). The plots show the interaction effect of two independent variables by holding the other two at their central points.

7.3.3 Creep and Recovery Behavior

A linear response surface model (Eq. 13) was fitted to *Q(t)%* for YPPI-supplemented salad dressings as shown below:

$$Y_5 = -57.24 + 3.73x_3 \qquad \text{(Eq. 13)}$$

The *Q(t)%* values increased linearly with increasing oil content (Fig. 7.4A). This result is in good agreement with the finding of Guerrero et al. (1998) which showed that a higher oil content significantly increased the elastic characteristics of mayonnaise.

The regression model of *Q(t)%* for DCPI-supplemented dressings (with R^2 of 0.954) is given by Eq. 14.

$$Y_5 = 119.32 - 15.17x_1 + 15.01x_2 - 8.34x_3 + 0.8627x_1x_3 - 0.4269x_2x_3 + 0.1234x_3^{\,2} \qquad \text{(Eq. 14)}$$

The *Q(t)%* value increased with an increase in the proportion of both oil (x_3) and DCPI (x_1), as shown in Fig. 7.4B, C. The *Q(t)%* value also increased slightly with egg yolk content at lower oil concentrations (Fig. 7.4D). Based on the sum of squares, the importance of the variables can be ranked as follows: linear effect of oil, linear effect of DCPI, the interactions between protein and oil, the quadratic term for oil, the interactions between egg yolk and oil, and the least influential linear term (egg yolk). The lowest and

highest *Q(t)%* values among the formulated dressings in this study were obtained for the formulations in run 15 (3% protein, 0% egg yolk, and 20% oil) and run 7 (8% protein, 5% egg yolk, and 50% oil), respectively, for both YPPI- and DCPI-supplemented salad dressings.

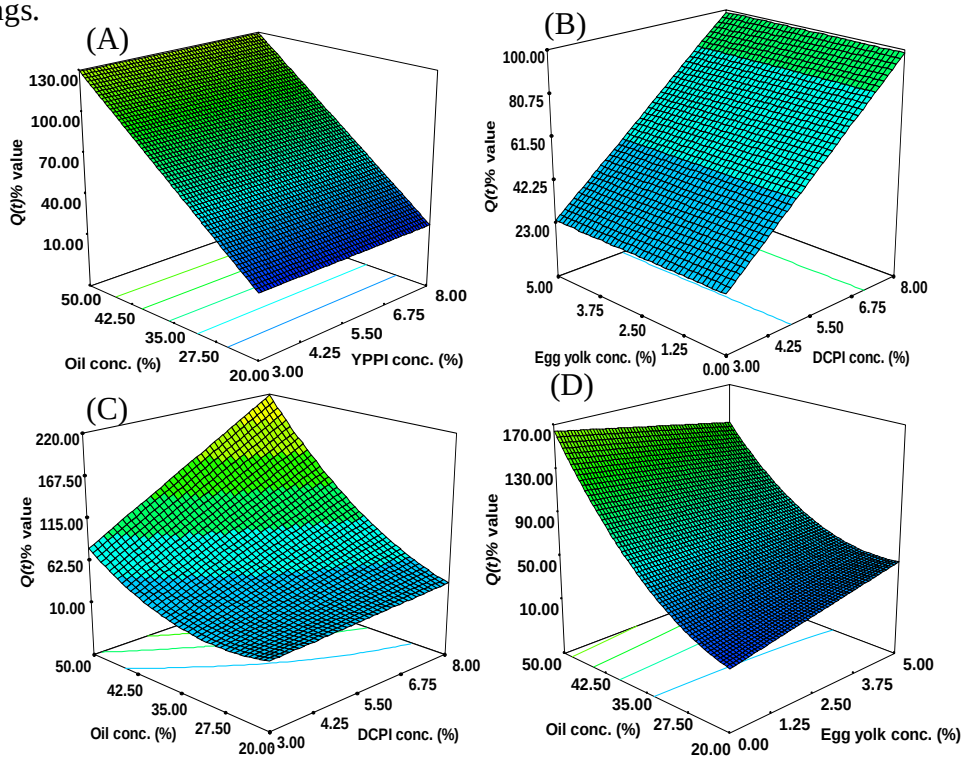


Fig. 7.4 (A). Response surface plots for *Q(t)%* for salad dressings supplemented with yellow pea protein isolates (YPPI). (B-D). Response surface plots for *Q(t)%* for salad dressings supplemented with Desi chickpea protein isolates (DCPI). The plots show the interaction effect of two independent variables by holding the other two at their central points.

7.3.4 Linear Viscoelasticity (G_N^0)

Linear models fitted to G_N^0 for dressings supplemented with GLPI (R^2=0.827) and YPPI (R^2=0.748) are presented below in Eq. 15 and Eq. 16, respectively.

$$Y_5 = -350.86 + 58.04x_1 - 30.24x_2 + 12.19x_3 - 1.70x_1x_3 + 1.30x_2x_3 \qquad \text{(Eq. 15)}$$

$$Y_5 = -427.58 + 58.73x_1 + 15.60x_2 + 16.24x_3 - 2.11x_1x_3 \qquad \text{(Eq. 16)}$$

An increase in G_N^0 was observed with increasing egg yolk (x_2) and oil (x_3) contents (Fig. 7.5C, 7.5F) and when the dressing had a higher GLPI or YPPI (x_1) content at a low oil concentration (Fig 7.5B, 7.5E). Based on the sum of squares, the effect of the independent variables on G_N^0 could be ranked as follows: oil > egg yolk > protein (Table

7.6). Additionally, interaction terms between the variables had a significant influence on G_N^0 (Fig. 7.5B, 7.5E).

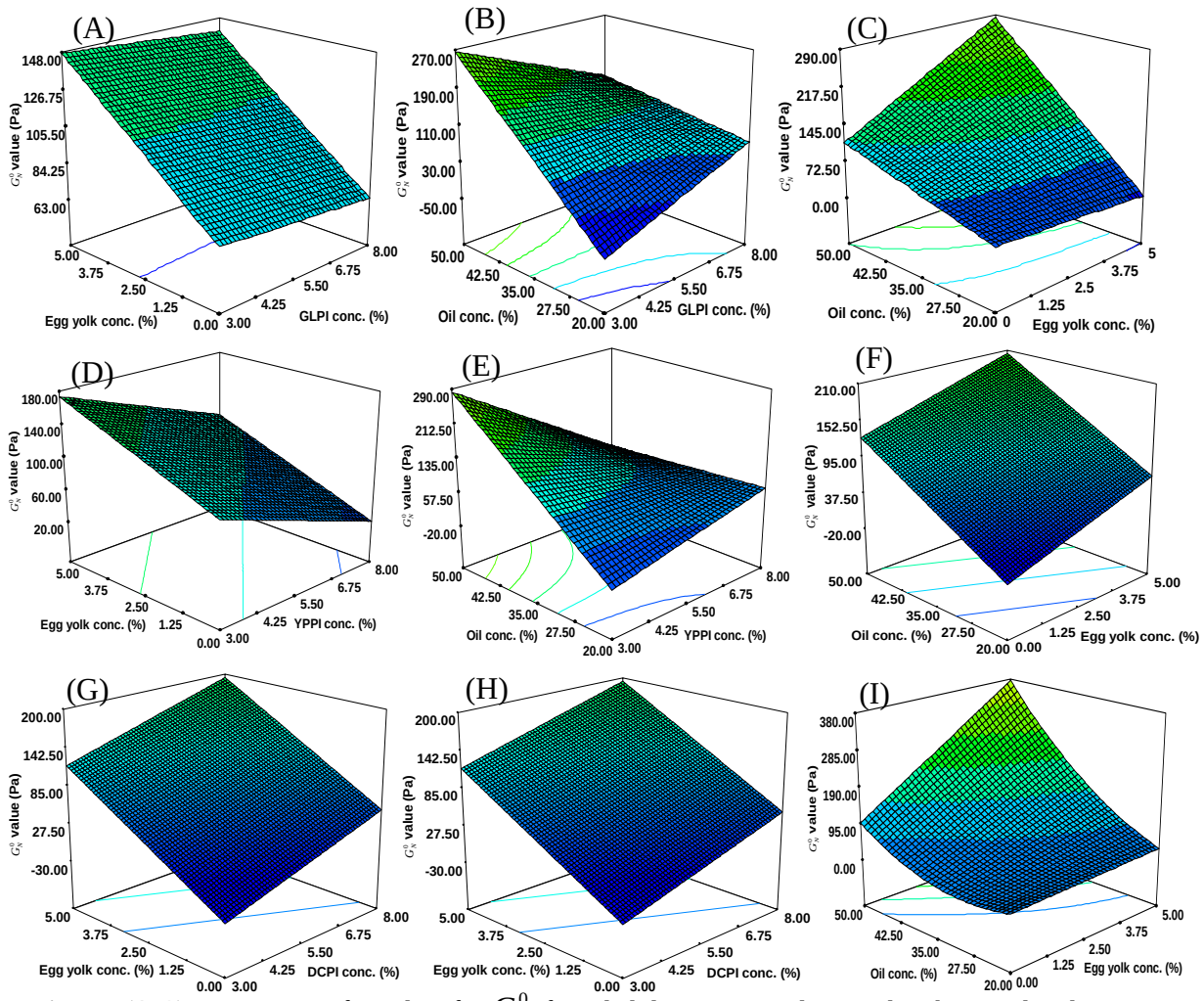


Fig. 7.5 (A-C). Response surface plots for G_N^0 for salad dressings supplemented with green lentil protein isolates (GLPI); (D-F). Response surface plots for G_N^0 for salad dressings supplemented with Desi chickpea protein isolates (DCPI). (G-I). Response surface plots for G_N^0 for salad dressings supplemented with yellow pea protein isolates (YPPI). The plots show the interaction effect of two independent variables by holding the other two at their central points.

For DCPI-supplemented salad dressings, a second-order model was fitted to the experimental data, as shown below:

$$Y_5 = 92.11 + 15.53x_1 - 33.01x_2 - 12.159x_3 + 1.7704x_2x_3 + 0.21158x_3^2 \qquad \text{(Eq. 17)}$$

The G_N^0 value increased steadily with increasing DCPI (x_1), egg yolk (x_2) and oil content (x_3) (Fig. 7.5G, 7.5H, 7.5I). Synergistic interactions were observed for each set of two variables. Based on the sum of squares, the effect of oil was more pronounced than

that of egg yolk, followed by DCPI. The shear sensitivity (i.e., a shear at which a significant breakdown of the structure is produced) of dressings with higher x_1, x_2, and x_3 values may have, thus, decreased because of the enhanced entanglement of the network that formed (i.e., higher G_N^0 values). The findings for oil and protein content are in good agreement with the results of other studies (Gallegos et al. 1992; Moros et al. 2002) which indicated that G_N^0 or the viscoelasticity of oil-in-water emulsions increases with an increase in oil and protein concentrations.

7.3.5 Textural Characteristics (Firmness)

The statistical models of the firmness of salad dressings prepared with YPPI and DCPI are presented in Eq. 18 and 19, respectively.

$$Y_7 = 0.588 - 0.28x_1 + 0.0003x_3 + 0.003x_1x_3 + 0.02x_1^2 \quad \text{(Eq. 18)}$$

$$Y_7 = 0.5336 - 0.03x_1 - 0.031x_3 + 0.002x_1x_3 + 0.00049x_3^2 \quad \text{(Eq. 19)}$$

Firmness of the dressings prepared with the two protein isolates showed a similar increasing trend with an increase in oil (x_3) and YPPI/DCPI (x_1) contents (Fig. 7.6A, 7.6B). The impact of linear terms for oil was more pronounced than that of pulse proteins for both isolates, based on the sum of squares (Table 7.6). The finding is consistent with the rheological behavior observed.

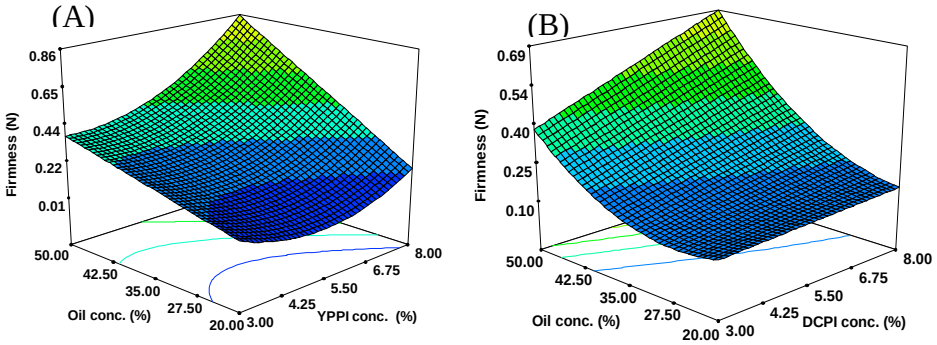
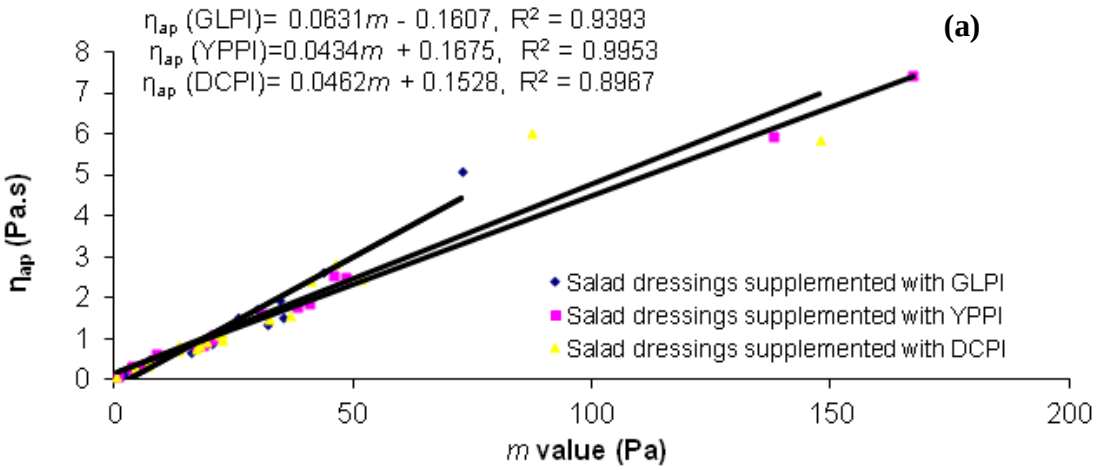


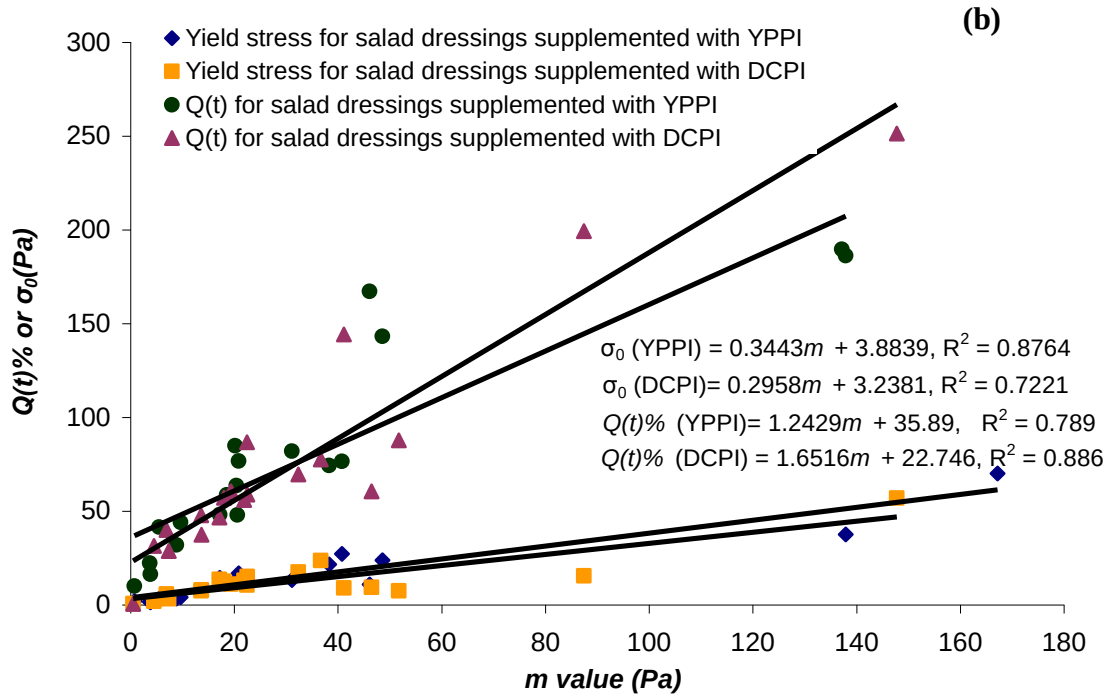
Fig. 7.6 (A). Response surface plots for firmness for salad dressings supplemented with yellow pea protein isolates (YPPI). (B). Response surface plots for *firmness* for salad dressings supplemented with Desi chickpea protein isolates (DCPI). The plots show the interaction effect of two independent variables by holding the other two at their central points.

7.3.6 Relationships Among the Rheological and Textural Parameters

Fig. 7.7 (A, B) shows the relationships among the rheological parameters obtained in this study for dressings prepared with GLPI, YPPI, and DCPI. Linear regressions between η_{ap} and m, σ_0 and m, as well as $Q_{(t)}\%$ and m were performed to identify the

relationships. The linear relationships between firmness and *m*, together with firmness and η_{ap}, were also fitted (R^2>0.90) and are presented in Fig 7.7C. These parameters (η_{ap}, *m*, σ_0, $Q_{(t)}$%, and firmness) obtained from rheological and textural tests can all be explained on the basis of the development of the entangled network among the polymeric molecules and the nature of particle-particle interaction in the salad dressing system. Thus, a high correlation was observed in this study (Fig. 7.7).





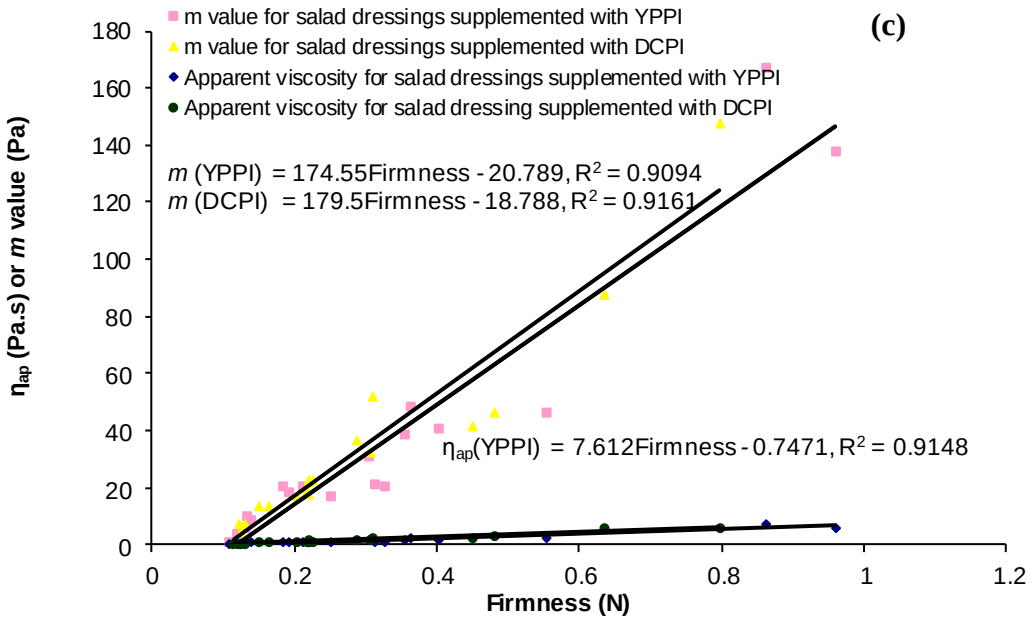


Fig. 7.7 (a, b) Correlation of consistency coefficient (*m* value) as a function of apparent viscosity (η_{ap}), recoverable strain ($Q_{(t)}$%) and yield stress (σ_0) firmness for dressings supplemented with pulse protein isolates. (c) Correlation of the firmness as functions of consistency coefficient (*m* value) and apparent viscosity (η_{ap}). GLPI: salad dressings supplemented with green lentil protein isolates; YPPI: salad dressings supplemented with yellow pea protein; DCPI: salad dressings supplemented with Desi chickpea protein isolates.

7.3.7 Color Characteristics

The color of salad dressings as affected by pulse protein, oil and egg yolk was studied using a tristimulus coordinate system. The *a* value*s, which measure greenness (-ve) and redness (+ve), showed an increasing trend (Fig. 7.8A, 7.8D) for dressings prepared with higher levels of egg yolk and protein (GLPI, YPPI, and DCPI). The 3D plots for DCPI-supplemented dressings are not shown since they exhibited a similar trend to the dressings prepared with YPPI for the three variables studied. The *a* value*, however, decreased with higher oil content especially at higher concentrations of pulse protein (Fig. 7.8B, 7.8E) and egg yolk (Fig. 7.8C, 7.8F). Regression models of *a** values are presented in Eq. 20, 21 and 22. R^2 values of 0.97, 0.89, and 0.98 were obtained for dressings supplemented with GLPI, YPPI, and DCPI, respectively.

$$Y_8 = -0.975 + 0.12x_1 + 0.057x_2 + 0.026x_3 - 0.002x_1x_3 - 0.007x_2^{\ 2} - 0.00029x_3^{\ 2} \quad \text{(Eq. 20)}$$

$$Y_8 = -1.18 + 0.142x_1 + 0.022x_2 + 0.039x_3 - 0.002x_1x_3 - 0.0005x_3^{\ 2} \quad \text{(Eq. 21)}$$

$$Y_8 = -1.43 + 0.149x_1 + 0.167x_2 + 0.03x_3 - 0.014x_1x_2 - 0.0015x_1x_3 - 0.0015x_2x_3 - 0.00031x_3^2$$ (Eq. 22)

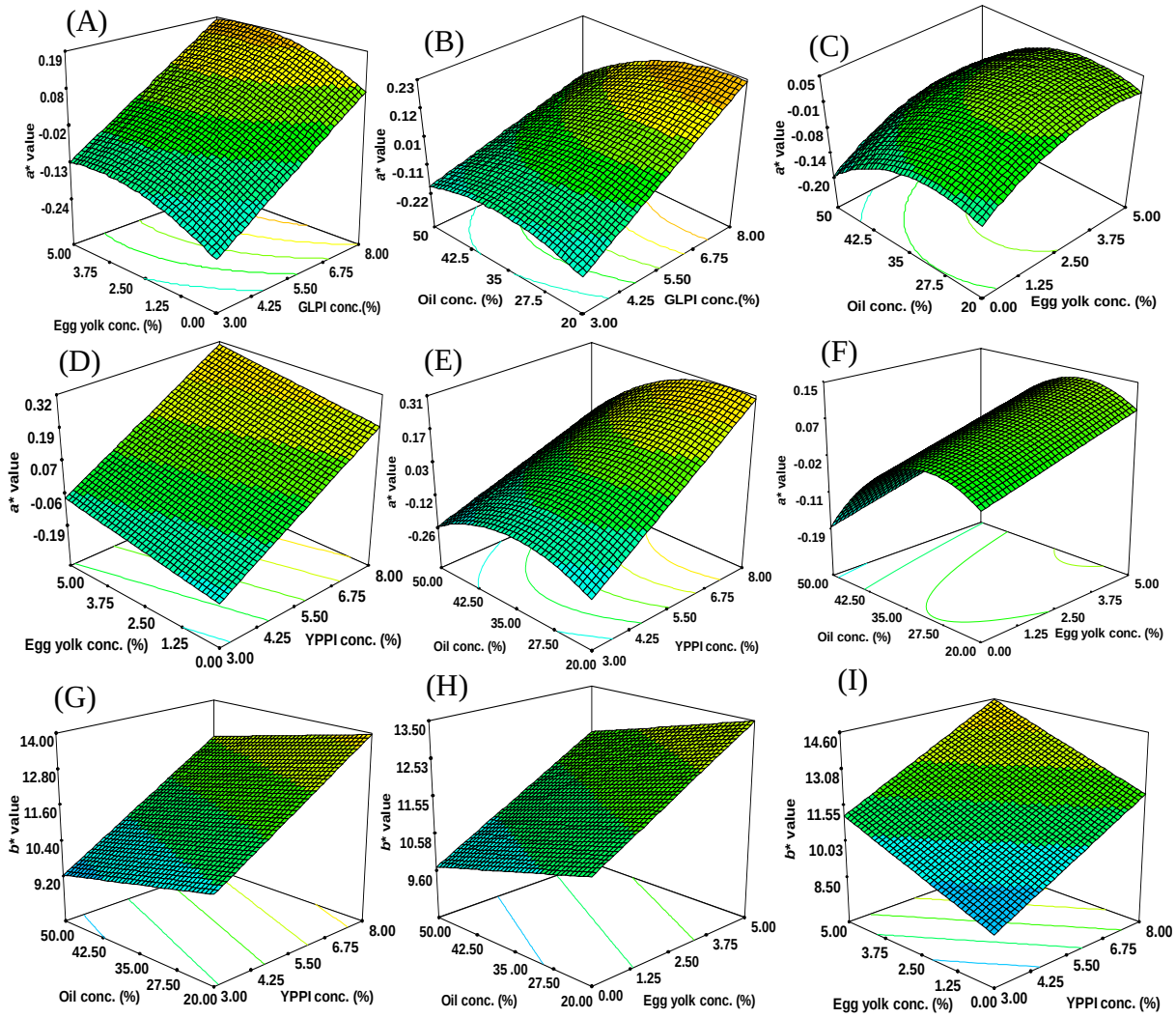


Fig. 7 8 (A-C). Response surface plots for *a* value* for salad dressings supplemented with green lentil protein isolates (GLPI); (D-F). Response surface plots for *a* value* for salad dressings supplemented with yellow pea protein isolates (YPPI). (G-I). Response surface plots for *b* value* for salad dressings supplemented with yellow pea protein isolates (YPPI). The plots show the interaction effect of two independent variables by holding the other two at their central points.

The *b* value* is a measure of the blueness (-ve) and yellowness (+ve) of a material. Linear regression models used to describe dressings prepared with YPPI and DCPI are presented in Eq. 23 and 24.

$$Y_9 = 8.04 + 0.688x_1 + 0.50x_2 - 0.04x_3 \qquad \text{(Eq. 23)}$$

$$Y_9 = 6.544 + 0.4152x_1 + 0.548x_2 - 0.029x_3 \quad \text{(Eq. 24)}$$

For *b** value, the higher the YPPI/DCPI and egg yolk contents in the dressings, the more yellow and more intense the color (Fig. 7.8I). The RSM plots for the *b** values of dressings prepared with DCPI exhibited a trend similar to that for YPPI. In general, the more oil incorporated in the sample, the less yellow and less intense the sample was (Fig. 7.8G, 7.8H).

Chroma, which relates to the color intensity of samples, gives a better description of the spatial position of the measured color. The regression models generated for the chroma of salad dressings prepared with YPPI and DCPI are shown in Eq. 25 and 26.

$$Y_{10} = 8.02 + 0.69x_1 + 0.50x_2 - 0.04x_3 \qquad \text{(Eq. 25)}$$

$$Y_{10} = 6.66 + 0.414x_1 + 0.547x_2 - 0.029x_3 \qquad \text{(Eq. 26)}$$

The response surface plots for chroma showed a similar trend to those for *b** values; hence, they are not shown.

7.3.8 Particle size distribution

A linear response surface model (Eq. 31) was fitted to the Sauter mean diameter *D(3,2)* of the droplets in the dressings made with DCPI.

$$Y_{10} = -86.895 + 39.757x_1 - 14.11x_2 + 4.54x_3 - 0.748x_1x_3 \text{ (Eq. 31)}$$

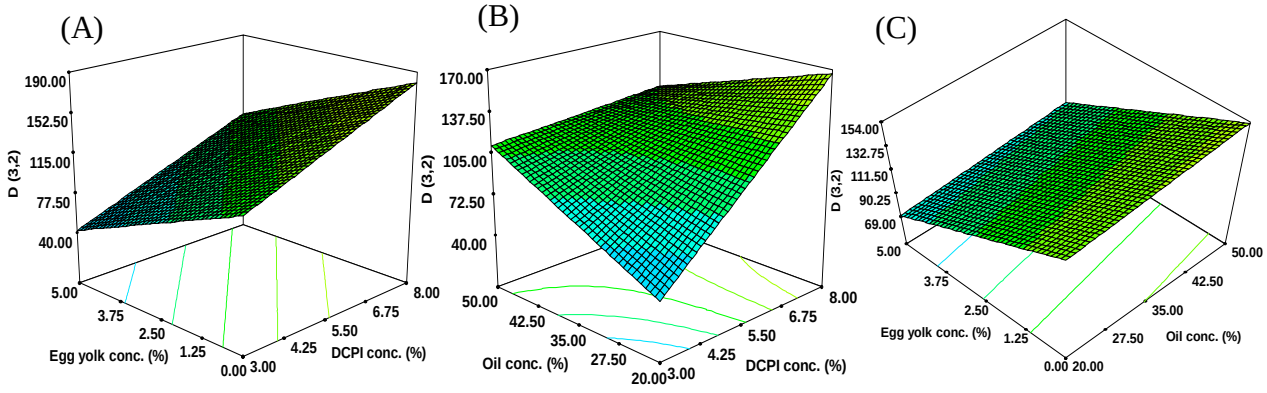


Fig. 7.9 (A-C). Response surface plots for *D(3,2)* value for salad dressings supplemented with Desi chickpea protein isolates (DCPI). The plots show the interaction effect of two independent variables by holding the other two at their central points.

An increase in DCPI (x_1) greatly increased the *D(3,2)* value, especially with low oil (x_3) and egg yolk (x_2) contents, whereas *D(3,2)* decreased with an increase in egg yolk content (Fig. 7.9A, 7.9B, 7.9C). Furthermore, an increase in oil content at lower emulsifier (egg yolk and DCPI) levels yielded higher *D(3,2)* values (Fig. 7.9B, 7.9C). Based on the sum of squares, the effect of the independent variables on *D(3,2)* can be

ranked as follows: protein > egg yolk >oil. The interaction between protein and oil was also significant ($P < 0.01$). Worrasinchai et al. (2006) reported that the droplet size of a full-fat mayonnaise is greater than that of reduced-fat samples. However, an increase in the amount of emulsifiers could decrease the occurrence of this phenomenon (Raymundo et al. 2002). This may explain why fat exerted an effect on *D(3,2)* at lower egg yolk and pulse protein isolate contents, as observed in Fig. 7.9B and, 7.9C).

7.3.9 Optimization and Validation

Multiple-response optimization was applied within the experimental range of the independent variables studied (x_1, x_2 and x_3) for selected dependent variables to be (a) maximized or minimized (Table 7.2); (b) targeted using average values obtained from commercial dressings (Table 7.3); or (c) optimized by keeping the dependent variables as the target values while minimizing the oil content (Table 7.4). The values of the optimum concentration and the predicted responses were determined by superimposing all contour plots for each salad dressing. The first three solutions with relatively high desirability were selected and are summarized in Tables 7.2, 7.3, and 7.4.

The first optimization was carried out to identify dressings with the highest rheological and textural parameters, acceptable color (with minimum a^* value and maximum b^* value), minimum water activity, and minimum particle size (see Table 7.2). Among the rheological parameters, the n value was kept to a minimum as it represents the most pronounced non-Newtonian fluid behavior. Minimum water activity was chosen as it is beneficial as a control mechanism for microorganisms. Salad dressings containing the highest levels of egg yolk (5% w/w), oil (50%, w/w) and YPPI/DCPI (8%, w/w), or a fairly high GLPI content (6% w/w) were predicted to have the highest viscoelastic properties, the most pronounced shear-thinning behavior, the highest color intensity, the yellowest and greenest tint, the lowest water activity, and the smallest Sauter diameter. The desirability for all three pulse protein-supplemented dressings studied was above 0.7. This indicates that the regression models were generally adequate and acceptable for predicting the physical properties of formulated salad dressings within the experimental range. The use of such optimization techniques can provide useful information for designing suitable food emulsions, such as spoonable salad dressing and mayonnaise.

Table 7.2 Results of optimization by desirability function for salad dressings supplemented with green lentil protein isolates (GLPI), yellow pea protein isolates (YPPI), and Desi chickpea protein isolates (DCPI) for selected dependent variables to be either maximized or minimized.

Independent (x) and dependent (Y) variables	Constraints	Lower limit	Upper limit	Optimum formulations			Validation
				1	2	3	
GLPI (x_1) %	in the range	3	8	6.03	5.99	6.08	6.0
Egg yolk content (x_2) %	in the range	0	5	5.00	5.0	5.0	5.0
Oil content (x_3) %	in the range	20	50	50.0	50.0	50.0	50.0
a^* value	minimize	-0.32	0.26	-0.07	-0.07	-0.068	-0.08 ±0.03
m value (Pa.s^n)[a]	maximize	1.74	72.81	61.56	61.30	61.86	64.23 ±1.03
G_N^0 value (Pa) [a]	maximize	1.37	364.5	269.08	270.17	267.78	290.95 ±4.56
a_w value	minimize	0.948	0.97	0.95	0.95	0.95	0.956 ±0.001
η_{ap} value (Pa.s) [a]	maximize	0.11	5.10	3.54	3.53	3.55	4.09 ±0.31
Desirability				0.7165	0.7165	0.7164	
YPPI (x_1) %	in the range	3	8	8.0	7.97	7.93	8.0
Egg yolk content (x_2) %	in the range	0	5	5.0	5.0	5.0	5.0
Oil content (x_3) %	in the range	20	50	50.0	50.0	50.0	50.0
a^* value	minimize	-0.37	0.39	0.049	0.048	0.046	0.03 ±0.02
b^* value	maximize	8.26	15.21	13.93	13.91	13.88	13.79 ±0.77
n value[a]	minimize	0.18	0.47	0.18	0.18	0.18	0.27 ±0.007
m value (Pa.s^n)[a]	maximize	0.76	167.19	140.14	139.39	138.61	119.92 ±0.55
G_N^0 value (Pa) [a]	maximize	3.35	390.2	88.55	90.17	91.86	89.13 ±3.28
Yield stress (Pa)	maximize	1.37	70.0	64.40	63.89	63.36	58.23 ±2.13
$Q(t)$%[a]	maximize	10.0	186.22	129.12	129.42	129.42	113.23 ±4.32
Firmness	maximize	0.11	0.96	0.86	0.85	0.84	0.70 ±0.04
η_{ap} value (Pa.s) [a]	maximize	0.10	7.44	6.62	6.59	6.55	7.30 ±0.17
Chroma	maximize	8.27	15.21	13.91	13.88	13.86	13.79 ±0.13
Desirability				0.70	0.69	0.69	
DCPI (x_1) %	in the range	3	8	8	8	7.97	8.0
Egg yolk content (x_2) %	in the range	0	5	5.0	4.96	5.0	5.0
Oil content (x_3) %	in the range	20	50	50.0	50.0	50.0	50.0
a^* value	minimize	-0.51	0.22	-0.04	-0.04	-0.04	-0.12 ±0.08
b^* value	maximize	6.19	11.68	11.14	11.12	11.13	10.99 ±0.43
n value[a]	minimize	0.16	0.49	0.28	0.28	0.28	0.30 ±0.01
m value (Pa.s^n)[a]	maximize	0.48	147.74	107.74	107.78	107.03	91.40 ±4.12
G_N^0 value (Pa) [a]	maximize	2.49	466.70	414.87	414.96	414.52	379.45 ±3.26
$Q(t)$%[a]	maximize	0.5	251.33	202.66	202.94	201.98	189.73 ±5.63
Firmness	maximize	0.12	0.80	0.69	0.69	0.69	0.73 ±0.13
a_w value	minimize	0.95	0.97	0.95	0.95	0.95	0.96 ±0.002
η_{ap} value (Pa.s) [a]	maximize	0.067	6.037	5.47	5.47	5.46	5.51 ±0.15
Chroma	maximize	6.21	11.68	11.24	11.22	11.23	10.99 ±0.23
D(3,2)	minimize	21.68	209.78	88.34	88.84	88.28	76.18 ±7.08
Desirability				0.7463	0.7452	0.7451	

Table 7.3 Results of optimization by desirability function for salad dressings supplemented with green lentil protein isolates (GLPI), yellow pea protein isolates (YPPI), and Desi chickpea protein isolates (DCPI) for selected dependent variables using averaged target values from commercial salad dressings.

Independent (x) and dependent (Y) variables	Constraints	Lower limit	Upper limit	Optimum formulations			Validation
				1	2	3	
GLPI (x_1) %	in the range	3	8	6.03	5.99	5.97	6.0
Egg yolk content (x_2) %	in the range	0	5	0.07	0.05	0.03	0.07
Oil content (x_3) %	in the range	20	50	50.0	50.0	50.0	50.0
a^* value	target=-0.28	-0.32	0.26	-0.17	-0.17	-0.18	-0.27 ±0.04
m value (Pa.s^n)[a]	target=28.09	1.74	72.81	33.55	33.29	33.11	20.21 ±5.40
G_N^0 value (Pa) [a]	target=98.35	1.37	364.5	98.34	98.35	98.35	99.05 ±1.02
a_w value	target=0.95	0.948	0.97	0.95	0.95	0.95	0.959 ±0.004
η_{ap} value (Pa.s) [a]	target=1.44	0.11	5.10	1.44	1.43	1.41	0.90 ±0.004
Desirability				0.8954	0.8951	0.8948	
YPPI (x_1) %	in the range	3	8	4.29	4.30	4.32	4.0
Egg yolk content (x_2) %	in the range	0	5	0.73	0.76	0.77	0.7
Oil content (x_3) %	in the range	20	50	43.67	43.63	43.54	44.0
a^* value	target= -0.28	-0.37	0.39	-0.13	-0.13	-0.13	-0.32 ±0.07
b^* value	target = 8.26	8.26	15.21	9.50	9.52	9.51	9.74 ±0.52
n value[a]	target = 0.22	0.18	0.47	0.25	0.25	0.25	0.23 ±0.003
m value (Pa.s^n)[a]	target= 28.09	0.76	167.19	32.95	32.97	32.86	27.15 ±3.74
G_N^0 value (Pa) [a]	target= 98.35	3.348	390.2	149.71	149.60	148.48	139.40 ±2.98
Yield stress (Pa)	target= 13.87	1.369	70	11.59	11.31	11.63	12.22 ±0.04
$Q(t)$%[a]	target= 85.70	10	186.22	105.80	105.64	105.29	75.03 ±1.56
Firmness	target = 0.21	0.11	0.96	0.32	0.32	0.32	0.19 ±0.01
η_{ap} value (Pa.s) [a]	target = 1.44	0.10	7.44	1.44	1.44	1.44	1.29 ±0.21
Chroma	target = 8.45	8.27	15.21	9.48	9.50	9.52	9.75 ±0.53
Desirability				0.86	0.86	0.86	
DCPI (x_1) %	in the range	3	8	3.82	3.84	3.89	4.0
Egg yolk content (x_2) %	in the range	0	5	1.99	1.95	1.86	2.0
Oil content (x_3) %	in the range	20	50	46.85	46.45	45.53	47.0
a^* value	target = -0.277	-0.51	0.22	-0.17	-0.17	-0.17	-0.29 ±0.07
b^* value	target = 7.847	6.19	11.68	7.85	7.85	7.85	7.93 ±0.6
n value[a]	target = 0.224	0.16	0.49	0.22	0.22	0.22	0.21 ±0.01
m value (Pa.s^n)[a]	target = 28.1	0.48	147.74	37.63	37.03	35.62	33.33 ±1.26
G_N^0 value (Pa) [a]	target = 98.35	2.49	466.70	145.36	139.35	126.09	135.8 ±2.81
$Q(t)$%[a]	target = 85.7	0.5	251.33	85.70	84.23	80.81	91.60 ±7.95
Firmness	target = 0.207	0.12	0.80	0.36	0.35	0.33	0.21 ±0.002
a_w value	target = 0.953	0.95	0.97	0.96	0.96	0.96	0.959 ±0.001
η_{ap} value (Pa.s) [a]	target = 1.442	0.067	6.037	2.23	2.16	2.02	1.60 ±0.001
Chroma	target = 8.45	6.21	11.68	7.96	7.96	7.96	7.94 ±0.13
D(3,2)	target = 31.57	21.68	209.78	115.7	115.7	115.7	97.28 ±5.76
Desirability				0.88	0.88	0.88	

Table 7.4 Results of optimization by desirability function for salad dressings supplemented with green lentil protein isolates (GLPI), yellow pea protein isolates (YPPI), and Desi chickpea protein isolates (DCPI) for selected dependent variables with an averaged target value of commercial salad dressings and minimal amount of oil.

Independent (x) and dependent (Y) variables	Constraints	Lower limit	Upper limit	Optimum formulations			Validation
				1	2	3	
GLPI (x_1) %	in the range	3	8	6.48	6.46	5.22	5.2
Egg yolk content (x_2) %	in the range	0	5	1.03	1.01	5.00	5.0
Oil content (x_3) %	minimize	20	50	41.59	41.76	32.19	32.0
a^* value	target=-0.28	-0.32	0.26	-0.014	-0.017	0.01398	0.04 ±0.03
m value (Pa.s^n)[a]	target=28.09	1.74	72.81	26.02	26.13	22.68	23.98 ±0.48
G_N^0 value (Pa) [a]	target=98.35	1.37	364.5	98.34	98.35	116.42	96.77 ±4.51
a_w value	target=0.95	0.948	0.97	0.9565	0.9564	0.96197	0.96 ±0.006
η_{ap} value (Pa.s) [a]	target=1.44	0.11	5.10	1.4423	1.4423	1.444	1.00 ±0.03
Desirability				0.666	0.666	0.660	
YPPI (x_1) %	in the range	3	8	5.38	5.39	5.37	5.4
Egg yolk content (x_2) %	in the range	0	5	1.72	1.69	1.76	1.7
Oil content (x_3) %	in the range	20	50	38.29	38.29	38.29	38.2
a^* value	target= -0.28	-0.37	0.39	0.0134	0.0133	0.0133	-0.12 ±0.26
b^* value	target = 8.26	8.26	15.21	10.977	10.966	10.99	10.61 ±0.20
n value[a]	target = 0.22	0.18	0.47	0.228	0.229	0.228	0.248 ±0.016
m value (Pa.s^n)[a]	target= 28.09	0.76	167.19	30.92	31.06	30.78	25.89 ±2.41
G_N^0 value (Pa) [a]	target= 98.35	3.348	390.2	102.57	101.82	103.33	97.65 ±2.73
Yield stress (Pa)	target= 13.87	1.369	70	13.63	13.595	13.66	10.88 ±2.15
$Q(t)$%[a]	target= 85.70	10	186.22	85.70	85.71	85.71	53.11 ±3.23
Firmness	target = 0.21	0.11	0.96	0.29	0.29	0.289	0.22 ±0.03
η_{ap} value (Pa.s) [a]	target = 1.44	0.10	7.44	1.44	1.44	1.44	1.11 ±0.07
Chroma	target = 8.45	8.27	15.21	10.95	10.94	10.96	10.61 ±0.24
Desirability				0.7839	0.7839	0.7839	
DCPI (x_1) %	in the range	3	8	4.73	4.7	4.65	4.7
Egg yolk content (x_2) %	in the range	0	5	1.89	1.88	1.86	1.89
Oil content (x_3) %	in the range	20	50	41.1	41.21	41.38	41.0
a^* value	target = -0.277	-0.51	0.22	-0.10	-0.10	-0.11	-0.26 ±0.18
b^* value	target = 7.847	6.19	11.68	8.34	8.32	8.28	8.42 ±0.06
n value[a]	target = 0.224	0.16	0.49	0.214	0.214	0.214	0.21 ±0.01
m value (Pa.s^n)[a]	target = 28.1	0.48	147.74	30.36	30.42	30.53	27.30 ±1.38
G_N^0 value (Pa) [a]	target = 98.35	2.49	466.70	98.35	98.35	98.35	108.50 ±6.08
$Q(t)$%[a]	target = 85.7	0.5	251.33	76.11	76.08	76.00	76.18 ±7.08
Firmness	target = 0.207	0.12	0.80	0.29	0.29	0.29	0.23 ±0.01
a_w value	target = 0.953	0.95	0.97	0.96	0.96	0.96	0.961 ±0.001
η_{ap} value (Pa.s) [a]	target = 1.442	0.067	6.037	1.68	1.68	1.69	1.319 ±0.02
Chroma	target = 8.45	6.21	11.68	8.449	8.428	8.39	8.42 ±0.41
D(3,2)	target = 31.57	21.68	209.78	115.70	115.70	115.70	114.64 ±7.38
Desirability				0.7958	0.7958	0.7957	

Salad dressing formulated based on the set of combinations of pulse protein, egg yolk, and oil indicated in Table 7.3 was predicted to have similar physical properties to the tested commercial dressing. An additional constraint (i.e., minimizing the oil content) was applied during the optimization, with the aim of achieving a low-fat salad dressing (less than 50% oil). Thus, as shown in Table 7.4, the oil content was optimized as 32.0%, 38.2%, and 41.0% for salad dressings prepared with GLPI, YPPI, and DCPI, respectively.

These contents are lower than the optimized oil levels shown in Table 7.3. The dependent responses obtained nonetheless came close to the values obtained for the tested commercial dressings (Table 7.3).

The desirable dressings based on the predicted optimum formulations identified in the optimization process for each pulse protein-supplemented dressings were prepared and evaluated. The adequacy of the response surface models was checked by comparing the responses (physical properties) between the experimental and the predicted values. Generally, as observed in Tables 7.2, 7.3, and 7.4, the response values obtained from the validation test were quite close to the predicted values derived from the multiple regression models. However, the predicted *Q(t)%* values were generally higher than those obtained in the validation tests for salad dressings prepared with YPPI (Table 7.3, 7.4). The validation tests confirmed the overall adequacy of the response surface models employed in predicting the variation in physicochemical emulsion properties as a function of the main emulsion components.

7.4 Conclusions

The use of egg yolk as an emulsifier in the formulation of salad dressings may be a concern for those with allergies and high cholesterol. Proteins prepared from pulses may be promising value-added replacements for such reduced egg-yolk emulsion-type food products. This study investigated the impacts of using varied levels of pulse proteins to replace egg yolks at different oil concentrations. The response surface results and the validated modeling prediction data provide practical information that could be used by the food industry to develop pulse protein-supplemented salad dressings targeting the low cholesterol, hypo-allergenic markets.

Table 7.5 Analysis of variance for the fit of experimental data to the response surface model for salad dressings supplemented with yellow pea protein isolates (YPPI).

Source	*n* value for YPPI dressing				*m* value (Pa.s^n) for YPPI dressing				η_{ap} (Pa) for YPPI dressing				Yield stress (Pa) for YPPI dressing				*Q(t)* % value for YPPI dressing			
	Coefficient	Sum of squares	DF	*p* value	Coefficient	Sum of squares	DF	*p* value	Coefficient	Sum of squares	DF	*p* value	Coefficient	Sum of squares	DF	*p* value	Coefficient	Sum of squares	DF	*p* value
Model	0.61	0.083	5	<0.0001	132.44	31789.1	4	<0.0001	4.89	61.39	5	<0.0001	33.62	4583.783	6	<0.0001	-57.24	31357.8	1	<0.0001
Linear																				
P	-0.016	0.016	1	0.0006	-13.63	7591.94	1	<0.0001	-0.53	12.74	1	<0.0001	-14.9	1403.277	1	<0.0001				
E	-0.092	0.016	1	0.0006					0.17	1.77	1	0.04	-2.89	370.2	1	0.0003				
O	-0.006	0.027	1	<0.0001	-8.53	15993	1	<0.0001	-0.34	33.276	1	<0.0001	-4.23	1760.863	1	<0.0001	3.73	31357.8	1	<0.0001
Quadratic																				
*P*P*													0.81	121.2	1	0.014				
*E*E*	0.0074	0.011	1	0.0024																
*O*O*					0.10	2624.14	1	0.0057	0.0045	4.76	1	0.0024								
Interaction																				
*P*E*													0.97	292.7	1	0.0008				
*P*O*					0.7	5580.11	1	0.0003	0.028	8.86	1	0.0002	0.24	635.4969	1	<0.0001				
*E*O*	0.0011	0.013	1	0.0014																
		0.0093	9				10			4.084	9			160.2	8			11645	13	
Lack of fit				0.0839 NS		3221.63		0.055NS				0.051 NS				0.064 NS				0.0888 NS
Pure error		0.00094	4			229.53	4			0.31	4			15.3	4	3.82		856.339	4	
R^2		0.89				0.90				0.93				0.96				0.7200		
Adj R^2		0.85				0.87				0.91				0.94				0.70		
Adeq precision		17.31				18.009				20.657				29.57				12.73		

*Coefficients are based on actual data; *P*, *E*, and *O* refer to pulse protein, egg yolk, and oil, respectively. YPPI dressing denotes salad dressing prepared with yellow pea protein isolates. **N**S = nonsignificant.

Table 7.6 Analysis of variance for the fit of experimental data to the response surface model for salad dressings supplemented with yellow pea protein isolates (YPPI).

G_N^0 value (Pa) for YPPI dressing				Firmness (N) for YPPI dressing				*a** value for YPPI dressing				*b** value for YPPI dressing				Chroma for YPPI dressing			
Coefficient	Sum of squares	DF	*p* value	Coefficient	Sum of squares	DF	*p* value	Coefficient	Sum of squares	DF	*p* value	Coefficient	Sum of squares	DF	*p* value	Coefficient	Sum of squares	DF	*p* value
-427.58	127827	4	0.0004	0.5875	0.90	4	<0.0001	-1.18	0.57	5	<0.0001	8.04	49.51	3	<0.0001	8.02	49.47	3	<0.0001
58.73	14205.2	1	0.05	-0.28	0.19	1	0.0009	0.14	0.38	1	<0.0001	0.69	29.58	1	<0.0001	0.69	29.56	1	<0.0001
15.60	15218	1	0.043					0.022	0.029	1	0.037	0.50	15.85	1	0.0002	0.50	15.83	1	0.0001
16.24	48370.9	1	0.0014	0.00038	0.54	1	<0.0001	0.039	0.067	1	0.0036	-0.043	4.07	1	0.0228	-0.043	4.07	1	0.022
				0.022	0.09	1	0.013												
								-0.00049	0.058	1	0.006								
-2.11	50032.7	1	0.0012	0.0027	0.08	1	0.014	-0.0018	0.038	1	0.02								
	38356.4	10			0.13	10			0.064	9				11	0.1783		8.70	11	
			0.13 NS				0.16 NS				0.062 NS		8.36		NS				0.058 NS
	4694.49	4			0.02	4			0.0054	4			1.14	4			0.58	4	
	0.74806				0.86				0.89				0.84				0.84		
	0.6761				0.82				0.85				0.81				0.81		
	13.19				15.78				16.03				19.81				20.03		

*Coefficients are based on actual data; *P*, *E*, and *O* denote pulse protein, egg yolk, and oil, respectively. YPPI dressing denotes salad dressing prepared with yellow pea protein isolates. **N**S = nonsignificant.

Connecting Statement to Chapter 8

In Chapters 5, 6 and 7, the thickening agent xanthan gum was used to stabilize salad dressing emulsions supplemented with pulse fractions. The work described in this chapter explored the possibility of using different types of gums and combinations of gums (including xanthan gum, mixtures of xanthan gum and gum arabic, xanthan gum and propylene glycol alginate (PGA), xanthan gum and pectin, as well as xanthan gum and guar gum) to stabilize lentil flour-supplemented dressings. The level of the supplemented lentil flours (3.5%) was chosen based on the results of the consumer acceptance tests described in Chapter 6. The effect of different levels of gums (0.2%–1.5%) on the rheological, textural, color and particle size characteristics of the supplemented salad dressings was evaluated using response surface methodology. The effect of reductions in the fat content (7.5%–35%) of the dressing formulations on the responses of interest was also examined, in light of the current market trend toward reducing the fat and cholesterol contents of food products. The formulation was optimized using commercial dressing products as a target, and the models were validated under the optimized conditions. This chapter addressed the sixth objective discussed in the "objective of study" section of Chapter 1. The results of this study will be presented as follows:

Ma, Z., Boye, J. I., B. K., Prasher, S. O., Physical properties of lentil-supplemented salad dressings as affected by type and level of hydrocolloids: a response surface methodology study. *Food and Bioprocess Technology*, (to be submitted)

Chapter 8. Physical Properties of Lentil-Supplemented Salad Dressings as Affected by Type and Level of Hydrocolloids: A Response Surface Methodology Study

Abstract

Studies on the nutritional and health benefits of pulses have stimulated interest in using whole pulses and pulse fractions in the development of novel food products. This study focused on the supplementation of salad dressings with lentil flours (3.5 w/w%). The effects of using different types and concentrations of gums as well as combinations of gums (including xanthan gum [XG], mixtures of XG and gum arabic [GA], XG and propylene glycol alginate [PGA], XG and pectin [PE], and XG and guar gum [GG]) on the physical properties (i.e., rheology, texture, color and particle size distribution) of lentil flour-supplemented salad dressings were systematically examined. Response surface methodology was used to study the main effect of the two independent variables (x_1, oil concentration; x_2, gum concentration) on the generated responses and to optimize emulsion composition using commercial salad dressing parameters as a standard. An increase in both gum and oil concentrations enhanced emulsion firmness and viscosity. Large droplets formed in the presence of higher gum concentrations at lower oil content. The validation test showed the overall adequacy of the final response surface models employed to predict properties of the lentil-supplemented salad dressings based on assumed formulations. The results will be useful for the selection of gum blends in the development of novel pulse-based salad dressing formulations.

8.1 Introduction

Growing consumer demand for low-fat and cholesterol-free food products have increased interest in the use of plant-derived food ingredients. Pulses (including dry bean, pea, chickpea, and lentil) are the second largest source of human food and animal feed worldwide (Berrios 2006). Due to their unique nutritional and functional properties, pulse ingredients such as whole flours, protein, starch and fiber fractions

are being explored in various novel value-added processed food products, including bread, meat products, yogurt, pasta and salad dressings (Petitot et al. 2010; Sosulski & Wu 1988; Zare et al. 2011).

Salad dressings encompass a broad range of oil-in-water emulsion products, which vary in fat content (20–65%) and viscosity. Emulsions are thermodynamically unstable systems, and the incorporation of emulsifiers and/or thickening agents is critical for obtaining a stable emulsion with acceptable quality. Emulsifiers are able to decrease the interfacial tension between the oil and water phases, and are able to prevent droplet aggregation by forming a protective coating around the droplets. Thickeners can impart long-term stability by thickening the emulsion system (i.e., reducing the movement of the system) and by forming viscous, ordered networks in the continuous phase to prevent oil separation (Dickinson & Stainsby 1988). Non-starch polysaccharides, one of the most widely used thickeners in food applications, are able to impart textural attributes and mouthfeel to food systems. Most of these are hydrophilic, except for gum arabic and propylene glycol alginate, which are amphiphilic and can prevent droplet aggregation by steric and/or electrostatic forces. The degree of texture modification associated with these hydrocolloids is dependent on the gum concentration used, the molecular weight of the polysaccharides and their functional groups, as well as on the degree of interaction between mixed gums (Ahmed et al. 2005). Mixtures of hydrocolloids may act synergistically to increase viscosity or antagonistically to reduce it. Their interactions have been studied extensively in an effort to generate new functionality or to manipulate the texture and rheology of food systems, with the ultimate goal of replacing expensive polysaccharides by cheaper alternatives (Cairns et al. 1987).

Xanthan gum is the extracellular anionic heteropolysaccharide produced by fermentation of the bacterium *Xanthomonas campestris*. Xanthan consists of pentasaccharide repeating units formed by a (1-4)-β-D-glucan backbone linked to a charged trisaccharide side chain (β-D-mannopyrannosyl-(1-4)-α-D-glucopyrannosyl-

(1-2)- β-D-mannopyrannosyl-6-O-acetate) at the 3 position on alternate glucose residues (Williams & Phillips 2009). Xanthan gum exhibits pseudoplasticity and thixotropy and xanthan gum solutions have high yield stress making them useful for stabilizing salad dressings (Parker et al. 1995). Gum arabic is a natural exudate obtained from the stems of *Acacia senegal.* Structurally, it is a high molecular weight charged heteropolysaccharide consisting of branched galactan heteropolymers. Hydrolysis results in D-galactose with lesser amounts of L-arabinose, D-glucoronic acid and L-rhamnose, along with a small amount of 4-*O*-methyl-D-glucoronic acid (Fennema 1985; Williams & Phillips 2009). Gum arabic solutions are the least viscous of the natural food-grade polysaccharides (Fennema 1985). The structure of gum arabic comprises an approximately 2% protein component which is covalently linked to the polysaccharide moiety (Akiyama et al. 1984). Propylene glycol alginate is a derivative of alginic acid with an average molecular mass ranging from 30,000 to 200,000 Daltons. It is a surface active biopolymer which has both hydrophobic and hydrophilic groups, and could therefore cause a reduction in the surface tension of the oil and water surfaces (Pettitt et al. 1995; Yilmazer et al. 1991). Pectin is a naturally occurring polysaccharide which is present in the primary cell walls of almost all terrestrial plants. It is usually extracted from citric fruits and apples. Pectins are a group of heteropolysaccharides which contain at least 65% by weight of galacturonic acid-based units, which may be partially esterified with a methoxyl group. Pectins are often classified according to their degree of esterification (DE): the ones with DE of up to 50% are classified as high methoxyl pectins (HMP), and those with DE of less than 50% DE are classified as low methoxyl pectins (LMP) (Thakur et al. 1997; Guimarães et al. 2008). Guar gum is a galactomannan polysaccharide which is formed by galactose and mannose molecules. It is obtained from the endosperm of the seed of *Cyamopsis tetragonolobus*. The principal backbone of guar gum is a chain of (1-4)-*β*-D-mannopyranosyl units, with single (1-6)-*α*-D-galactopyranosyl units linked to the principal chain (McCleary et al. 1981; Casas et al. 2000). The interactions between

xanthan and guar gum have been studied extensively (Casas et al. 2000; Tako & Nakamura 1985; Wang et al. 2002), with several types of evidence supporting the existence of intermolecular binding between xanthan and galactomannans.

Salad dressings offer an opportunity to expand the utilization of pulse ingredients. Although several studies have examined the thickening effect of different hydrocolloids and combinations of hydrocolloids used in food product applications (such as gravies, dairy products, food drinks and pet foods), no systematic studies have evaluated the impact of using different types and concentrations of gums or combinations of gums on the physical properties of salad dressings made using pulse ingredients. Owing to the complex composition and structure of food systems, food hydrocolloids may exhibit a wide range of structural transitions and rheological properties under different conditions and at various concentrations in food emulsions. The present study was, therefore, undertaken to investigate the influence of different types and levels of single or hydrocolloid mixtures (including xanthan gum [XG], mixtures of XG and gum arabic [GA], XG and propylene glycol alginate [PGA], XG and pectin [PE] and XG and guar gum [GG]) on the physical properties (i.e., rheological, textural, color and particle size characteristics) of lentil flour-supplemented salad dressing, with the aim of assessing the feasibility of using such pulse ingredients in salad dressing formulations.

8.2 Materials and methods

8.2.l Raw Materials

Whole green lentil flour was provided by the Canadian International Grains Institute (Winnipeg, MB, Canada). Spray-dried egg yolk powder was obtained from Canadian Inovatech Inc. (Winnipeg, MB, Canada). The following gums were kindly provided by Tic Gums (Belcamp, MD, USA): xanthan gum (TIC Pretested® Ticaxan® Xanthan Powder), gum arabic (TIC Pretested® Gum Arabic FT Powder), PGA (TICA-algin® PGA LV powder), guar gum (TIC Pretested® Guar gum 8/24 powder), pectin (TIC Pretested® pectin HM Slow Set Powder). Potassium sorbate was provided

by Nealanders International Inc. (Mississauga, ON, Canada). All other ingredients used in the preparation of the salad dressings were purchased from a local supermarket. All other chemicals used were of analytical grade.

8.2.2 Salad Dressing Sample Preparation

Salad dressings were prepared using different combinations of oil (7.5%–35.0%) and gums (0.2%–0.5%) as described in Table 8.1. The five different types and combinations (ratios) of gums used were XG; XG: GA=0.5:1; XG: PGA= 0.5:1; XG: PE=1:1; XG: GG=1:1. The other ingredients in the recipe were as follows (expressed as a percentage [w/w]): whole green lentil flour 3.5%, vinegar 7.0% (with 5% acetic acid), lemon juice 5.0%, salt 1.0%, and sugar 3.5%. The gums, or combinations of them with appropriate concentrations (0.2%, 0.35%, and 0.5%), and sugar were prepared in advance by mixing the desired amount of dry sample with deionized water while continuously dispersing the gum and sugar solution with a magnetic stirrer at ambient temperature for 2 h. The resulting dispersions were stored overnight to ensure complete hydration prior to adding other ingredients. All other ingredients except oil were then added and mixed until homogeneous. Sodium benzoate (0.02 wt%) was added as an antimicrobial agent. Lastly, canola oil was added and emulsification was achieved using an Ultra-Turrax homogenizer (Model T25, Janke & Kunkel, Ika-Labortechnik, Staufen, Germany) equipped with a S25-18G dispersing tool at 13,800 rpm for 3 min. All measurements were performed the day the samples were prepared.

8.2.3 Texture Profile Analysis

Texture profile analysis was performed with a TA-XT2 texture analyser (Stable Micro System, UK). Salad dressing samples were placed in cylindrical bottles (60 mm diameter × 10 mm height) and were punctured with a cylindrical probe (25 mm diameter × 35 mm height) in a 5000 g load cell at a crosshead speed of 1.0 mm/s. Firmness values were measured and were taken as the height of the peak force during the first compression cycle from the force *vs*. time texturograms.

8.2.4 Rheological Measurements

Rheological measurements were performed with an AR 1000 rheometer (TA Instruments, New Castle, DE, USA) equipped with a plate/cone system. Steady-state flow tests and dynamic oscillatory tests were conducted using a stainless steel parallel plate with a diameter of 4 cm. The gap setting was 1 mm. One tablespoon of sample was placed at the center of the circular plate, and excess sample was removed from the edges of the plate. The linear viscoelastic (LVE) range was determined by performing amplitude sweeps at 1 Hz frequency over a strain range from 0.01% to 1000%. The steady-state flow tests were performed at increasing shear rates (0.02 to 300 s^{-1}). Experimental flow curves were fitted using the power law:

$$\eta = m\gamma^{(n-1)}$$

where n is the flow behavior index (dimensionless), η is the shear viscosity (Pa.s), m is the consistency coefficient ($Pa.s^n$), and γ is the shear rate (s^{-1}). Dynamic oscillatory tests were then performed within the LVE range at 0.1% strain with angular frequency increasing from 0.1 to 100 rad/s. Storage modulus (G′, Pa) and loss modulus (G″, Pa) *vs.* angular frequency (rad/s) were measured for all samples.

8.2.5 Color Measurements

The color of the salad dressing samples was measured with the L^*, a^*, b^* tristimulus system using a Minolta CM-503c spectrophotometer (Minolta Co. Ltd., Osaka, Japan). A fixed amount of salad dressing was poured into the measuring cup, which was then surrounded with a black paper strip. In this color system, L^* is a measure of lightness to darkness (0=black and 100=white), a^* is a measure of redness (+ve) to greenness (−ve), and b^* is a measure of yellowness (+ve) to blueness (−ve). The data were also characterized in terms of chroma (C) and color difference (ΔE) to highlight differences between the samples:

$$C = (a^2 + b^2)^{1/2};\ \Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$$

where ΔL, Δa, Δb are the color differences when compared with the color parameters of milk.

8.2.6 Droplet Size Distribution

The particle size of the salad dressings was analyzed by laser light-scattering using the Mastersizer 2000 MU particle size analyzer (Malvern Instruments Ltd, Worcestershire, UK) with the Hydro 2000MU accessory using distilled water as a dispersant at the speed of 2000 rpm. In keeping with the procedure described by Worrasinchai, Suphantharika et al. (2006), the results were reported as the specific weighted mean diameter (or Sauter mean diameter *D[3,2]*) and the volume-weighted mean diameter (*D[4,3]*), respectively,

$$D[3,2] = \sum n_i d_i^3 / \sum n_i d_i^2 \ , \ D[4,3] = \sum n_i d_i^4 / \sum n_i d_i^3$$

where n_i is the number of droplets of diameter d_i. The specific surface area (m^2/ml) was also calculated using the following equations:

$$Specific\ surface\ area = \frac{6 \times oil\ \ fraction}{D[3,2]}$$

8.2.7 Statistical Analysis

Response surface methodology (RSM) was used to study the main effect of the component variables on the physical properties of lentil flour-supplemented salad dressings prepared with XG, XG-GA, XG-PGA, XG-PE, and XG-GG. A RSM Miscellaneous experimental design with 3^2 full factorials and 5 center runs was used with two independent variables (x_1, oil concentration; x_2, gum concentration) at three coded levels (-1, 0, +1) and five replicates at the center point. A complete design consisted of 14 experimental runs for dressings formulated with each gum. The experimental conditions studied are given in Table 8.1.

The effect of the two independent variables on the following responses was modeled and optimized using RSM: firmness (Y_1), flow behavior index *n* (Y_2), consistency coefficient *m* (Y_3), apparent viscosity η_{ap} (Y_4), plateau modulus G_N^0 (Y_5), *L** value (Y_6), *a** value (Y_7), *b** value (Y_8), ΔE (Y_9), chroma (Y_{10}), *D[3,2]* (Y_{11}), *D[4,3]* (Y_{12}), and specific surface area (Y_{13}). All the response surface plots were generated by keeping one variable constant at the center point and varying the other two variables

within the experimental range. The regression model selected for predicting individual *Y* variables is given by the following equation:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii}{}^2 x^2_{ii} + \sum\sum \beta_{ij} x_i x_j + \varepsilon$$

where *Y* is the predicted response, β_0 is the constant, β_i , β_{ii} , β_{ij} are the regression coefficients, and x_i, x_j are the levels of independent variables (Myers et al. 2009). One-way analysis of variance (ANOVA) was used to evaluate the significant terms in the model for each response. The adequacy of models was ensured by removing the non-significant terms (p>0.05) using a step-wise "backward" multiple reduction algorithm. The overall optimization was carried out within the range of experimental conditions. The responses obtained for five types of commercial salad dressing were used as the target values to predict the optimal formulation during optimization procedure. The optimal levels of the two independent variables leading to the desired properties were obtained for each gum or combination of gums. Design Expert, version 7 (Stat-Ease Inc., Minneapolis, MN, USA), was used to design the experiments, generate the response surfaces and optimize the variables.

Table 8.1 Miscellaneous full experimental design with coded and actual variables and values of experimental data for formulating lentil-supplemented salad dressings prepared using different gum combinations.

Run no.	Oil concentration (%, w/w)	Gum concentration (%, w/w)
1	35 (+1)	1.5 (+1)
2	21.25 (0)	0.85 (0)
3	7.5 (-1)	0.85 (0)
4	21.25 (0)	0.85 (0)
5	21.25 (0)	0.85 (0)
6	7.5 (-1)	0.2 (-1)
7	21.25 (0)	0.85 (0)
8	35 (+1)	0.85 (0)
9	7.5 (-1)	1.5 (+1)
10	21.25 (0)	1.5 (+1)
11	21.25 (0)	0.85 (0)
12	21.25 (0)	0.2 (-1)
13	21.25 (0)	0.85 (0)
14	35 (+1)	0.2 (-1)

Code 0 is for center point of the parameter range studied, 1 for factorial points.

8.3 Results and Discussion

8.3.1 Regression Models

The associated R^2 and adjusted R^2 for selected response (firmness, Y_1) for the 14 × 5 experiments carried out using the Miscellaneous experimental design shown in Table 8.1 were computed. Coefficients for the linear, quadratic, and interaction terms of each model were calculated and tabulated for this response in Table 8.2. Some responses could not be fitted satisfactorily to mathematical models due to lack of fit or poor fit with low R^2 or adjusted R^2 values. The discussion therefore only addresses responses which exhibited statistically significant predicted models (P<0.05) with non-significant lack of fit (P>0.05), as well as relatively high R^2 and adjusted R^2 values.

8.3.2 Regression Models of Firmness

Fig. 8.1 shows the response surfaces of firmness for the dressing emulsions prepared with different gums as a function of the component variables (x_1, oil; x_2, gum). As shown, firmness (Y_1) for all gums and combination thereof exhibited a similar increasing trend in response to increasing oil concentrations (x_1) at higher gum content. For dressings prepared with XG-GA, XG-PGA, and XG-PE (Fig. 8.1b, 8.1c, 8.1d), an increase in the gum concentrations (x_2) yielded higher firmness (Y_1) values at higher oil content (x_1). Whereas dressings with XG and XG-GG showed an increasing trend in Y_1 with an increase in gum content (x_2) at all oil concentrations within the experimental range studied (Fig. 8.1a, 8.1e). The results are consistent with those of Raymundo et al. (2002) which showed that firmness increased with increasing protein, xanthan gum, and oil concentrations in a low-fat oil-in-water emulsion reportedly due to the formation of a firmer, adhesive gel-like structure. The significant second-order regression models with R^2 of 0.982, 0.849, 0.987, 0.956, and 0.974 for dressings prepared with XG, XG-GA, XG-PGA, XG-PE, and XG-GG are presented in Eq. 1, 2, 3, 4, 5, respectively.

$$Y_1 = 0.103 - 0.0014x_1 - 0.031x_2 + 0.0117x_1x_2 + 0.0961{x_2}^2 \quad \text{(Eq. 1)}$$

$$Y_1 = 0.165 - 0.0017x_1 - 0.176x_2 + 0.0049x_1x_2 + 0.076{x_2}^2 \quad \text{(Eq. 2)}$$

$$Y_1 = 0.266 - 0.012x_1 - 0.359x_2 + 0.0111x_1x_2 + 0.0002{x_1}^2 + 0.203{x_2}^2 \quad \text{(Eq. 3)}$$

$$Y_1 = 0.223 - 0.0036x_1 - 0.472x_2 + 0.0129x_1x_2 + 0.27{x_2}^2 \quad \text{(Eq. 4)}$$

$$Y_1 = 0.153 - 0.019x_1 + 0.168x_2 + 0.016x_1x_2 + 0.0004{x_1}^2 \quad \text{(Eq. 5)}$$

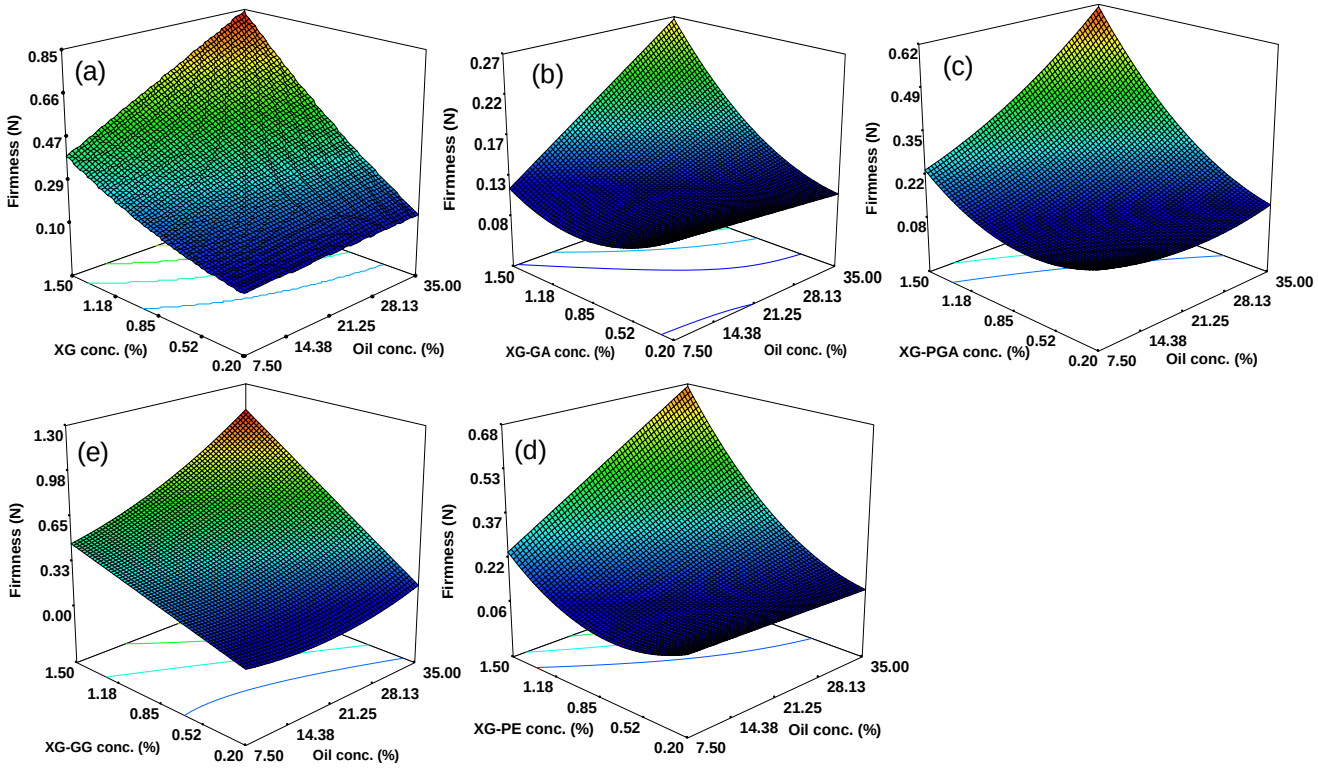


Fig. 8.1 Response surface plots of firmness (N) for dressings prepared with (a) xanthan gum (XG), (b) xanthan gum-gum Arabic (XG-GA), (c) xanthan gum-propylene glycol alginate (XG-PGA), (d) xanthan gum-pectin (XG-PE), and (e) xanthan gum-guar gum (XG-GG).

8.3.3 Regression Models of Rheological Parameters

The regression models for responses, including flow behavior index (*n value*), consistency coefficient (*m value*), apparent viscosity (η_{ap}), and plateau modulus (G_N^0), are discussed below.

8.3.3.1 Regression model of the flow behavior index (*n* value)

The flow behavior index (*n*) obtained using the power law model was less than 1 (0.1 to 0.7) for all the salad dressings indicative of pseudoplastic behavior. An acceptable food emulsion with high viscosity and good mouth feel should have a low *n* value as gum solutions with high *n* value tend to be slimy in the mouth (Szczesniak & Farkas 1962).

The multiple regression equations for dressings prepared with XG, XG-PGA, XG-PE, and XG-GG (R^2 values of 0.9668, 0.755, 0.651, and 0.963, respectively) are presented in Eq. 6, 7, 8, and 9, respectively.

$$Y_2 = 0.506 - 0.003 x_1 - 0.608 x_2 + 0.003 x_1 x_2 + 0.232 x_2^{\,2} \quad \text{(Eq. 6)}$$

$$Y_2 = 0.475 - 0.159 x_2 \quad \text{(Eq. 7)}$$

$$Y_2 = 0.554 - 0.277 x_2 \quad \text{(Eq. 8)}$$

$$Y_2 = 0.588 - 0.007 x_1 - 0.567 x_2 + 0.006 x_1 x_2 + 0.158 x_2^{\,2} \quad \text{(Eq. 9)}$$

From the above equations, it is evident that for dressings prepared with XG-PGA or XG-PE, the flow behavior index (Y_2) was dependent (P<0.05) solely on gum concentration (x_2) (Eq. 7, 8). Quadratic equations (Eq. 6, 9) were obtained for dressings prepared with XG and mixed XG-GG.

As shown in Fig. 8.2, the statistical surface representing Y_2 was dependent on oil (x_1) and gum content (x_2) for dressings formulated with XG and XG-GG (Fig. 8.2a, 8.2d). As predicted by the statistical model, n value was dependent only on gum concentration (x_2) for XG-PGA and XG-PE. (Fig. 8.2b, 8.2c). In general, for all dressings, an increase in the concentration of x_2 was accompanied by an increase in pseudoplasticity, as evidenced by a decrease in the values of the flow behavior index. This suggests that the magnitude of change in apparent viscosity corresponding to the change in shear rate tended to increase as the gum concentration increased in the continuous phase of the emulsion. For the dressing with XG alone, the tendency for the n value to decrease was less pronounced when the gum concentration was between 1.18% and 1.50%, which suggests that the pseudoplastic behavior remained quite stable above a threshold XG concentration in the salad dressing system.

In a dressing system, gums may interact with proteins from egg yolk and pulses, with starch from pulses, as well as with each another. The extent of these interactions, which is dependent on the gum concentration, polysaccharide molecular weight and the presence of functional groups of polysaccharides, may explain the differences in the rheological behavior of the dressing systems prepared with different gums.

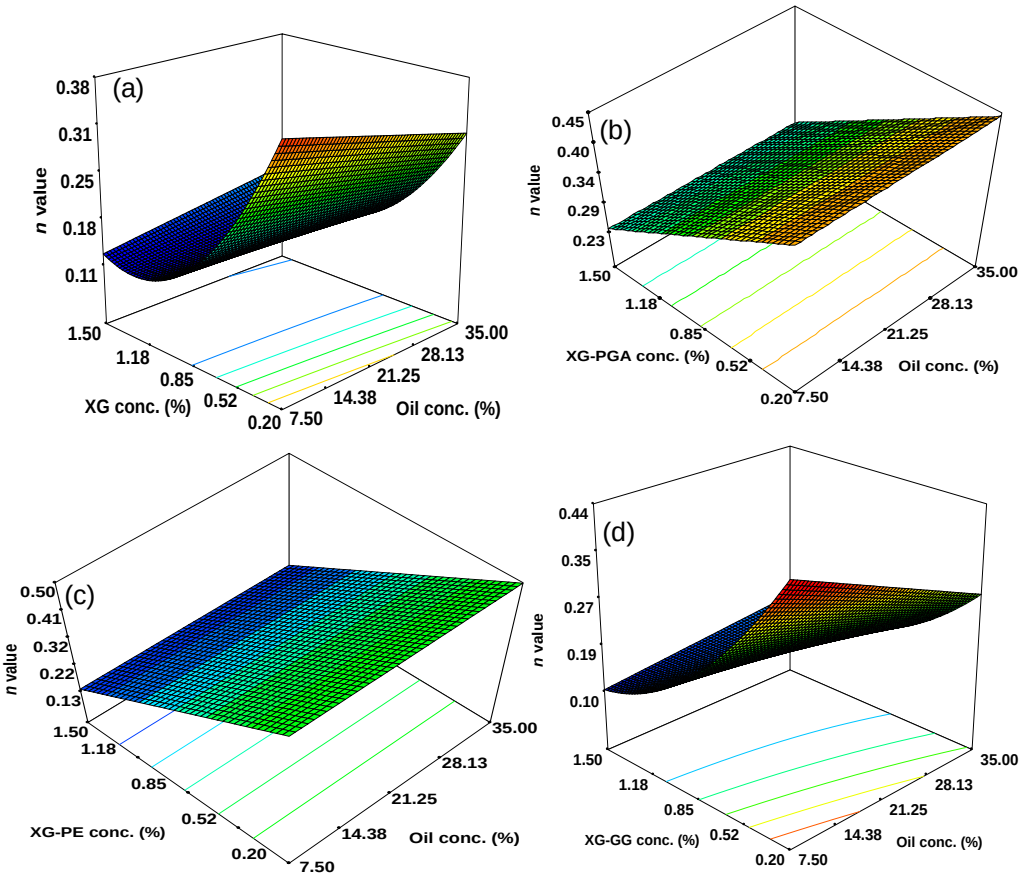


Fig. 8.2 Response surface plots of power law model flow behavior index (*n* value) for dressings prepared with (a) xanthan gum (XG), (b) xanthan gum-propylene glycol alginate (XG-PGA), (c) xanthan gum-pectin (XG-PE), and (d) xanthan gum-guar gum (XG-GG).

8.3.3.2 Regression models of the consistency coefficient (*m* value)

The secondary-order polynomial models fitted to the *m* value (Y_3) are presented in Eq. 10, 11, 12, 13, and 14 for dressings prepared with XG, XG-GA, XG-PGA, XG-PE, and XG-GG (R^2 values of 0.995, 0.977, 0.989, 0.986, and 0.993, respectively.

$$Y_3 = -0.906 - 0.175x_1 + 0.432x_2 + 1.408x_1x_2 + 20.899x_2^2 \quad \text{(Eq. 10)}$$

$$Y_3 = 7.308 - 0.752x_1 - 12.306x_2 + 0.531x_1x_2 + 0.0162x_1^2 + 7.406x_2^2 \quad \text{(Eq. 11)}$$

$$Y_3 = 13.715 - 1.223x_1 - 33.92x_2 + 1.12x_1x_2 + 0.025x_1^2 + 25.183x_2^2 \quad \text{(Eq. 12)}$$

$$Y_3 = 22.081 - 1.937x_1 - 46.443x_2 + 1.797x_1x_2 + 0.035x_1^2 + 29.839x_2^2 \quad \text{(Eq. 13)}$$

$$Y_3 = 8.166 - 0.172x_1 - 79.796x_2 + 2.536x_1x_2 + 78.926x_2^2 \quad \text{(Eq. 14)}$$

All the dressings had similar response surface plots for the *m* value (Y_3) as that shown in Fig. 8.3(a). A synergistic effect between gum(s) and oil can be observed in

the response plots, with maximum *m* values achieved at the highest combination level for the two variables. This behavior was most pronounced for dressings prepared with XG and XG-GG. This observation is supported by earlier reports on hydrocolloids solutions indicating that the magnitude of the consistency coefficient increased with an increase in the concentration of the GA-GG combination, as well as the GA-XG combination (Ahmed et al. 2005). Marcotte et al. (2001) also reported that *m* value increased with increasing gum content for carrageenan, pectin, gelatin, starch and xanthan gum. The increase in the viscous nature of the salad dressings (*m* value) with increasing oil content, especially at high gum concentration(s), is suggestive of the formation of a more compact network structure. Overall, the magnitude of the consistency coefficient (Y_3) was highest for dressings prepared with XG-GG and XG and lowest for XG-GA within the range of concentrations studied. The dressings prepared with XG-PE and XG-PGA had intermediate values.

8.3.3.3 Regression models of the apparent viscosity (η_{ap})

The apparent viscosity (η_{ap}) at a shear rate of 46.16s^{-1}, which as reported is based on the shear rate of the perceived in-mouth thickness of normal fluids, was calculated according to the power law model (Baines & Morris 1988). Multiple regression equations with R^2 of 0.995, 0.855, 0.931, 0.994, and 0.998 for dressings prepared with XG, XG-GA, XG-PGA, XG-PE, and XG-GG are presented in Eq. 15, 16, 17, 18, and 19, respectively.

$$Y_4 = -0.23 + 0.0057x_1 + 0.449x_2 + 0.0366x_1x_2 + 0.623{x_2}^2 \quad \text{(Eq. 15)}$$

$$Y_4 = -0.152 + 0.0032x_1 + 0.151x_2 + 0.018x_1x_2 \quad \text{(Eq. 16)}$$

$$Y_4 = 0.279 - 0.081x_1 + 0.675x_2 + 0.039x_1x_2 + 0.002{x_2}^2 \quad \text{(Eq. 17)}$$

$$Y_4 = 0.623 - 0.0696x_1 - 0.97x_2 + 0.065x_1x_2 + 0.0014{x_1}^2 + 0.806{x_2}^2 \quad \text{(Eq. 18)}$$

$$Y_4 = 0.916 - 0.0796x_1 - 2.452x_2 + 0.118x_1x_2 + 0.0015{x_1}^2 + 2.217{x_2}^2 \quad \text{(Eq. 19)}$$

All the response surface plots were similar to that shown in Fig. 8.3(b,c), with an increase in gum(s) content and oil components yielding a linear (Fig. 8.3b) or non-linear increase (Fig. 8.3c) in η_{ap} (Y_4). The results indicate that the perceived mouth

feel thickness (i.e., η_{ap} at 46.16s^{-1}) increased with increasing fat and hydrocolloid concentrations. The effect of oil on η_{ap} (Y_4) is in good agreement with the findings reported by Wendin et al. (1997), specifically that an increase in fat content increased perceived thickness, fattiness, and toughness during sensory analysis. The flow behavior of emulsions is determined by the colloidal nature of the continuous phase as well as by the average particle size distribution (Coia & Stauffer 1987). An increase in oil (x_1) and hydrocolloid content (x_2) can lead to an increased degree of chain entanglement (i.e., hydrogen bonding with hydroxyl groups) and the distortion in the velocity pattern of the liquid by hydrated molecules of the solute in the emulsion system (Zhang et al. 2011; Azoubel et al. 2005). It is, thus, likely that as the gum concentration increased, larger numbers of high molecular weight molecules formed in the emulsion, increasing the resistance to flow and, therefore, the apparent viscosity of the emulsion.

8.3.3.4 Regression models of the plateau modulus (G_N^0)

Both the storage modulus (G′) and the loss modulus (G″), as measured during the dynamic oscillatory tests, were frequency-dependent and they both increased with increasing frequency (results not shown). G′ was significantly greater than G″ across the tested frequency range for all samples indicative of a predominantly elastic character. A plateau region observed in the oscillation curves at high frequencies may reflect a gel-like structure of a flocculated emulsion with the development of an entangled network (Franco et al. 1997; Raymundo et al. 2002). The statistically significant second-order polynomial models with R^2 of 0.931 and 0.903 for dressings prepared with XG or XG-PGA are presented in Eq. 20 and 21, respectively.

$$Y_5 = 96.826 - 216.49x_2 + 284.53x_2^{\ 2} \quad \text{(Eq. 20)}$$

$$Y_5 = 19.643 - 3.219x_1 - 0.915x_2 + 1.843x_1x_2 + 0.078x_1^{\ 2} \quad \text{(Eq. 21)}$$

Fig. 8.3(d,e) shows the three-dimensional response surface plots for the independent variables and their interactions with the predictive model for the plateau modulus (G_N^0). G_N^0 (Y_5) increased with an increase in gum content, especially for

XG (Fig. 8.3d) which showed significant increases at all oil concentrations within the experimental range studied. For the dressings prepared with XG-PGA, higher oil concentrations resulted in higher increases in G_N^0 (Fig. 8.3e). G_N^0 is a measure of the intensity of the entangled network that develops between the adsorbed and non-adsorbed protein molecules (Franco et al. 1997). The results are supported by the study of Raymundo et al. (2002) and Gallegos et al. (1992), who found that the G_N^0 values of mayonnaise increased with increasing xanthan gum and oil content in commercial mayonnaise and low-fat oil-in-water emulsions based on different formulations, due to an increase in both viscoelastic functions (i.e., G′ and G″).

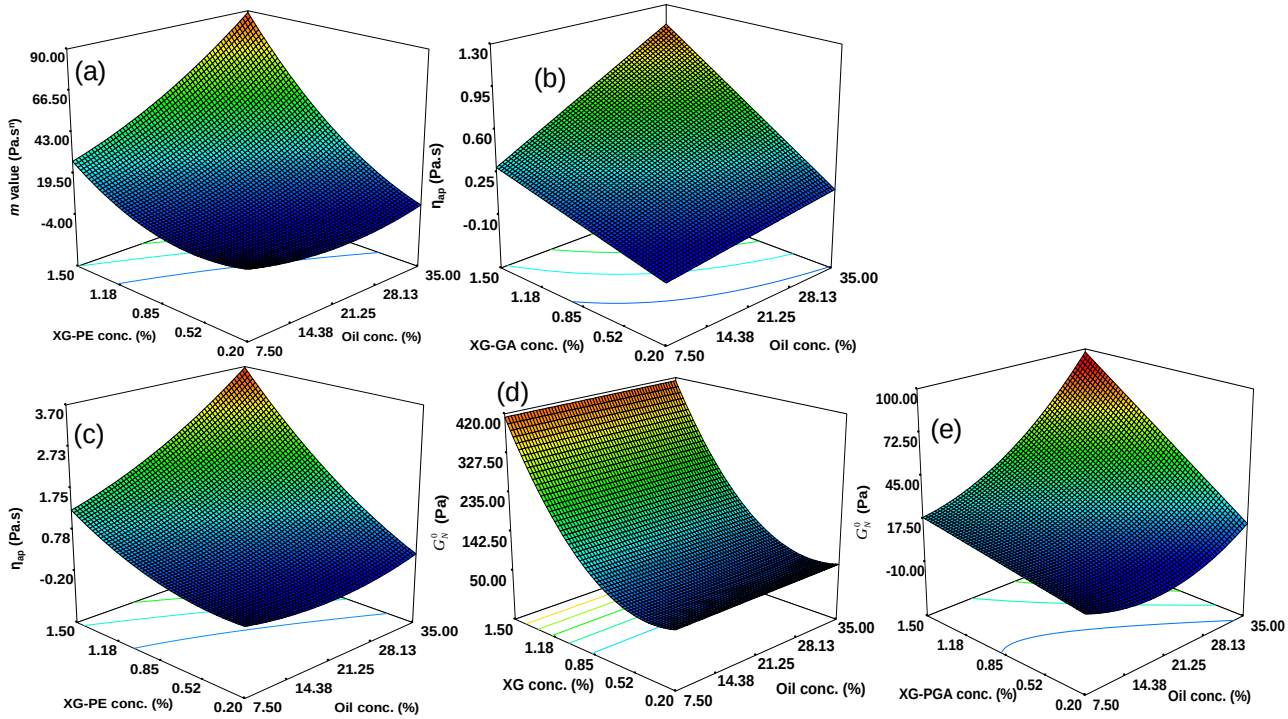


Fig. 8.3 Response surface plots of (a) power law model consistency coefficient (*m* value) for dressings prepared with xanthan gum-pectin (XG-PE), (b) power law model apparent viscosity (η_{ap}) obtained at shear rate of 46.16s^{-1} for dressings prepared xanthan gum-gum Arabic (XG-GA), (c) power law model η_{ap} for dressings prepared xanthan gum-pectin (XG-PE), (d) plateau modulus (G_N^0) obtained during dynamic oscillatory testing for dressings prepared with xanthan gum (XG), (e) G_N^0 for dressings prepared with xanthan gum-propylene glycol alginate (XG-PGA).

8.3.4 Regression Models of Color Parameters (*L**, *a**, and *b** values)

The appearance of salad dressings is very important for consumer acceptability and color has a major impact on perceived appearance. The multiple regression models applied to the *L** value (Y_6) with R^2 of 0.645, 0.696, 0.887, 0.866, and 0.980

for dressings prepared with XG, XG-GA, XG-PGA, XG-PE, and XG-GG, respectively, are given by the following equations:

$$Y_6 = 47.457 + 0.316x_1 + 7.584x_2 \quad \text{(Eq. 22)}$$

$$Y_6 = 43.727 + 0.419x_1 + 6.452x_2 \quad \text{(Eq. 23)}$$

$$Y_6 = 46.777 + 0.484x_1 + 5.753x_2 \quad \text{(Eq. 24)}$$

$$Y_6 = 43.568 + 0.495x_1 + 7.147x_2 \quad \text{(Eq. 25)}$$

$$Y_6 = 55.486 + 0.741x_1 - 5.343x_2 + 0.12x_1x_2 - 0.014x_1^2 \quad \text{(Eq. 26)}$$

In general, the dressings prepared with XG, XG-PGA, and XG-PE yielded response surface plots similar to that shown in Fig. 8.4a for dressings with XG-GA. The positive coefficients for the two independent variables (x_1, x_2) in the equation (Eq. 22-25) indicate that the response variable Y_6 (L^* value) increased with an increase in both variables (oil, x_1; and gum, x_2). In the case of dressings prepared with XG-GG, as observed in Eq. 26, both the oil (x_1) and gum (x_2) concentrations individually and the quadratic terms of x_1, as well as the interaction terms of x_1 and x_2, significantly influenced the lightness of the lentil supplemented dressings which explains the difference in the response surface plot (Fig. 8.4b).

Fig. 8.4 (c-g) and Eq. (27-31) present the response surfaces and statistical models of a^* values (Y_7) for dressings prepared with XG, XG-GA, XG-PGA, XG-PE, and XG-GG, respectively.

$$Y_7 = -0.982 + 0.009x_1 + 0.788x_2 - 0.442x_2^2 \quad \text{(Eq. 27)}$$

$$Y_7 = -1.567 + 0.017x_1 + 1.084x_2 - 0.434x_2^2 \quad \text{(Eq. 28)}$$

$$Y_7 = -0.941 + 0.048x_1 - 0.001x_1^2 \quad \text{(Eq. 29)}$$

$$Y_7 = -1.860 + 0.067x_1 + 1.327x_2 - 0.0158x_1x_2 - 0.001x_1^2 - 0.453x_2^2 \quad \text{(Eq. 30)}$$

$$Y_7 = -0.881 + 0.011x_1 + 0.463x_2 - 0.321x_2^2 \quad \text{(Eq. 31)}$$

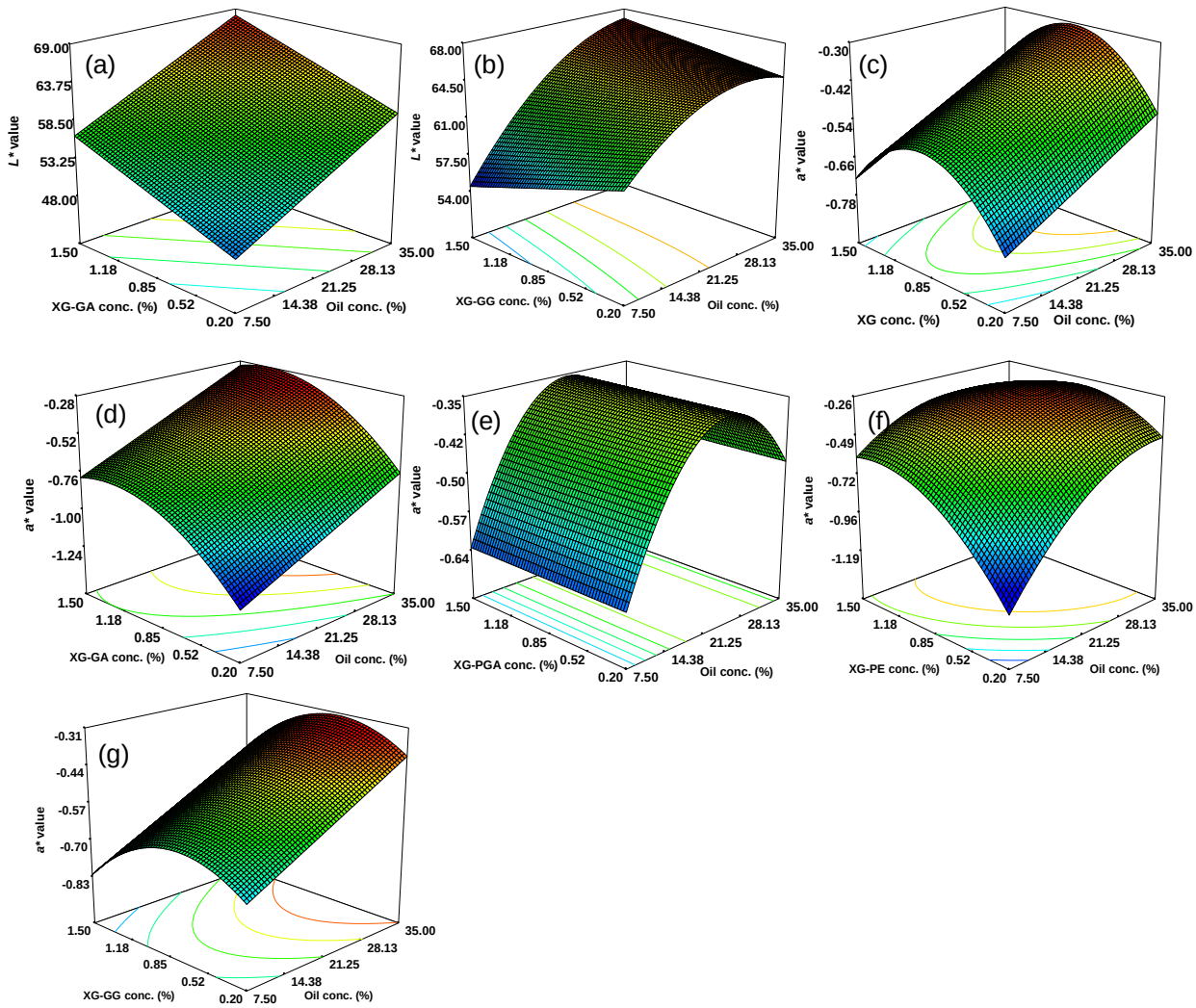


Fig. 8.4 Response surface plots of (a) L^* value for dressings prepared with xanthan gum-propylene glycol alginate (XG-PGA), (b) L^* value for dressings prepared with xanthan gum-guar gum (XG-GG), (c) a^* value for dressings prepared with xanthan gum (XG), (d) a^* value for dressings prepared with xanthan gum-gum Arabic (XG-GA), (e) a^* value for dressings prepared with xanthan gum- propylene-glycol alginate (XG-PGA), (f) a^* value for dressings prepared with xanthan gum-pectin (XG-PE), (g) a^* value for dressings prepared with xanthan gum- guar gum (XG-GG).

In general, the a^* value (Y_7) increased with oil concentration for dressings with XG (Fig. 8.4c), XG-GA (Fig. 8.4d), XG-PE (Fig. 8.4f), and XG-GG (Fig. 8.4g). The effect of oil on Y_7 for the dressing with XG-PGA was quite different (Fig. 8.4e) (i.e., the a^* value increased with an increase in oil up to an oil content of 24.7% but decreased afterwards).

The effects of the oil and gum concentrations on b^* values (Y_8) are shown in Fig. 8.5 (a, b). The multiple regression equations for dressings prepared with XG-PE and XG-GG are presented below (Eq. 32, and 33, respectively).

$$Y_8 = 1.372 + 0.354x_1 + 7.917x_2 - 0.152x_1x_2 - 0.004{x_1}^2 - 1.994{x_2}^2 \quad \text{(Eq. 32)}$$

$$Y_8 = 7.131 + 0.18x_1 - 0.004{x_1}^2 \quad \text{(Eq. 33)}$$

The regression equations and the three-dimensional plots for ΔE values (Y_9) for dressings prepared with XG-PGA and XG-GG are presented in Eq. (34, 35) and Fig. 8.5 (c, d), respectively.

$$Y_9 = 7.506 + 0.142x_1 + 1.782x_2 \quad \text{(Eq. 34)}$$

$$Y_9 = 7.044 + 0.318x_1 - 0.005{x_1}^2 \quad \text{(Eq. 35)}$$

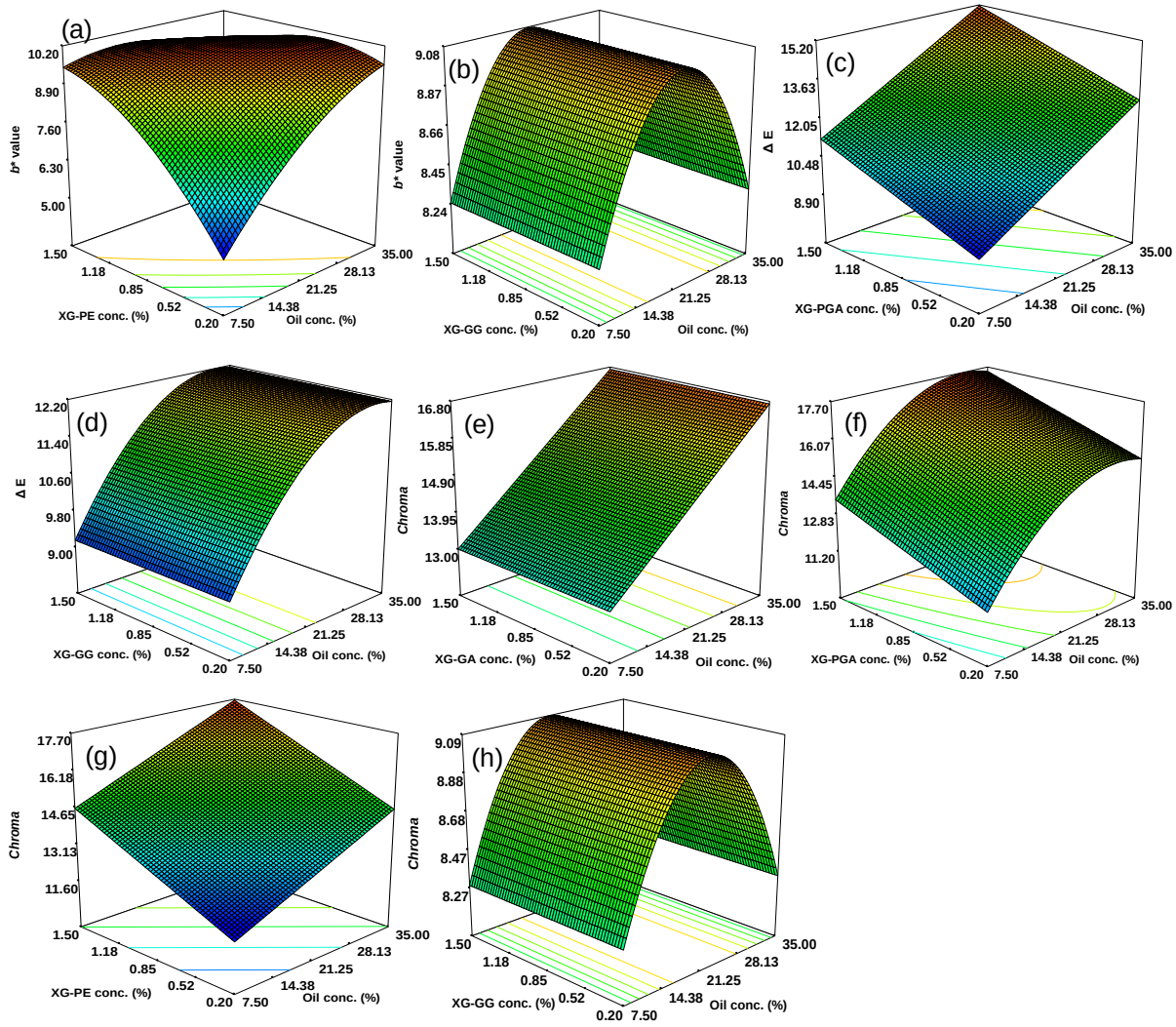


Fig. 8.5 Response surface plots of (a) *b** value for dressings prepared with xanthan gum-pectin (XG-PE), (b) *b** value for dressings prepared with xanthan gum-guar gum (XG-GG), (c) ΔE value for dressings prepared with xanthan gum-propylene glycol alginate (XG-PGA), (d) ΔE value for dressings prepared with xanthan gum-guar gum (XG-GG), (e) chroma for dressings prepared with xanthan gum-gum Arabic (XG-GA), (f) chroma for dressings prepared with xanthan gum-propylene glycol alginate (XG-PGA), (g) chroma for dressings prepared with xanthan gum-pectin (XG-PE), (h) chroma for dressings prepared with xanthan gum-guar gum (XG-GG).

An increase in oil/gum concentration resulted in higher b^* (Y_8) values at lower gum/oil content for dressings prepared with XG-PE (Fig. 8.5a). The Y_8 values increased with an increase in oil up to an oil content of 21.25% but decreased afterwards for dressings prepared with XG-GG (Fig. 8.5b). An increase in oil content generally yielded greater ΔE value (Y_9) for dressings with XG-PGA and XG-GG (Fig. 8.5c, 8.5d).

Chroma, which represents the color intensity of samples, gives a better description of the spatial position of the measured color. Fig. 8.5 (e-h) and Eq. (36-39) present the response surfaces and multiple regression equations for chroma (Y_{10}) for dressings prepared with XG-GA, XG-PGA, XG-PE, and XG-GG, respectively.

$$Y_{10} = 11.986 + 0.136x_1 \quad \text{(Eq. 36)}$$

$$Y_{10} = 7.749 + 0.488x_1 + 1.704x_2 - 0.008x_1^2 \quad \text{(Eq. 37)}$$

$$Y_{10} = 10.366 + 0.109x_1 + 2.325x_2 \quad \text{(Eq. 38)}$$

$$Y_{10} = 7.19 + 0.177x_1 - 0.004x_1^2 \quad \text{(Eq. 39)}$$

As can be observed in Fig. 8.5(f, g), chroma (Y_{10}) increased with oil (x_1) and gum (x_2) concentrations for dressings with XG-PE and XG-PGA, whereas for dressings prepared with XG-GA the response was solely dependent on oil content (Fig. 8.5e). For dressings with XG-GG (Fig. 8.5h), the effect of oil on chroma (Y_{10}) was different; it increased up to an oil content of 21.25% and then decreased.

Thus, the effect of the gums was complex and varied for different gums and combinations thereof. The color characteristics of an emulsion are the result of interactions between light waves and the emulsion. Color is generally governed by the unique composition and structure of food emulsions, and can be modified by the presence of droplets or other particulate matter (McClements 2005f; Chantrapornchai et al. 1998). As reported previously (McClements et al. 1998; Chantrapornchai et al. 1998), the blueness and greenness of an emulsion system are closely related to the droplet size and the droplet concentration of the emulsion system. Due to the interactions that occur between mixed gums and other ingredients, the conformation

that the mixed gums adopt will depend on the particular environmental conditions. This may explain the variation observed in the generated three-dimensional plots and the regression models of color parameters for the dressings prepared with different gums.

8.3.5 Regression Models of Particle Size Parameters

The significant mathematical models (R^2 of 0.935, 0.736, 0.816 and 0.941) of *D[3, 2]* for dressings prepared with XG, XG-GA, XG-PE, as well as XG-GG, are presented in Eq. 40, 41, 42, and 43, respectively.

$$Y_{11} = 23.952 - 0.405x_1 - 10.893x_2 + 4.214x_2^{\ 2} \quad \text{(Eq. 40)}$$

$$Y_{11} = 6.856 + 0.0664x_1 + 9.071x_2 - 0.374x_1x_2 \quad \text{(Eq. 41)}$$

$$Y_{11} = 10.659 - 0.062x_1 + 3.365x_2 - 0.21x_1x_2 \quad \text{(Eq. 42)}$$

$$Y_{11} = 6.847 - 0.407x_1 + 10.212x_2 - 0.287x_1x_2 + 0.01x_1^{\ 2} \quad \text{(Eq. 43)}$$

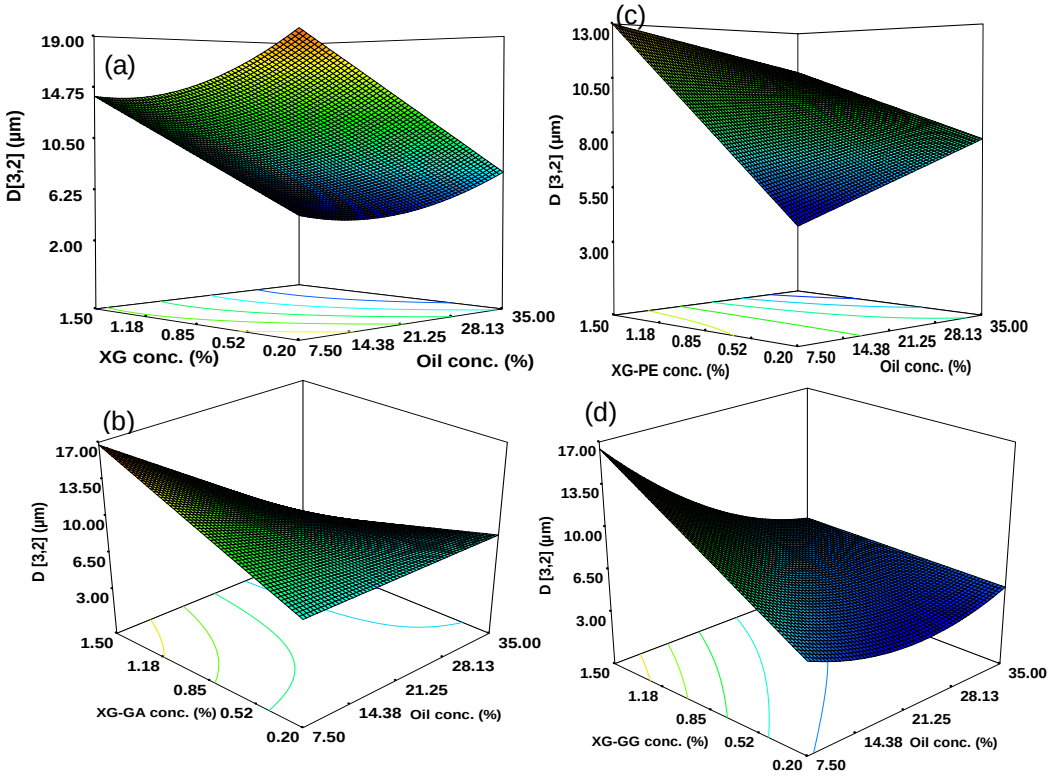


Fig. 8.6 Response surface plots of Sauter diameter D[3,2] for dressings prepared with (a) xanthan gum (XG), (b) xanthan gum-gum Arabic (XG-GA), (c) xanthan gum-pectin (XG-PE), and (d) xanthan gum-guar gum (XG-GG).

The response surface plots (Fig. 8.6) show that an increase in gum concentration generally led to an increase in the Sauter mean diameter *D[3,2]* (Y_{11}), especially at

lower oil concentrations for XG-GA, XG-PE, and XG-GG (Fig. 8.6b, 8.6c, 8.6d). Thus, larger droplets formed in response to increased gum concentrations at lower oil contents. A possible explanation for the observed trends is that the increased viscosity induced by the higher gum concentrations may have reduced the efficiency of the homogenization process and led to the formation of larger oil droplets. An increase in oil occurring at a higher gum content yielded lower values of *D[3,2]* for XG-GA, XG-PE, and XG-GG (Fig. 8.6b-d), while an increased tendency of droplet size with oil content was observed at lower gum concentrations for dressings prepared with XG-PE (Fig. 8.6c). The dressings prepared with XG showed a slightly increased *D[3,2]* (Y_{11}) with increasing oil concentration particular at higher gum content (shown in Fig. 8.6a).

Fig. 8.7 (a-e) and Eq. (44-48) present the three-dimensional plots and multiple regression equations for the volume-weighted mean diameter *D[4,3]* (Y_{12}), with R^2 of 0.828, 0.745, 0.692, 0.952 and 0.819 for dressings prepared with XG, XG-GA, XG-PGA, XG-PE, and XG-GG, respectively.

$$Y_{12} = 205.89 - 4.67x_1 \quad \text{(Eq. 44)}$$

$$Y_{12} = 38.014 - 0.143x_1 + 90.634x_2 - 3.189x_1x_2 \quad \text{(Eq. 45)}$$

$$Y_{12} = 55.968 - 0.755x_1 - 35.443x_2 + 24.404x_2^{\,2} \quad \text{(Eq. 46)}$$

$$Y_{12} = 41.362 - 0.446x_1 + 75.123x_2 - 2.071x_1x_2 \quad \text{(Eq. 47)}$$

$$Y_{12} = 28.865 - 29.085x_2 + 65.402x_2^{\,2} \quad \text{(Eq. 48)}$$

In general, an increase in oil concentration led to a decrease in *D[4,3]* (Y_{12}), except in the case of the dressing prepared with XG-GG (Fig. 8.7e), which was dependent only on gum content, as evidenced by the single variable regression found between gum concentration (x_2) and the response Y_{12} (Eq. 48). An increasing trend in *D[4,3]* (Y_{12}) in response to increased gum content, especially at lower oil concentrations for XG-GA, XG-PGA, XG-PE was also observed in Fig. 8.7.

The regression models generated for specific surface area (Y_{13}) with R^2 of 0.991, 0.988, 0.972, 0.988, and 0.839 for dressings prepared with XG, XG-GA, XG-PGA, XG-PE, XG-GG are presented in Eq. 49, 50, 51, 52, 53, respectively.

$$Y_{13} = 0.085 - 0.01x_1 - 0.058x_2 + 0.0068x_1x_2 + 0.00044x_1^{\,2} \quad \text{(Eq. 49)}$$

$$Y_{13} = 0.07 - 0.001x_1 - 0.08x_2 + 0.0057x_1x_2 + 0.0002x_1^{\,2} \quad \text{(Eq. 50)}$$

$$Y_{13} = -0.03 + 0.0092x_1 - 0.043x_2 + 0.0058x_1x_2 \quad \text{(Eq. 51)}$$

$$Y_{13} = 8.617 - 0.588x_1 - 7.585x_2 + 0.687x_1x_2 + 0.03x_1^{\,2} \quad \text{(Eq. 52)}$$

$$Y_{13} = -0.058 + 0.013x_1 \quad \text{(Eq. 53)}$$

The response surface plots (not shown) for all five dressings showed an increase in response (Y_{13}) with increasing oil (x_1) concentration, as well as an increased trend in Y_{13} with gum content (x_2) particularly at higher oil concentrations (x_1).

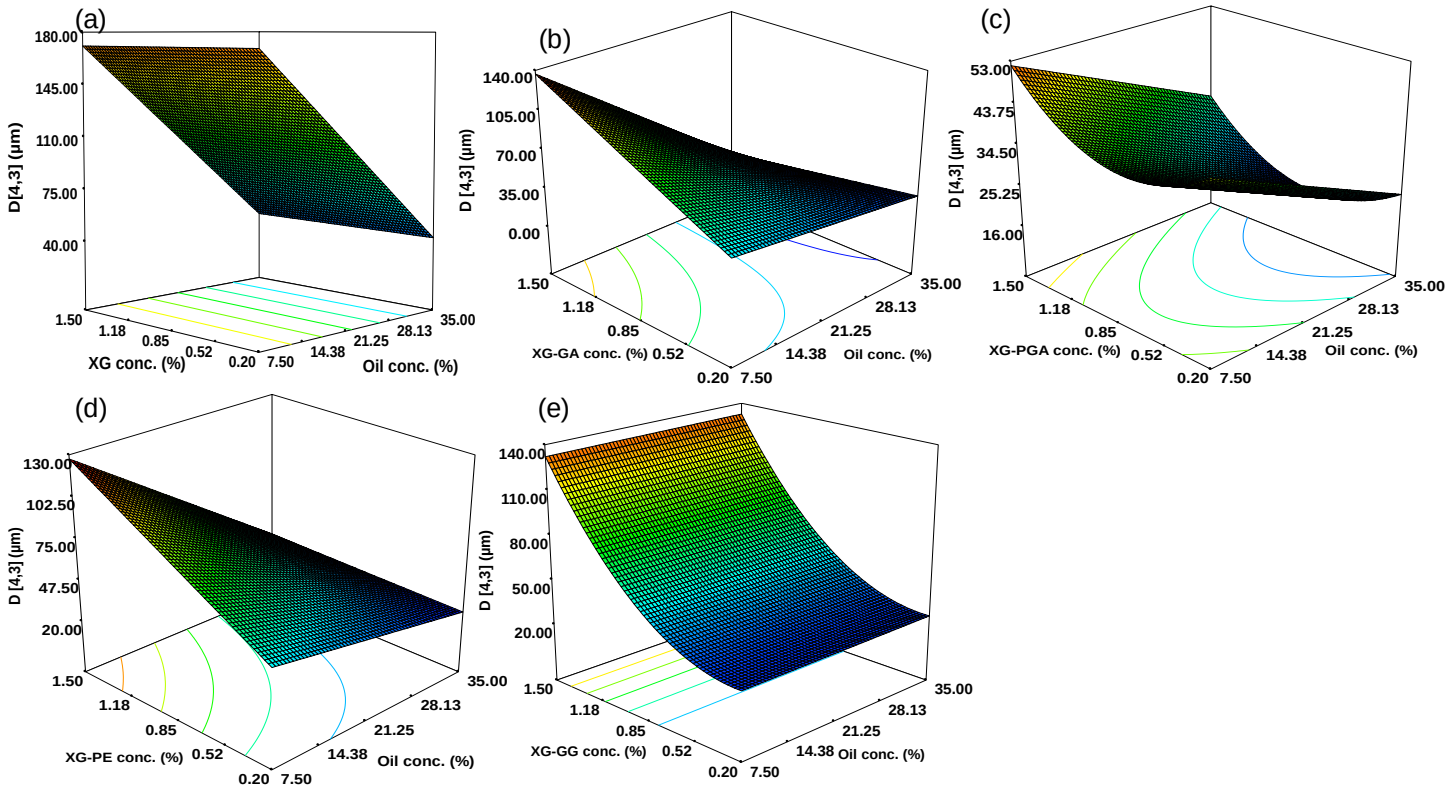


Fig. 8.7 Response surface plots of volume-weighted mean diameter D[4,3] for dressings prepared with (a) xanthan gum (XG), (b) xanthan gum-gum Arabic (XG-GA), (c) xanthan gum-propylene glycol alginate (XG-PGA), (d) xanthan gum-pectin (XG-PE), and (e) xanthan gum-guar gum (XG-GG).

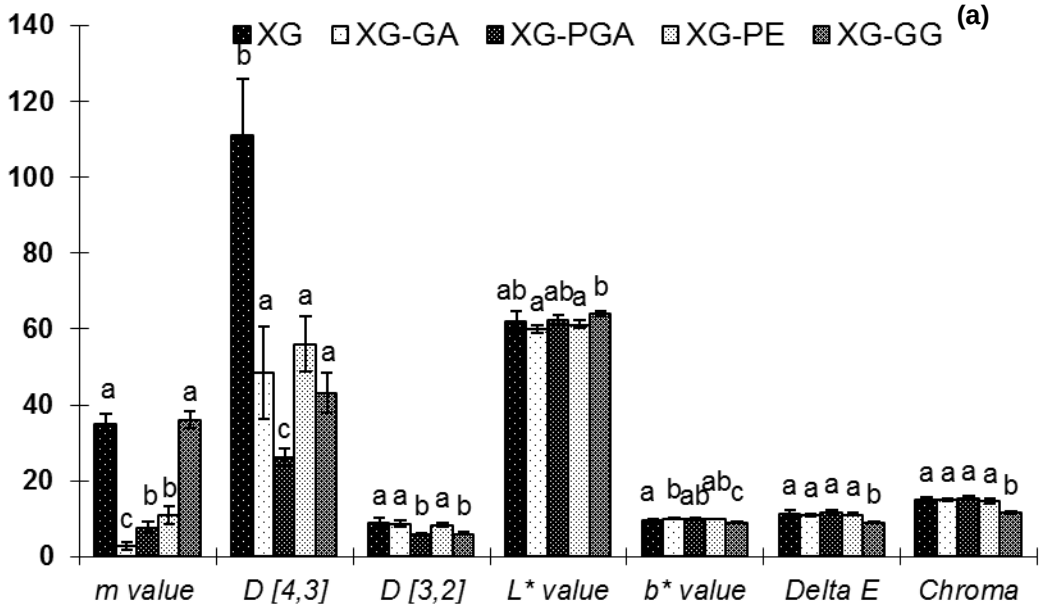
Table 8.2 Analysis of variance for the textural attribute of firmness for salad dressings prepared with five different gums.

Source	Firmness (N) for dressing (xanthan gum)				Firmness (N) for dressing (xanthan gum-gum Arabic)				Firmness (N) for dressing (xanthan gum-PGA)				Firmness (N) for dressing (xanthan gum-pectin)				Firmness (N) for dressing (xanthan gum-guar gum)			
	Coefficient	Sum of squares	DF	*p* value																
Model	0.10	0.50	4	< 0.0001	0.16	0.0259	4	0.0010	0.27	0.26	5	< 0.0001	0.22	0.33	4	< 0.0001	0.15	0.95	4	< 0.0001
Linear																				
O	-0.001397	0.084	1	< 0.0001	-0.0017	0.0067	1	0.0056	-0.012	0.048	1	< 0.0001	-0.0036	0.061	1	0.0002	-0.019	0.19	1	< 0.0001
G	-0.031	0.37	1	< 0.0001	-0.18	0.0081	1	0.0032	-0.36	0.13	1	< 0.0001	-0.47	0.17	1	< 0.0001	0.17	0.65	1	< 0.0001
Quadratic																				
*O***O*									0.00021	0.0046	1	0.0100					0.00044	0.024	1	0.017
*G***G*	0.096	0.0057	1	0.043	0.076	0.0035	1		0.20	0.0209	1	< 0.0001	0.27	0.044	1	0.0006				
Interaction																				
*O***G*	0.012	0.044	1	0.0001	0.0049	0.0076	1	0.0039	0.011	0.0397	1	< 0.0001	0.013	0.053	1	0.0003	0.016	0.082	1	0.0004
Lack of fit		0.0059	4	0.20NS		0.0046	4	0.038NS		0.0024	3	0.0669NS		0.012	4	0.068NS		0.02	4	0.053NS
Pure error		0.0033	5			6E-06	5			0.0009	5			0.00332	5			0.005	5	
R^2		0.9800				0.85				0.99				0.96				0.97		
Adj R^2		0.97				2E+11				0.9796				0.94				0.96		
Adeq precision		38.32				13.27				39.481				24.54				33.73		

*Coefficienst are based on actual data; *O* and *G* refer to oil and gum, respectively; PGA represents for propylene glycol alginate. NS=nonsignificant.

8.3.6 Comparison of Different Gums and Gum Combinations

Fig. 8.8 compares the responses obtained at the center point of the experimental design (i.e., 21.25% oil and 0.85% gum). As can be seen in Fig. 8.8a, the dressings prepared with XG and XG-GG exhibited the significantly highest *m* value (viscous nature), followed by XG-PE, and XG-PGA; the lowest *m* value was obtained for dressings prepared with XG-GA. The observation of the high viscous properties of XG and XG-GG were in accordance with their high pseudoplasticity, as discussed earlier. A previous study (Yaseen et al. 2005) on pure solutions of the hydrocolloids also reported that XG and GG showed the highest viscous properties (η′) in a concentration-dependent manner. The synergistic effect of the two polysaccharides (GG and XG) has been reported extensively in earlier studies. Guar gum can change the helix-coil equilibrium of xanthan gum to a more flexible conformation for efficient binding (Wang et al. 2002). The highly extensive molecular structure and mechanical inflexibility of PGA, as well as potential entanglement with XG, may explain the relatively high viscosity of dressing emulsions formulated with XG-PGA observed in this study.



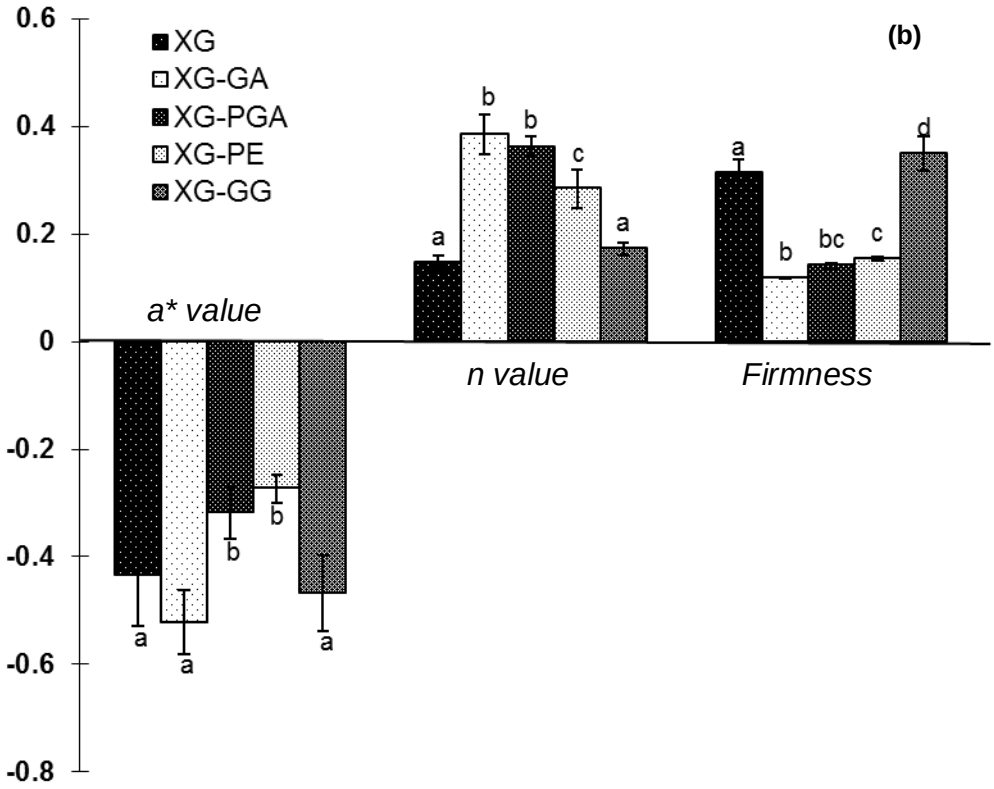


Fig. 8.8 The variation of different responses including (a) consistency coefficient (*m* value), *D[4,3]*, *D[3,2]*, *L** value, *b** value, *Delta E*, Chroma, and (b) *a** value, the flow behavior index (*n* value), firmness for dressings prepared with different gums based on the combination at the center point of the experimental design (21.25% of oil and 0.85% of gum). The results were the average value of six replications. For each parameter, mean values bearing different letters are significantly different ($P<0.05$) as per Tukey's multiple comparison test. In the graphic, XG: xanthan gum; XG-GA: xanthan gum-gum Arabic; XG-PGA: xanthan gum-propylene glycol alginate; XG-PE: xanthan gum-pectin; XG-GG: xanthan gum-guar gum.

The dressing prepared with XG showed the highest ($P<0.05$) volume-weighted mean diameter (*D[4,3]*), whereas the XG-PGA combination had the lowest *D[4,3]* value (Fig. 8.8a). For color, no major differences were observed for dressings prepared with the different gum combinations (Fig. 8.8a) except for dressings prepared with XG-PGA and XG-PE which exhibited significantly higher *a** value, indicative of a less greener hue (Fig. 8.8b). Dressings prepared with XG-GA and XG-PGA gave the highest flow behavior index (*n*) values ($P<0.05$), indicating that they had the lowest pseudoplasticity. In contrast, the dressings prepared with XG and XG-GG had the lowest *n* values (Fig. 8.8b). This observation is in agreement with the findings of Pettitt et al. (1995) and Ahmed, Ramaswamy et al. (2005) who reported that the addition of GA and PGA to fixed levels of XG decreased pseudoplasticity, as evidenced by a significantly increased flow index (*n*). In terms of firmness (Fig. 8.8b), dressings prepared with XG-GG gave the

highest (P<0.05) values, followed by dressings with XG, while dressings formulated with XG-GA and XG-PGA had the lowest values (P<0.05). The dressing prepared with XG-PE had an intermediate value.

8.3.7 Optimization and Validation Tests

A multiple-response optimization was applied within the experimental range of the independent variables studied (x_1 and x_2). For each response (Y) the mean values for tested commercial dressings were used as "target" as shown in Table 8.3. An optimum formulation for the composition of lentil supplemented dressing was obtained by superimposing all contour plots with the predicted equations of each response to yield the mean values for each independent variable (x) and the predicted values for each dependent variable (Y). A set of combinations of oil (x_1) and gum (x_2) concentrations was obtained as presented in Table 8.3.

Table 8.3 Results of optimization by desirability function for salad dressings supplemented with different gum combinations for selected dependent variables with an averaged target value of commercial salad dressings.

Independent (x) and dependent (Y) variables	Constraints	Lower limit	Upper limit	Optimum formulation	Validation
Oil content (x_1) %	in the range	7.5	35.0	25.18	25.18
Xanthan gum (XG) content (x_2) %	in the range	0.2	1.5	0.67	0.67
n value	target=0.22	0.12	0.39	0.18	0.16 ±0.01
m value (Pa.s^n)	target=28.09	0.41	115.56	28.09	25.46 ±0.01
η_{ap} value (Pa.s)	target=1.44	0.039	3.91	1.11	1.02 ±0.04
$G^0_{N'}$ value (Pa)	target=98.35	21.4	444.9	79.46	90.22 ±4.13
Firmness (N)	target=0.21	0.12	0.83	0.29	0.24 ±0.008
D [3,2]	target=8.35	3.87	20.69	8.35	21.13 ±0.66
D [4,3]	target=157.02	23.80	193.78	88.36	97.15 ±9.76
Desirability				0.76	
Oil content (x_1) %	in the range	7.5	35.0	24.5	24.5
Xanthan gum-Gum Arabic (XG-GA) content (x_2) %	in the range	0.2	1.5	1.5	1.5
m value (Pa.s^n)	maximize	0.11	27.11	16.32	10.51 ±0.76
η_{ap} value (Pa.s)	maximize	0.010	1.21	0.81	0.57 ±0.04
Firmness (N)	target = 0.21	0.12	0.30	0.19	0.15 ±0.01
L* value	target= 66.48	45.25	67.99	63.68	62.26 ±1.22
D [3,2]	target= 8.35	5.214	17.08	8.35	21.05 ±0.19
D [4,3]	maximize	21.99	139.25	53.23	63.59 ±2.37
Desirability				0.69	
Oil content (x_1) %	in the range	7.5	35.0	17.5	17.5
Xanthan gum- PGA (XG-PGA) content (x_2) %	in the range	0.2	1.5	1.46	1.46
n value	target = 0.22	0.19	0.45	0.24	0.22 ±0.003
m value	target = 28.09	0.34	64.40	32.81	38.71 ±2.78
η_{ap} value (Pa.s)	target = 1.44	0.04	2.84	1.44	2.02 ±0.12
$G^0_{N'}$ value (Pa)	maximize	0.36	88.44	33.01	50.75 ±4.25
Firmness (N)	target = 0.21	0.12	0.62	0.32	0.35 ±0.02
L* value	target = 66.48	50.31	69.86	63.66	65.43 ±2.06
a* value	target = -0.28	-0.69	-0.23	-0.40	-0.46 ±0.07

D [4,3]	maximize	17.01	55.31	43.07	57.27±7.48
Desirability				0.76	
Oil content (x_1) %	in the range	7.5	35.0	16.51	16.51
Xanthan gum-Pectin (XG-PE) content (x_2) %	in the range	0.2	1.5	1.21	1.21
n value	target= 0.22	0.16	0.73	0.22	0.18±0.002
m value	target= 28.09	0.09	91.96	22.81	30.89±0.05
η_{ap} value (Pa.s)	target= 1.44	0.02	3.72	1.15	1.61±0.01
Firmness (N)	target= 0.21	0.12	0.72	0.24	0.28±0.003
*L** value	target= 66.48	48.77	68.59	60.36	61.03±0.79
*a** value	target= -0.28	-1.10	-0.24	-0.37	-0.29±0.05
*b** value	target= 8.26	5.15	10.17	9.81	9.59±0.4
D [3,2]	target= 8.35	4.25	14.63	9.72	23.97±0.48
D [4,3]	maximize	24.89	125.04	83.35	35.44±6.19
Desirability				0.69	
Oil content (x_1) %	in the range	7.5	35.0	15.7	15.7
Xanthan gum-Guar gum (XG-GG) content (x_2) %	in the range	0.2	1.5	0.94	0.94
n value	target= 0.22	0.099	0.41	0.17	0.17±0.001
m value	target= 28.09	0.53	190.46	38.04	34.93±1.26
η_{ap} value (Pa.s)	target= 1.44	0.056	7.52	1.44	1.44±0.04
Firmness (N)	target= 0.21	0.11	1.09	0.35	0.31±0.005
*L** value	target= 66.48	54.66	67.08	61.65	59.81±0.65
*b** value	target= 8.26	7.62	9.3	8.93	9.15±0.15
Delta E	target= 10.81	8.83	12.80	10.83	10.75±0.23
D [3,2]	target= 8.35	3.92	17.17	8.35	22.21±1.92
D [4,3]	maximize	19.04	143.93	59.67	88.407±2.71
Span	target= 13.04	2.72	22.00	7.05	7.47±1.45
Desirability				0.65	

Dressings were prepared using the optimized formulations and the responses for these were tested. The adequacy of the response surface models was evaluated by comparing the responses for the predicted values and the experimental values. As can be seen in Table 8.3, the response values obtained from the validation tests were quite similar to the predicted values for dressing with each gum, except for the experimental values for *D[3,2]* and *D[4,3]* which were generally higher than the predicted values. In addition, for dressings prepared with XG-PE, the consistency coefficients (*m* value) obtained in the validation tests were different form the predicted ones; however, they were very similar to the targeted values. In general, the results showed that the regression models employed to predict the physical properties of the lentil-supplemented salad dressing emulsions were adequate.

8.4 Conclusions

The validation test showed the adequacy of the models used in predicting dressing behavior and further demonstrated that stable lentil flour-supplemented salad dressings could be prepared with a variety of gum blends and using different oil concentrations.

Perhaps, the most interesting aspect of the study is that the dressings developed had physical properties similar to those of the commercial dressings studied. Overall, the study provides timely and useful information for predicting the textural, rheological, color and particle size characteristics of emulsions that could be used in the development of novel lentil flour-supplemented salad dressings. As there are several types of pulses that can be similarly used in salad dressing preparations, the study further provides a model approach that could be translated to determine ideal conditions for the preparation of other pulse-supplemented salad dressings.

Chapter 9. General Conclusions and Recommendations

9.1 General Conclusion

The development of salad dressings supplemented with pulse flour and pulse fractions represents a novel avenue of research. The research described in this thesis expands scientific knowledge related to salad dressing supplementation with pulse fractions and the effects on the color, rheology, texture, particle size and sensory properties of the supplemented products. The research has economic significance for the development of commercial and industrial applications using pulse fractions with techno-functional and nutritional potential. The following summarizes the specific conclusions of this thesis.

9.1.1 Our preliminary results showed that thermal treatments (roasting and boiling) significantly influenced the functional properties of pulse flours. The flours subjected to the boiling treatment exhibited significantly higher (P<0.05) fat binding capacity, water holding capacity, and gelling capacity, while protein solubility was significantly reduced compared to raw and roasted pulse flours. Both thermal treatments resulted in a significant reduction (P<0.05) in trypsin inhibitor activity ranging from -95.6% to -37.8. The scanning electron microscope studies provided information on differences in the microstructure of flours resulting from thermal treatment which gave rise to significant differences in nutritional and functional properties, as mentioned above. Compared to the study reported in the literature, in which whole seeds were boiled prior to grinding, the heating of the flour solution prior to drying resulted in distinctly different microstructures, with a clear absence of intact starch granules, probably as a result of pre-gelatinization. In general, our results showed that thermally treated pulse flours may have good potential for use as value-added ingredients with enhanced functionality and improved nutritional quality.

9.1.2 During thermal processing (roasted flour, ground roasted seeds, pre-cooked seeds/slurry, pre-cooked freeze/spray-dried seeds), a complex sequence of enzymatic and chemical reactions can occur which resulted in variations in the volatile flavour profiles of selected pulses obtained using a headspace-solid phase

microextraction-gas chromatography-mass spectrometry method. The significant changes in flavour profile induced by cooking were reflected in a decrease in the total peak area, indicating a loss of volatile and/or hydrophilic compounds. The relative peak area of the compounds based on the same chemical family identified in the raw samples underwent either an increase or a decrease after thermal treatments. The formation of a variety of alkylated pyrazines during cooking and drying may be of importance as it may mask some of the beany flavour of pulses. Basic knowledge of the volatile profiles of different pulse varieties and the flavour changes associated with different types of thermal processing could facilitate better quality control of raw materials and help product developers meet flavour-delivery challenges.

9.1.3 Pulse flour supplementation of salad dressings significantly increased (P<0.05) the rheological parameters, including the consistency coefficient (m), yield stress (σ_0) and plateau modulus (G_N^0), pointing to a thickening effect associated with lentil flour supplementation. Pre-boiling of lentil flour significantly increased the thickening effect, which was evidenced by the significantly higher rheological properties observed in dressings supplemented with pre-cooked, ground and freeze/spray-dried seeds. The intensity of the scores for the evaluated attributes (i.e., overall flavour, legume flavour, vinegar, acidity and particle size) ranged from slight to moderate in the quantitative descriptive sensory analysis for dressings prepared with raw and thermally treated lentils. The rheological data were consistent with the sensory results related to firmness. Scanning electron microscopy revealed marked differences in the microstructure of the salad dressings. In addition, lentil supplementation significantly (P<0.05) increased the yellowness hue (b* value) and the total color intensity (chroma) of the salad dressings. All of the dressings maintained acceptable consistency and stability over 28 days of storage. This study demonstrates the potential for using lentil flours as an ingredient in salad dressings, and the results have economic significance as they provide useful information for the commercial and industrial use of pulses, especially thermal treated pulses, as techno-functional food ingredients.

9.1.4 The modelling of the effect of three ingredients (pulse flour, 3.5%–10.5%; egg yolk, 3%–7%; and oil, 20%–50%) on the physical properties of the pulse flour supplemented salad dressing showed that an increase in oil and pulse flour produced, an increase in the consistency coefficient (*m*) within the experimental range studied. The plateau modulus (G_N^0) also increased with the increase in oil and egg yolk. The combination of higher egg yolk or pulse flour at higher oil concentration tended to yield lower *Q(t)* values. An increase in pulse flour and oil content produced a linear decrease in lightness of the supplemented dressings; dressing with higher egg yolk and pulse flour contents produced a more yellow hue. Scanning electron microscope results, which were consistent with the rheological data, showed that the dressings prepared with lower oil and egg yolk contents had a less densely packed network and more loosely aggregated droplets compared with the samples containing higher oil and egg yolk concentrations. Sensory results from consumer acceptability testing of the selected salad dressing samples indicated that dressings with a low yellow pea flour content were significantly ($P<0.05$) preferred over those with high yellow pea flour content as well as those with chickpea flour and low oil content for all attributes except appearance and aroma. Dressings supplemented with high levels of yellow pea flour and chickpea flour with low oil content had significantly lower ($P<0.05$) mean scores for flavour and overall acceptability, indicating that neither formulation has potential for product development. The sensory results also indicate that salad dressings supplemented with whole green lentil, low yellow pea flour content, and chickpea with high oil content are quite promising; however, modifications to the flavour and flavour intensity of each treatment could help to improve overall acceptability scores. This study provides useful information to enable manufacturers to formulate an optimal pulse-supplemented salad dressing product with desirable appearance, and rheological and sensory properties.

9.1.5 An increase in the oil (20%–50%), egg yolk (0%–5%) or pulse protein content (3%–8%) of pulse (lentil, pea and chickpea) protein supplemented salad dressings generated higher values for the rheological and textural properties,

including the consistency coefficient (m), apparent viscosity (η_{ap}), yield stress (σ_0), recoverable strain ($Q(t)\%$), plateau modulus (G_N^0) and firmness These parameters were all closely related to the structural network that formed and the nature of the particle-particle interactions. The colour characteristics of the dressings varied depending on the formulation. Response surface methodology was used to optimize the formulations, and multiple regression equations were developed to describe the effects of the variables on the determined responses. The validation test showed an overall adequacy of the response surface models employed for predicting the properties based on assumed formulations. Overall, the results suggest that pulse protein-stabilized salad dressings with physical properties similar to those of commercial dressing can be designed. Additionally, dressings prepared with pulse proteins behaved similarly compared with those prepared with pulse flours.

9.1.6 An increase in gum content and gum blends (xanthan gum, xanthan gum and gum arabic, xanthan gum and PGA, xanthan gum and pectin, and xanthan gum and guar gum), as well as in oil content generated higher values for firmness, consistency coefficient (m), and apparent viscosity (η_{ap}), as a result of the increased degree of chain entanglement and increased resistance to flow linked to the presence of larger amounts of high molecular weight molecules. An increase in gum concentration was also accompanied by an increase in pseudoplasticity, as evidenced by a decrease in the flow behaviour index (n). The general trend of the effects of oil and gums on the colour characteristics (L^*, a^*, b^*, ΔE, and *chroma*) was complex and varied to different extents depending on the interactions between light waves and the unique composition and structure of food emulsions prepared with different gums. It was observed that large droplets generally formed in the presence of increased gum concentrations at lower oil content. Data at the center point of the central composite design showed that the dressings prepared with xanthan gum and xanthan gum/guar gum tended to show significantly higher ($P<0.05$) values for consistency coefficient (m), and firmness, $D[4,3]$, and a significantly lower value for the flow behaviour index (n). In contrast, dressing formulated with xanthan gum/gum arabic generally

exhibited the lowest value (P<0.05) for m and firmness, but the highest n value. The validation test for the overall adequacy of the models showed that it is possible to produce stable lentil flour-supplemented salad dressings by using a variety of gum blends with different oil contents, and that these dressings can have physical properties matching those of commercial dressing products. This study provides useful information for predicting the textural, rheological, colour and particle size characteristics of lentil flour-supplemented salad dressings based on assumed formulations.

9.2 Recommendation for Future Studies

This research work has produced several important findings. It also identifies some promising avenues for future research and product development, which can be summarized as follows:

1) Study of physical properties (i.e., rheology, particle size, colour and texture), scanning electron microscope observations, and sensory attributes of salad dressings supplemented with pulse fractions as affected by different homogenization methods (such as Ultra-Turrax homogenizer, polytron PT homogenizer, colloidal mills, Warring blender, and extrusion) as well as by various processing conditions (such as agitation speed and time).

2) Study of the changes in the volatile flavour profile (based on the total peak area of the volatile compounds and the total relative peak area based on the same chemical family) of salad dressing supplemented with pulse fractions as influenced by component concentrations (such as oil, pulse fraction, and egg yolk), using central composite design and response surface methodology.

3) Optimization of the formulation for pulse fraction-supplemented salad dressings with varying concentrations of spices and flavouring ingredients, sweeteners, and acidifying ingredients, and their impacts on the sensory and techno-functional properties (rheology, texture, particle size, color and physical stability). Consumer acceptability testing and quantitative descriptive analysis of reduced fat and reduced cholesterol salad dressings.

4) Use of fibre prepared from pulses to supplement salad dressing type emulsions, with the aim of helping to increase dietary fibre consumption in North America. Study on the impact of increased amounts of fibre on the nutritional, sensory, rheology, texture and particle size characteristics of dressing type emulsions.

5) Other rheological models could be applied during steady state flow tests to study the rheological behaviour induced by the addition of pulses and the reduction in oil content, such as the Carreau model, where the critical shear rate for the onset of the shear thinning behaviour (γ_0), the limiting viscosity for the first Newtonian region (η_0), and the slope of the shear thinning region (s) can be compared. Dynamic temperature ramp (4–40 ℃) could also be studied for the supplemented salad dressing emulsions, in order to monitor the rheological behaviour of dressing when it is taken out of the refrigerator for consumption and then put back again.

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