



Technological and Nutritional Studies on Sweet Lupine Seeds and its Applicability in Selected Bakery Products

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Abdelrahman Ragab Abdelrahman Ahmed

Abstract

Abstract

Legume seeds are an abundant source of proteins and, among them; lupine is one of the richest. Lupine seed deserves great interest due to its chemical composition and augmented availability in many countries in recent years. The aim of this research was to study the chemical and nutritional properties of sweet lupine seeds and effects of its addition at different concentration (5, 10 and 15 %) on the dough rheology and backing characteristics of wheat flour enrichment lupine flour and lupine fiber.

Lupine flour showed higher levels of moisture, crude protein, ash, crude fat and dietary fiber than the wheat flour. Conversely, wheat flour showed higher levels of starch. The lupine fiber showed higher levels ash, crude fat and dietary fiber than the wheat flour. Essential amino acids (lysine, threonine, isoleucine, phenylalanine and tryptophane) in lupine flour were higher than those in wheat flour except methionine content which was higher in wheat flour (1.7 g/kg). The lupine flour showed higher levels of total phenolic and total flavonoids than the wheat flour. Conversely, wheat flour showed higher levels of total flavonols. Results clearly indicate that lupine flour exhibited higher antioxidant activity with DPPH and ABTS than the wheat flour. The conventional rheological studies of dough (Farinograph) show clear differences between wheat flour, lupine flour, lupine fiber and their blends. With increasing concentration of lupine flour or fiber, the viscoelastic properties were decrease.

The dough blends demonstrated a deformation-dependent behavior and a distinctive linear viscoelastic behavior in the range of $10^{-4} \leq \gamma \leq 10^{-3}$. The curves slope of storage modulus G' and loss modulus G'' nearly parallel in frequency sweep measurements for all concentrations, G' was much greater than G'' this indicates the distinctive solid state characteristics of all dough's and demonstrated that dough promoted dispersion and not gel-like structure. The level of G' and G'' increase with increasing the lupine proportion. By the temperature sweep, simulated the bakery process, increase from 15 to 90 °C can be detected the changes of material and process as denaturation of proteins, pre-and gelatinization of starch granules and the immobilization of water. A sensory acceptability of the bread or cake is satisfactory up to 10 % concentration of lupine flour or fiber given. Even though deterioration in the structural formation and a weakening of the gluten formation in the dough system after the addition of lupine flour or fiber were detected that the blends have relatively good viscoelastic behavior (viscoelastic properties will be maintained by the dominance of wheat) to bake acceptable, protein enriched consumable and bread.

Finally, the addition of lupine flour or fiber reduced the blood glucose, total cholesterol and total lipid for diabetic rats. Lupine flour or fiber can be used successfully as hypoglycemic agents in bakery products. This could be utilized for the development of composite blends from locally produced lupine at small scale industry level as value-add products.

Keywords: Lupine; Nutrition Value; Chemical Composition; Rheological Properties; Baking Applications; Diabetic Rats

Abstract

Der Einsatz von Leguminosenprotein für die menschliche Ernährung stellt eine hochwertige natürliche Ressource dar. Die Lupineinhaltsstoffe sind auch für Entwicklungs- und Schwellenländer als eigenständige landwirtschaftliche Ressourcen verfügbar und sichern die Proteinversorgung mit hohem Gesundheitsbezug. Ziel dieser Arbeit ist es, die physio-chemischen und ernährungsphysiologischen Eigenschaften von Süßlupinen beim Einsatz mit einer Konzentration von 5, 10 und 15 % auf Weizenmehl zu untersuchen. Die Veränderungen der Teigrheologie und der Backeigenschaften bei Zugabe von Lupinenmehl und Lupinenfasern sowie die nutritive Bewertung sind Gegenstand der vorgelegten Arbeit.

Der Einsatz von LF als Zumischung zum WF bewirkt eine Veränderung des Wasserbindevermögens, von Rohprotein, Asche, Rohfett und dem Ballaststoffgehalt. Während Weizenmehl einen höheren Eiweißgehalt aufweist, besitzen Lupinenfasern höhere Anteile an Asche, Rohfett und Ballaststoffen. Ebenso erhöht im Süßlupinemehl ist der Anteil essentieller Aminosäuren wie Lysin und Threonin. Aus ernährungsphysiologischer Sicht ergänzen sich Weizen- und Süßlupineneiweiß im Aminosäurespektrum. Weiter weist LF einen höheren Gehalt von phenolischen Flavanoiden auf. Ebenso ist eine höhere antioxidative Aktivität am Beispiel von DPPH und ABTS als im WF vorhanden.

Bereits konventionelle rheologische Untersuchungen von Teigen mit dem Farinograph weisen deutliche Unterschiede zwischen WF, LF und L-fasern im untersuchten Konzentrationsbereich auf. Mit zunehmendem Anteil LF wird eine Abnahme der viskoelastischen Relation festgestellt. Die mit LF und -fasern angereicherten Teige zeigen ein linear- und viskoelastisches Verhalten nur in einem schmalen Bereich von $10^{-4} \leq \gamma \leq 10^{-3}$ auf. Der parallele Verlauf der G' und G'' - kurven mit Anstieg im Frequenzsweep weist für jede durchgeführte Messanstellung eine Dispersion nach. Die Backeigenschaften werden mit Hilfe von Temperatursweeps untersucht. Zusätzliche Kriechtestmessungen liefern Kennwerte zur Ruhescherviskosität, zum Gesamtschubmodul und zur Komplianz. Die Level von G' und G'' sinken generell bei Erhöhung der LF- Zugabe, was auf eine Inkompatibilität von Weizen- und Leguminoseneiweiß hindeutet. Als akzeptable Grenz-konzentration für die Implementierung wird eine Zumischung von 10 % anerkannt, um die Teig- und Backwarenstruktur zu sichern. Unter diesen Bedingungen ist auch die sensorische Akzeptanz von Backwaren wie Brot und Kuchen gesichert.

Die schlechtesten Strukturen werden bei Einsatz von L-fasern mit hohem Anteil an Polysacchariden bzw. Polyphenolen erhalten. Gerade der Einsatz von Lupinenfasermaterial als Ballaststoffmaterial bewirkt aus diesem Grunde eine Absenkung des Blutzuckergehaltes, des Gesamtcholesteringehaltes, des Lipidgehaltes sowie eine antidiabetische Wirkung, die bei umfangreichen Untersuchungen mit diabetischen Ratten nachgewiesen wurden. Die Ergebnisse dieser Arbeit können in Mehlmischungen aus lokal produzierten Lupinen in Schwellen- und Entwicklungsländern genutzt werden. Die problematische Eiweißversorgung gerade von ärmeren Bevölkerungsschichten kann so bei Beibehaltung bekannter Weizenbacktechnologien perspektivisch besser gesichert werden.

Schlüsselwörter: Lupine; Nährwert; chemische Zusammensetzung; rheologische Eigenschaften; Backanwendungen; diabetische Ratten

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List of Symbols

Symbol	Description	Unit
A	Area	m ²
a*	Redness	-
b*	Yellowness	-
C	Concentration	mg/g
d ₁₀	Round of 10 % of the particle spectrum	m
d ₅₀	Round of 50 % of the particle spectrum, Median	m
d ₉₀	Round of 90 % of the particle spectrum	m
DDT	Dough development time	min
F	Force	N
FU	Farinograph unit	FU
f	Frequency	Hz
G	Rigidity modulus	Pa
G'	Storage modulus	Pa
G''	Loss modulus	Pa
G*	Complex storage modulus	Pa
G ₀	Modulus of rigidity	Pa
h	Plate distance	m
J	Compliance	Pa ⁻¹
J _e	Elastic part of compliance,	Pa ⁻¹
J _{max}	Maximum viscoelastic compliance	Pa ⁻¹
L*	Lightness	-
m	Mass	kg
MTI	Mixing tolerance index	FU
N	Normality, express the concentration of a solution	-
R ²	Regression factor	-
V	Volume	m ³
x	Exponent used to determine the storage modulus	Eqe
y	Exponent used to determine the loss modulus	Eqe

List of Greek Symbols

Symbol	Description	Unit
γ	Deformation	-
$\dot{\gamma}$	Shear rate	s^{-1}
$\dot{\gamma}_3$	Steady-state shear rate	s^{-1}
γ_e	Elastic recovery	-
γ_{\max}	Maximum deformation	-
γ_v	Viscous recovery	-
γ_z	Destroyd deformation	-
δ	Loss angle	$^{\circ}$
$\tan \delta$	Loss factor	-
η	Viscosity	Pa.s
η_{app}	Apparent viscosity	Pa.s
η_0	Zero shear viscosity	Pa.s
η'	Real viscosity	Pa.s
η''	Imaginary viscosity	Pa.s
$ \eta^* $	Complex viscosity	Pa.s
λ	Relaxation time	s
τ	Shear stress	Pa
$\dot{\tau}$	Deviation stress rate	Pa/s
τ_0	Yield point	Pa
φ	Relative humidity	%
ω	Angular frequency	Hz

List of Abbreviations

Abbreviation	Clarification
AACC	American association of cereal chemistry
Ab	Absorbance
ABTS	2, 2'-azinobis (3-ethylbenzthiazoline- sulphonic acid)
ACNFP	Advisory Committee on Novel Foods and Processes
AHC	Australian Health Info Center
ANN	Artificial neural network
ANOVA	Analysis of variance
AOAC	Association of official analytical chemists
ARC	Center of excellence for integrative legume research
BSA	Bovine serum albumin
CS	Chemical score
Control A*	Normal rats fed on basal diet
Control B*	Diabetic rats fed on basal diet
DDT	Dough development time
DPPH	1,1-diphenyl-2-picrylhydrazyl
DW	Dry weight
EAA	Essential amino acid
EAAI	Essential amino acid index
FAO	Food and Agriculture Organization
FU	Farinograph unit
FER	Feed efficiency ratio
GAE	Gallic acid equivalents
HPLC	High-performance liquid chromatography
ICC	International Association for Cereal Chemistry
IDF	Insoluble dietary fibre
ISO	International Standard Organization
LF	Lupine flour
L-fiber	Lupine fiber
LSD	Least significant difference

List of Abbreviations

LVR	Linear viscoelastic region
MTI	Mixing tolerance index
P	Probability
PER	Protein efficiency ratio
QE	Quercetin equivalent
SAS	Statistical analysis system
SDF	Soluble dietary fibre
TDF	Total dietary fibre
TPC	Total phenolic content
TSM	Temperature sweep measurements
UDS	Universal dynamic spectrometer
WF	Wheat flour
WHO	World Health Organization

1. Introduction

1.1. Background of the study

Leguminosae is one of the three largest families of flowering plants, comprising nearly 700 genera and 18,000 species. The legumes used by humans are commonly called food legumes or grain legumes. The food legumes can be divided into two groups, the pulses and the oilseeds. Pulses group consists of dried seeds of cultivated legumes, which have been eaten for a long time (Asian Productivity Organization, 2003).

In general, cereals and legumes take a large place of human food consumption. Animal proteins being more expensive, especially people in developing countries depend largely on plant to fulfill their protein requirements. Grain legumes alone contribute to about 33 % of the dietary protein nitrogen needs of humans. Moreover, it is also a good source of minerals (Kirmizi and Guleryuz, 2007). Besides being a good source of nutrition, there is a considerable interest in the relationship between plant-based diets and the prevention of certain human diseases, in which increased levels of radicals are implicated. Likewise legumes seem to be responsible for improving health and can prevent chronic diseases (Frias et al., 2005). Cholesterol-free legumes in combination with their low sodium content form a good food stuff not only for people living in developing countries but also for those living in industrialized nations (Sebastiá et al., 2001).

Lupine has been used as a source of protein and oil since ancient times. Currently interest in a wider utilization of this legume seed is rising. This is mainly due to its similarity with soybeans as a high source of protein and to the fact that it can be grown in wider climatic range. Moreover; its adaptation to poor (i.e. leached) soil, makes it economically feasible (Sujak et al., 2006). Lupine is commonly consumed as a snack in the Middle East and is coming into use as a high-protein soy substitute in the other parts of the world (Kurzbaum et al., 2008).

Out of the many species of lupine, *Lupinus albus* native to Mediterranean area is agriculturally important (Kurzbaum et al., 2008). During the past 3000 years, *L. albus* has been used as a minor crop in the old and new world. Human movement and de-centralization has helped *L. albus* to diversify considerably in the primary and secondary centers of its origin. This diversification has helped for the development of interesting characteristics of the plant. These include cold and disease tolerant, having improved leaflet and seed size and shape, flower and

seed color, and degree of apical and branch dominance characteristics of the plant (Noffsinger and Van Santen, 2005).

Lupine flour is widely considered an excellent raw material for supplementing different food products owing to its high protein content (Sironi et al., 2005) and is largely used as eggs substitute, for example in cakes, pancakes, biscuits, or brioche (Tronc, 1999), and has been added to spaghetti (Rayas-Duarte et al., 1996), pasta, crisps (Lampart-Szczapa et al., 1997), and bread (Dervas et al., 1999). It has been also used as a butter substitute in cake, brioche, and croissant (Tronc, 1999). Lupine does not contain gluten, thus it is sometimes used as a functional ingredient in gluten-free foods (Scarafoni et al., 2009). Lupine kernel fiber has also a potential as a human food ingredient as it has been used in the production of fiber-enriched baked goods and pasta (Smith et al., 2006).

From the 1st step in the bread making process (blending of flour and water with other ingredients) to the final step (baking), the ingredients used undergo a number of physical and chemical changes (Faridi and Faubion, 1990) such as evaporation of water, formation of porous structure, volume expansion, protein denaturation, starch gelatinization, crust formation etc. take place during bread baking. Crumb structure of cereal products like bread is a very important factor determining the sensorial quality as may be quantified for example as texture or crispness as well as storage and staling properties (Regier et al., 2007).

1.2. Statement of the problem

Many researchers have paid more attention towards the possibility of using lupins as a human food (Petterson and Mackintosh, 1997) and their potential health benefits. Due to low glycemic index of lupine seeds, it was found that lupine kernel fibers have appetite suppression (Archer et al., 2004) and cholesterol lowering properties, that they lower blood glucose and insulin levels, and aid bowel health as a fecal bulking agent. However, little is known about their photochemistry and antioxidant activity (Hall et al., 2005).

Full understanding of the rheological behavior of flour dough is of great importance from the practical point of view. Dough rheology directly affects the baking performance of flours, and rheological analyses have been made in order to optimize dough formulation and dough quality. Although dough rheology has long been investigated, there remains a significant lack of

understanding. This lack of progress is due to the complexity of this biological system (Masi et al., 2001).

The nutritional quality of wheat protein is lower than that of proteins from pulses and oilseeds due to its low levels of lysine, methionine, and threonine (Kulp, 1988). Nevertheless, demand for wheat-based bakery products is increasing, particularly in developing countries where the major grain is wheat (Quail, 1996). The nutritional quality of these products could be improved by supplementation with non wheat proteins such as those from pulses, including lupine, which would increase the protein content and improve the essential amino acid balance of the baked product.

The aim of this research was to study the chemical and nutritional properties of sweet lupine flour and fiber and effects of its addition as food material sciences examination at different concentration (5, 10 and 15 %) on the dough rheology and backing characteristics. This could be utilized for the development of composite blends from locally produced lupine at small scale industry level as value-add products.

2. Tasks and Objectives

The aim of this study is investigate innovative bakery products with increased functionality and examine its composition; taste; texture and structure of conventional differ on the market with a balanced content of nutritionally valuable substances. The main focus is using of mixtures of wheat and lupine flours or fiber for qualitative and quantitative improvement to able to use in bread in particular, the rheological properties of dough for these flour mixtures in three different mixing ratios of lupine flour or fiber (5, 10, and 15 %).

These changes in dough structure due to different ingredients have significant impact on the further processing steps used in the selection of process technology, on the fermentation times and on the baking process and finally to the quality of the baked goods.

To investigate the comminution and the optimal wetting of lupine flour in the laboratory or small-scale of the entire technological process, we need to study the lupine flour and its blends themselves by hydrothermal milling.

Wheat and lupine flour were mixed. This dough was prepared under determinate condition and studied material science. The characterization of the rheological or baking properties of wheat flour mixed with lupine to share with conventional tests (farinograph) and dynamic tests (oscillatory, creep and temperature sweep) test, with modern measuring methods (air bearing technology) performed comparatively. The effect of adding of lupine flour and lupine fiber, and the influence of dough temperature on the processing behavior of the dough should be investigated and evaluate professionally. Sensory tests are the acceptance and the quality of the manufactured baked goods (bread and cake). Finally, effects of different sweet lupine seed derivatives (flour and fiber) at different concentration (5, 10 and 15 %) on diabetic rats were also studied.

The specific objectives of this research include also:

1. To access the antioxidant/antiradical activities of crude methanolic extracts, their phenolic fractions, their flavonoids and flavonols fractions from sweet lupine seeds and its fiber, which is the dominant variety grown in Egypt. The chemical constituents of the crude phenolic extracts were then characterized with HPLC.

Tasks and Objectives

2. To assess the effects of partial substitution of wheat flour with lupine flour or lupine fibre at different concentrations (5, 10 and 15 %) on dough rheological properties (fundamental and empirical rheology) and baking performance of final products (bread and cake)
3. To study the nutritional potential, (amino acids content, and biological effects) of lupine flour and fiber in final bakery products.

3. Review of literature

3.1. Taxonomy and classification

Lupins (*Lupinus* spp.) belong to the *Genisteae* family, *Fabaceae* or *Leguminosae* (Pastor-Cavada et al., 2009). Second to cereal crops, leguminosae is agriculturally important and one of the three largest families of flowering plants. Leguminosae has been divided into three sub-families named as Caesalpinieae, Mimosoideae and Papilionoideae (Phan et al., 2006). Lupine is the common name for members of the genus *Lupinus* of the legume family (Kurzbaum et al., 2008). From the genus *Lupinus* more than 400 species are known, from which only four are of agronomic interest (Reinhard et al., 2006): (*L. albus* L.: white lupine, *L. angustifolius* L.: blue or narrow-leaved lupine, *L. luteus* L.: yellow lupine and *L. mutabilis* L.: pearl or Tarrwi lupine) (Uzun et al., 2007). The first three species originate from the Mediterranean area, including Turkey, while *L. mutabilis* belongs to South America (Mülayim et al., 2002). These species are known as sweet lupins due to their low levels (0.003 %) of bitter-tasting and potentially toxic alkaloids (Wasche et al., 2001) and, therefore, there is no risk of toxicity for animals and humans (Martínez-Villaluenga et al., 2006a).

The name lupine is derived from the Latin word *Lupus*, meaning 'wolf'. The Romans believed that lupins robbed the soil nutrients in the same way that wolf would steal domestic animal (ARC, 2009). It is known as lupines in the United States, as turmus in the Middle East and Tawari in Latin America. The plant is characterized by having various flowering spikes in large range of colors (Figure 1) (Kurzbaum et al., 2008).



L. angustifolius

L. albus

L. luteus

L. mutabilis

Figure 1: Flowers of different lupine species

Commonly, four lupine species are reported as cultigens in the world (Figure 1). These include *L.albus L*, *L.langustifolius L.*, *L.leutus Land L.mutabilis L.* (Kurzbaum et al., 2008). Trivially, these species are called white lupine, narrow-leaved (blue) lupine, yellow lupine and pearl lupine respectively (ARC, 2009). Out of these four species the focus area of this research is on *Lupinus albus L*. This species is also called white lupine in most part of the world. In this document we will use its scientific name *L. albus L* consistently to refer the crop. The lupine seed is produced in pods which develop on the main stem of the lupine plant (Figure 2). Pods contain between three and seven seeds and these seeds vary in size, color, appearance and composition depending on the species of lupine. Among them the seeds of *L. albus* are the largest. They have a circular flattened shape and are cream in color (AHC, 2009).



Figure 2: Pods of *L. albus* seeds

3.2. Centers of origin

Four different centers of origin have been proposed for the genus lupinus. These include the Mediterranean region (including northern Africa), North America, South America, and East Asia. Today, approximately 90 % of the recognized species are found in alpine, temperate and subtropical zones of North and South America, which ranges from Alaska to Southern Argentina and Chile. The remaining species are native to the Mediterranean region and Africa. But due to their larger seeds, most of the economically important species come from the Mediterranean region (Figure 3) (ARC, 2009).

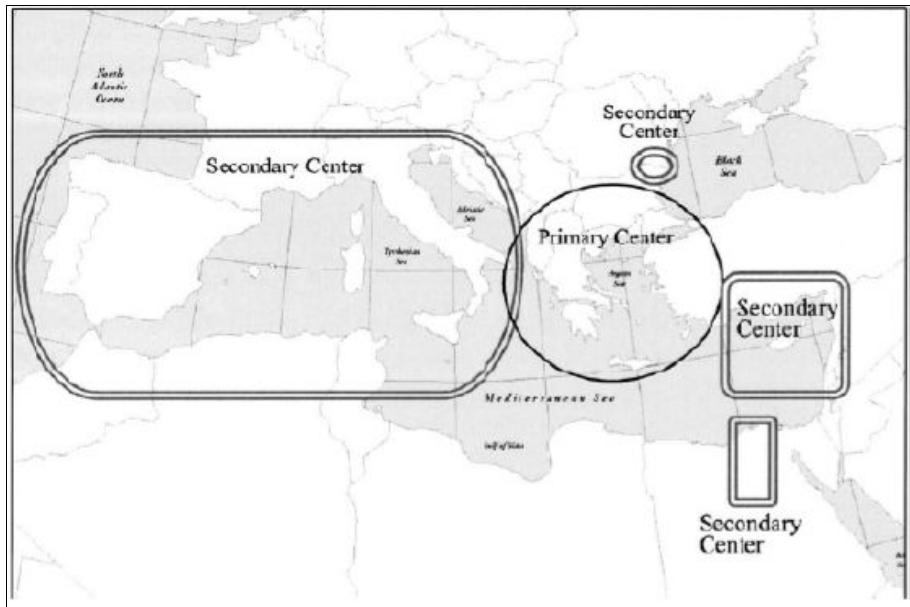


Figure 3: Primary (single line circle) and secondary (double line circles) centers of origin for *L. albus* in the Mediterranean Region (Noffsinger and Van Santen, 2005).

In places where no other crops can be grown profitably, Lupins could be considered as a model for low input plants. Among the common species *L. albus* L., *L. luteus* L. and *L. angustifolius* L. are Old World species whereas; *L. mutabilis* is a new world species originating from South America (Cowling et al., 1998).

3.3. Production and utilization

3.3.1. Worldwide production of *L. albus*

The world production of legume seeds, 'the poor man's meat' as developed-country producers call them, was about 58 million tons in 1994 (FAO estimations). Of this, the major part, 40 million tons, was produced by developing countries, especially India and China with only 8.5 % consumed outside the country in which it was produced. Only Argentina, Mexico, the USA and China export significant quantities of legume grain, while Europe is the main importing continent (Heiser, 1996). In some European countries, pickle is produced from lupine seeds (Vasilakis and Doxastakis, 1999). White lupine, which has been consumed as a food in a narrow area for a long time, was accepted for human consumption by the Australian government in 1987 and by the United Kingdom government in 1996 (Swam, 2000). The average price of lupine seeds is about 185 \$/ton (GrainPool, 2003). In Australia, 1.6 million tones of lupine seed is

produced annually, representing 80 % of the total world production (Pollard et al., 2002). The other important lupine producers are Poland, France and South America.

3.3.2. History of *L. albus* utilization

Legume seeds are protein valuable foods which have been present in the Mediterranean diet since ancient times. Among them, lupins are high protein crops (Frias et al., 2005). Wild and partially domesticated lupine species were grown thousands of years ago both in the Mediterranean region and in the South American Andes before the Incan Empire. The cultivation of *L. albus* was well known to the ancient Greeks and Romans and its cultivation has been mentioned by early writers including the poet Virgil and Pliny the Elder (AHC, 2009). It was in the twentieth century that the old bitter types of lupine were replaced by ‘sweet’ low alkaloid types. Before this major development, bitter lupins were spread in southern Europe and North Africa. They were also introduced in northern Europe when Frederick the Great of Prussia sent for lupine seeds from Italy in 1781 to improve the poor soils in north Germany (Frias et al., 2005). Before 1926, lupines had been used as side rates only. The issue of natural existence of low alkaloid lupines was raised by E. Bauer and A. Pryanishnikov. However; works in this field were held back by the absence of reliable and rapid methods of quantifying alkaloids in plants. In 1928, Reinhold von Sengbusch from the Central German Institute of Genetics proposed a method which was applied to analyze alkaloid in plants (Maknickiene and Asakaviciute, 2008). Lupine is an economically and agriculturally valuable plant (Gulewicz et al., 2008). Its seeds are employed as a protein source for animal and human nutrition in various parts of the world, not only for their nutritional value, but also for their adaptability to marginal soils and climates. Human consumption of lupins has increased in recent years (De Cortes Sánchez et al., 2005).

3.3.3. Some Common Lupine Based Food Types

L. albus seeds meet the requirements as alternative home prepared diets with high nutritional value and reasonable price among leguminous plants (Zraly et al., 2007). Lupines and lupine products have traditionally formed part of the human diet. Food products available on different markets of Europe are lupine snacks, lupine pasta, lupine bread and cookies, lupine coffee and some vegetarian instant meals (Figure 4) (AHC, 2009).

Lupinus albus flour is added for nutritive value and also provides functional properties in bakery and pastry products, protein concentrates and other industrial products, as well as the elaboration of lactose free milk and yoghurt analogues (De Cortes Sánchez et al., 2005).



Figure 4: Some model foods containing lupine protein

L. albus flour has characteristics of improving the micro distribution of water in dough and mixtures. Products could then resist freezing and thawing better, the preparation of bread dough could be easier, shrinking could be limited, and emulsifying power will be good, for a yellow color development, to change some of rheological parameters, like crispness and smoothness. *L. albus* flours are largely used as eggs substitute, for example in cakes, pancakes and biscuit. The flour can also be used as a butter substitute in cakes (Lacana, 1999).

3.4. Chemical and Nutritional Composition of lupine grains

Legumes represent, together with cereals, the main plant source of proteins in human diet. They are also rich in dietary fibre and carbohydrates (Rochfort and Panozzo, 2007). Minor compounds of legumes are lipids, polyphenols, and bioactive peptides (Pastor-Cavada et al., 2009).

Lupine is a good source of nutrients, not only proteins but also lipids, dietary fibre, minerals, and vitamins (Martínez-Villaluenga et al., 2009) (Figure 5). Lupine generally contains about twice the amount of proteins found in those legumes that are commonly consumed by humans.

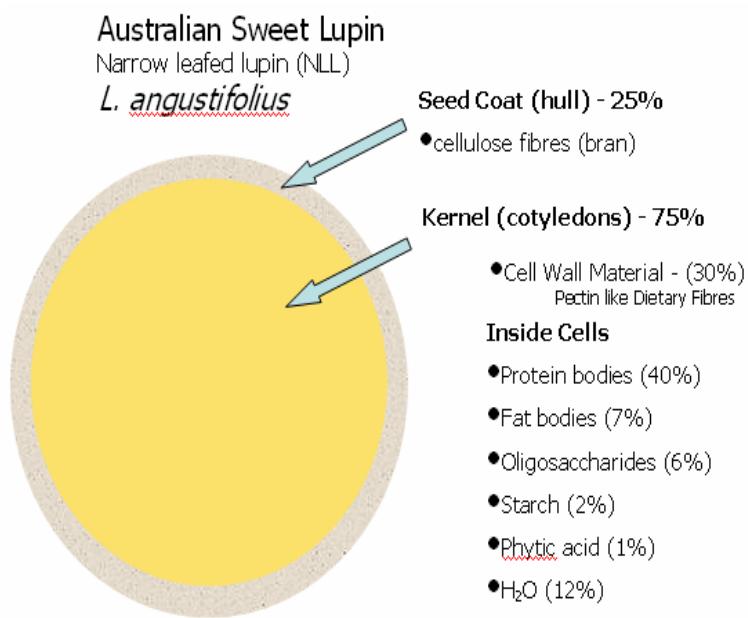


Figure 5: Seed coat and cotyledon composition of other species of Lupinus genus

Lupins have a typical dicotyledon structure. Their thick seed coat (hull or testa) comprises about 30 % of the seed weight. This is considerably higher than for most domesticated grain species. The thick seed coat is mostly cellulose and hemicellulose, means that it is important to consider the composition and nutritional value of their cotyledons (kernel). Within the cotyledons (kernels), energy is mostly stored in form of thickened cell wall material, about 25 % of the cotyledons, and oil bodies, comprising from 6 to 14 % of the cotyledons in domestic species.

There is virtually no starch (2 %) in any of the lupine species. This is in marked contrast to crops such as field peas and chickpeas, which can have 50-70 % of the cotyledon weight as starch and have low protein and oil content, and the soybean with 15-20 % oil and high protein content. Their crude protein content ranges from about 28 to 42 %. There are variations in the protein content between species and cultivars as a result of the characteristics of the growing conditions and soil types (Martínez-Villaluenga et al., 2006a) from 28 % in to 48 % (Capraro et al., 2008). Proximate analyses for whole grain of the major domesticated species, and the Andean lupine, are shown in Figure (6).

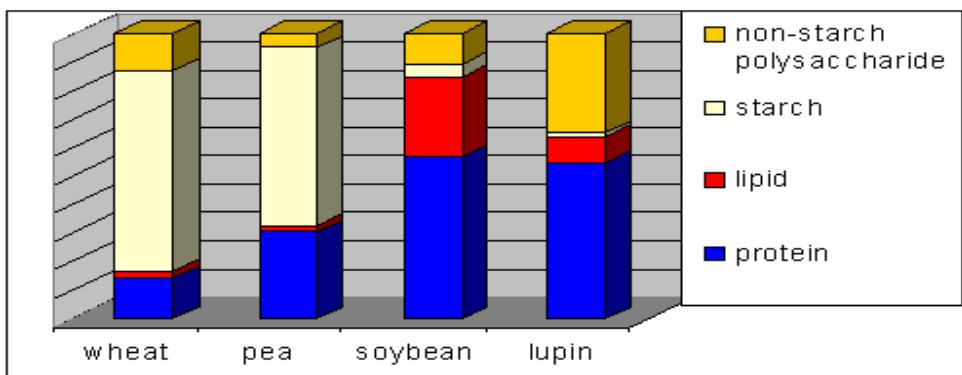


Figure 6: Comparative whole grain content of the major domesticated species

3.4.1. Crude protein

Legumes play an important role in human nutrition since they are rich sources of protein, calories, certain minerals and vitamins. In African diets legumes are also, the major contributors of protein and calories for economic and cultural reasons (El Maki et al., 2007). Analyses of nutritional values of *Lupinus albus* have shown that the bio-availability of the constituents is comparable to those of processed soybeans (Joray et al., 2007). Grain legumes are main sources of vegetable protein, among which *L. albus* is known to have seeds with the highest protein content like soybean (Sujak et al., 2006). Based on this fact *L. albus* seeds have been employed as a protein source for animal and human nutrition in various parts of the world (De Cortes Sánchez et al., 2005).

The requirements with regard to chemical composition, nutritional value and product safety were laid down by the Advisory Committee on Novel Foods and Processes (ACNFP) in 1996 for certified lupins (sweet lupins). Based on the strength of this certification, these products were recommended as feedstuffs and food ingredients (e.g. lupine flours for baked goods) (AHC, 2009).

3.4.2. Amino acids content

Legume proteins are rich in lysine and deficient in sulphur containing amino acids, whereas cereal proteins are deficient in lysine, but have adequate amounts of sulphur amino acids (Eggum and Beame, 1983). As a member of legume family lupine bean protein is rich in lysine and deficient in sulfur containing amino acids (Phan et al., 2007). In contrast its arginine content is markedly higher (Zraly et al., 2007). And also the value of leucine is satisfactory for most of the species of lupines. Apart from the highest level of amino acids within the crude protein, it was

found to have a better and nutritionally more beneficial amino acid composition and the highest essential amino acid level (EAA) (Sujak et al., 2006). It is also characterized by a higher essential amino acid index (EAAI) as well as chemical score (CS) of restrictive amino acids, and the highest protein efficiency ratio (PER), expressed in terms of the availability of leucine and tyrosine as compared to blue and yellow lupine variety (Sujak et al., 2006). Currently, there are only few companies in Europe that produce *L. albus* protein ingredients for food use. The products available are toasted and non-toasted lupine flour, grits, granulates, fiber and protein concentrates from the non-defatted seed. Lupins and lupine products were considered to be traditional foods even before the introduction of the Novel Food Decree (1997) (AHC, 2009).

3.4.3. Crud fiber

Pulses, the edible seeds of leguminous crops, are a rich food source of dietary fibers that promote various beneficial physiological effects for human health. Canada is a major world producer and exporter of pulses, but the whole seeds have a low market value. Milling and fractionation of pulse seeds can isolate important dietary fibre components for incorporation into commercial food products to enrich their fibre content and/or serve as functional ingredients. Expanding pulse utilization through such applications can serve to enhance human health while increasing the market value of the crops.

The dietary fibre is composed of total dietary fibre (TDF), which includes both soluble (SDF) and insoluble dietary fibre (IDF). In terms of health benefits, both kinds of fibre complement with each other. A well balanced proportion is considered when there is 70-50 % insoluble and 30-50 % soluble DF (Grigelmo-Miguel et al., 1999).

Lupine kernel fibre is a novel food ingredient containing both soluble and insoluble fractions (Hall et al., 2005). It is extracted from the kernel of Australian sweet lupine (*L. angustifolius*), a legume grown in large quantities in Australia and considered to be underutilized as a human food source (Petterson, 1998). Currently, it is being used mainly as an animal feed. The dietary fibre content of Australian sweet lupine kernels is higher than that of most other legumes, making up approximately 40 % of the kernel weight (Guillon and Champ, 2002). Lupine kernel fibre has shown potential for the manufacture of palatable, fibre-enriched products such as baked goods and pasta (Clark and Johnson, 2002).

3.4.4. Crude fat

The fat level in lupine is ranked third after ground nut (*Arachis hypogaea L.*) and soybean (*Glycin max*) among legumes (Uzun et al., 2007). The lipid contents of *L. albus* are similar to other species of the genus lupinus like *L. campestris* (Jimenez-Martinez et al., 2003). The mean value of crude fat in *L. albus* grown in different parts of the world is 13 % (Phan et al., 2006).

The oil extracted from *L. albus* seed consist various types of fatty acids. The fatty acids of the oil from the raw seed are composed of more of unsaturated fatty acid and small percentage of saturated fatty acids. This means *L. albus* can be a potential source of considerable amount of useful vegetable fat. Among the unsaturated fatty acids, majority oleic and linolenic acids are found (Uzun et al., 2007). The high content of ω -6 and ω -3 fatty acids, make the crop a healthy alternative edible oil source (Joray et al., 2007).

3.5. Chemical and nutritional composition of wheat grains

Wheat kernels have three main parts: the endosperm, the germ, and the bran (Figure 7). While whole wheat flour contains all three parts of the kernel, white flour is milled from the endosperm. Whole wheat flour is considered a whole grain product because it contains the entire wheat kernel. The endosperm makes up the bulk of the kernel. It is the whitest part, partly because it contains mostly starch typically 70–75 %. The starch is embedded in chunks of protein. (See, 2008)

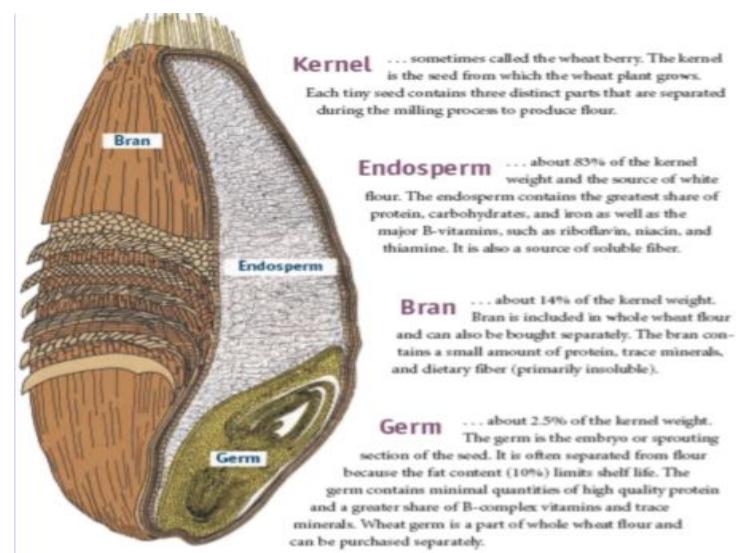


Figure 7: Longitudinal section of grain of wheat

3.5.1. Crude protein

Wheat flour contains two types of proteins, gliadin and glutenin. The prolamins of wheat (gliadin) that comprise 40–50 % of the proteins are extremely sticky and inelastic and responsible for the cohesiveness of doughs. On the other hand, the glutelins were also named glutenins, provides resistance to extension (Singh and MacRitchie, 2001). The prolamins and glutelins combined during mixing to form the elastic protein gluten complex resulting in viscoelastic dough. The dough has the ability to form thin sheets that will be able to retain gas and produce a light baked product (Gujral and Rosell, 2004). Wheat flour can be made from whole wheat or the germ and bran can be separated from the endosperm and then ground into flour. The strong flour has protein content in the range of 10.5-14.5 % and the weak flour has less than 8.5 % protein. According to Cauvain, (2003), hard wheat with strong gluten and high protein content in the flour has better ability to trap and retain carbon dioxide gas and resulted in higher volume of bread.

3.5.2. Amino acids content

Lysine is the limiting amino acid in wheat (Kent and Evers, 1994). The shortage of energy, protein and essential amino acids are the main problems of human nutrition in developing and under developed countries. The nutritional quality can be improved by increasing protein content and limiting amino acids especially lysine. Protein content of wheat can be significantly increased through breeding. Unfortunately, a negative correlation exists between lysine expressed as a percent of protein and percent protein in common and durum wheat. Therefore, lysine as percent of protein can be used as a measure of protein quality while lysine as percent of sample is a function of both the protein and lysine (percent of protein) concentrations of a sample (Pogna et al., 1994). The nutritional importance of wheat in human diet and in animal feed necessitates pursuing study for detailed and accurate knowledge of the amino acid composition of wheat proteins and its products. Such information is especially required to develop specific food recommendations and feed formulations.

3.5.3. Crude fiber

The bran (outer layers of wheat grain) is made up of several layers, which protect the main part of the grain. Bran is rich in B vitamins and minerals; it is separated from the starchy endosperm during the first stage of milling. In order to protect the grain and endosperm material, the bran comprises water-insoluble fibre. More than half the bran consists of fibre components (53 %). Chemical composition of wheat bran fibre is complex, but it contains, essentially,

cellulose and pentosans, polymers based on xylose and arabinose, which are tightly bound to proteins. These substances are typical polymers present in the cell walls of wheat and layers of cells such as aleurone layer. Proteins and carbohydrates each represent 16 % of total dry matter of bran. The mineral content is rather high (7.2 %). The two external layers of the grain (pericarp and seed coat) are made up of dead empty cells. The cells of the inner bran layer- aleurone layer are filled with living protoplasts. This explains the rather high levels of protein and carbohydrate in the bran. There are large differences between the levels of certain amino acids in the aleurone layer and those in flour. Glutamine and proline levels are only about one half, while arginine is treble and alanine, asparagine, glycine, histidin and lysine are double those in wheat flour (Cornell, 2003).

3.5.4. Crude fat

Lipids are present only in a small extent in cereals but they have a significant effect on the quality and the texture of foods because of their ability to associate with proteins due their amphipatic nature and with starch, forming inclusion complexes. Lipids are minor components of wheat flour with essential function in wheat end-use quality. Total lipids account for 3–4 % of the wheat kernels and about 45 % of these are located in the starchy endosperm (Chung, 1986). The content of lipids in wheat flour varied between 1.5 % and 2.0 % and most of them are contributed from the endosperm whilst the others are from germ and aleurone in tissue fragments and as oil adhering to the flour particle surface. Lipids in wheat or wheat flour can be grouped into three categories: non-starch lipids, starch lipids and starch surface lipids, according to their location in flour components and their extraction methods (Pomeranz, 1988). Non-starch lipids, especially the free lipids, including non-polar and polar lipids, have attracted more interest since their contribution to end-use quality of wheat flour has been recognised.

3.6. Phenolic compounds and active components

Leguminous seeds are an important source of nutrient compounds such as protein, starch, dietary fiber, and minerals, particularly in third-world countries. Incorporation of leguminous seeds into the human diet in developing countries can offer protective effects against chronic diseases (Leterme, 2002). Legumes contain a number of bioactive substances including phenolics that can diminish protein digestibility and mineral bioavailability (Sandberg, 2002). On the other hand, phenolic compounds such as flavonoids, phenolic acids, lignans, and tannins have antioxidant properties, and these are very important from nutritional and technological points of

view. Various evidences suggest that oxidative stress is closely associated with a diverse assortment of diseases such as cancer and cardiovascular disease. The antioxidant capacity of legumes depends on the biological variety of the plant, and is observed over broad ranges. Technological processing and seed germination can impact the levels of natural endogenous antioxidants (e.g. phenolics, tocopherols; vitamin C) in leguminous seeds. An important point of consideration is the high content of phenolic antioxidants present in seed coats.

3.6.1. Content of total phenolic and tannins in leguminous seeds

The total phenolic content (TPC) in leguminous seeds or extracts prepared from such plant materials is one of the main parameters dictating the potential antioxidant capacity of seeds or the antioxidant activity of extracts there from. Determination of the TPC in legumes includes an extraction step followed by a colorimetric reaction under alkaline conditions between the extracted phenolic constituents and Folin-Ciocalteu's phenol reagent. The results of the assay are reported as the quantity of equivalents of standard compounds (i.e. typically gallic acid or catechin) per mass unit of raw material or extract.

The type of solvent used for extraction of various classes of phenolic compounds from legumes is very broad and typical examples include water, methanol, ethanol, methanol/water, ethanol/water, and acetone/water (Turkmen et al., 2005). Details pertaining to the application of different solvents for the extraction of phenolics from plant material have been reviewed by Naczk and Shahidi, (2006). A comparative study of phenolic profiles and antioxidant activities of legumes, as affected by extraction solvents, has been reported by Xu and Chang, (2007); the results of their study showed that 50 % (vol/vol) acetone extracts exhibited the highest TPC for yellow pea, green pea, and chickpea. Amarowicz et al., (1995) reported that an acetone/water system extracted greater quantities of phenolic compounds from lentil seeds compared with methanol/water or ethanol/ water systems. In the acetonnic extract, thin-layer chromatography revealed the presence of tannins of higher molecular weight that were not present in ethanolic and methanolic extracts. For some preparations, an absolute value is given based on the reference, but in other cases, a range is provided. It is clear that wide variations exist in the TPC, depending on the source of the leguminous seed as well as on how it has been processed or extracted. Condensed tannins (i.e. proanthocyanidins) are flavan-3-ol-based biopolymers that, at high temperature in alcohol solutions of strong mineral acids, release anthocyanidins and catechins as end groups. Several studies have reported on the antioxidant and antiradical activity of tannins

(Amarowicz, 2007). The most common methods used for condensed tannin analysis include the vanillin/HCl method, the bovine serum albumin (BSA) precipitation method and the proanthocyanidin method after n-butanol/HCl hydrolysis. The results are generally presented as catechin equivalents per mass unit (i.e. the vanillin/HCl method) or as absorbance units at 500 nm (i.e. the vanillin/HCl method), 510 nm (i.e. the BSA precipitation method) or 550 nm (i.e. the proanthocyanidin method) per mass unit.

3.6.2. Phenolic composition of leguminous seeds

The dominant phenolic compounds present in leguminous seeds are flavonoids, phenolic acids, and procyanidins. Seeds with colored coats are also rich in anthocyanidins (Choung et al., 2003). The content of total flavonoids in seeds from six legumes, green pea, yellow pea, chickpea, lentil, red kidney, and black bean ranged from 0.08 to 3.21 mg catechin equivalents/g (Xu and Chang, 2007); the highest quantity of total phenolics was determined in seeds of red kidney and black bean. Flavonoids present in leguminous seeds belong to flavanols, flavan-3-ols, flavones, and anthocyanidins (Amarowicz et al., 2008). The majority of them, however, are present as glycosides in the seeds. Diaz-Batalla et al., (2006) also detected isoflavones in germinated beans. In the study of Sosulski and Dabrowski, (1984), the phenolic constituents in defatted flours and hulls of ten leguminous species, mung bean, smooth field pea, yellow lentil, small faba bean, pigeon pea, navy bean, white lupine, baby lima bean, chickpea, and cow pea, were fractionated into free acids, soluble esters, and residue compounds. The flours contained only soluble esters; hydrolysis of these revealed the presence of trans-ferulic, trans-p-coumaric, and syringic acids in nearly all of the species examined. The lowest amount of phenolic acids was found in mung bean, field bean, lentil, faba bean, and pigeon pea, with 2–3 mg of phenolic acids per 100 g of flour. Navy bean, lupine, lima bean, and cowpea were characterized as possessing the highest level of phenolic acids. The hulls contained p-hydroxybenzoic, protocatechuic, syringic, gallic, trans-p-coumaric, and trans-ferulic acids in the soluble ester fraction. Madhujith et al., (2004) reported vanillic, caffeic, p-coumaric, ferulic, and sinapic acids as the main phenolic acids identified in bean hull extracts.

3.6.3. Antioxidant activity of lupine seeds or their extracts

Earlier research (Sosulski and Dabrowski, 1984) indicated that lupine (*Lupinus albus* L.) flour contains only soluble esters (71 mg phenolics/kg flour) comprised of transferulic, p-hydroxybenzoic, syringic and trans-p-coumaric acids (55, 17, 15 and 13 % of the soluble ester

fraction, respectively), while the hulls constituting 12.6 % of the whole seed phenolics consists mainly of trans-ferulic and p-hydroxybenzoic acids (60 and 40 % of the soluble ester fraction, respectively). However, recent HPLC analysis (Lampart-Szczapa et al., 2003b) revealed the presence of procatechuic, p-hydroxybenzoic, vanillin, p-coumar and ferulic acids in lupine hulls with only trace amounts of ferulic acid present in dark hulls.

Total phenolic and procyanidin contents of aqueous acetone (70 % v/v) extracts of lupine cultivars (*Lupinus albus* L., *Lupinus angustifolius* L., *Lupinus luteus* L., *Lupinus mutabilis* L. and *Lupinus hispanicus* L. species) varied from 7 to 70 g/kg (expressed in gallic acid) and 70 to 530 mg/kg (expressed as +catechin) seed, respectively. Significant ($p<0.01$) differences in total phenolics and proanthocyanidin contents were observed among cultivars of the same species (*L. albus*) grown in one location and one cultivar grown in six locations in Portugal. The total phenolic content in the cotyledons of lupine cultivars Mirela and Wersal were 1878 and 336 mg/kg, respectively and in the hulls 288 and 184 mg/kg, respectively when extracted with 80 % aqueous ethanol (Lampart-Szczapa et al., 2003b).

Biological activity and quality attributes have been associated with phenolic content of lupine. Thus, antibacterial activity displayed by lupine hulls was dependent on the content of total phenolic compounds. However, antioxidant activity of lupine cultivars was independent of hull color or content of polyphenols (Lampart-Szczapa et al., 2003a). Lupine seed flour, on the other hand, exhibits antioxidant activity (evaluated by the β -carotene bleaching method) that correlates with the presence of total phenolics (13.6 and 20.7 % polyphenolic content) when extracted with cold and hot methanol, respectively. A weak antioxidant activity (10 and 6 % hydroperoxide inhibition at 500 and 5000 ppm extract levels, respectively) was reported for an aqueous methanolic extract (80 %) of *L. angustifolius* that had total phenolic content of 4.7 ± 0.1 mg gallic acid equivalents, (GAE)/g dry matter (Kähkönen et al., 1999).

3.7. Milling/Particle size analysis

3.7.1. Milling

Milling is a complex industrial process which involves a set of grinding and sieving operations, the objectives of which are to break the grain, separate the starchy endosperm from brans and reduce it to flours. The milling properties of wheat grains and the end-use of the resulting flour are mainly determined by hardness. For example hard wheat requires more energy

than soft wheat during flour milling (Kilborn et al., 1982) and using hard or soft wheat flour affects the formation of cookies (Abboud et al., 1985). Variations in wheat hardness mainly result in different particle sizes for the meal, and hard wheat breaks into larger particles than soft wheat. This property is the basis of the most popular tests for measuring wheat hardness, ie the Particle Size Index (PSI) or the Near Infrared Reflectance Index (Williams and Sobering, 1986a, b).

The process of particle size reduction improves ingredient performance during mixing and, in most cases; the nutritive value of an ingredient can be improved or more nearly realized. There are many ways to reduce the particle size of ingredients. Two of the most common pieces of equipment used are the hammer mill and the roller mill (Figure 8).

The initial reduction of cereal grains begins by disrupting the outer protective layer of the seed (hull), exposing the interior. Continued size reduction increases both the number of particles and the amount of surface area per unit of volume. It is this increased surface area that is of primary importance. A greater portion of the grain's interior is exposed to digestive enzymes, allowing increased access to nutritional components such as starch and protein. The enhanced breakdown of these nutritional components improves absorption in the digestive tract. Size reduction is also used to modify the physical characteristics of ingredients resulting in improved mixing, pelleting, and, in some instances, handling or transport.

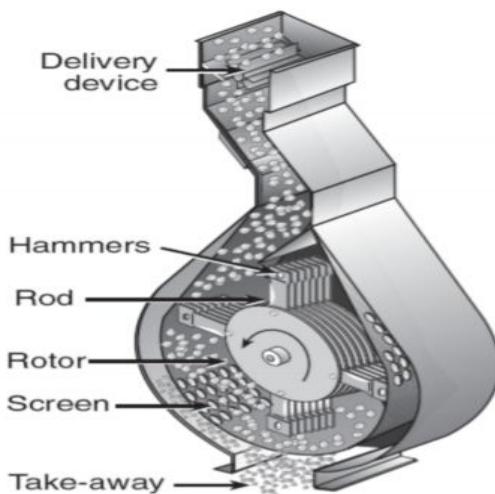


Figure 8: Hammer mill

3.7.2. Hammer mills

Hammer mills accomplish size reduction by impacting a slow moving target, such as a cereal grain, with a rapidly moving hammer. The target has little or no momentum (low kinetic energy), whereas the hammer tip is travelling at a minimum of 16,000 feet per minute (4,880 m/min) and perhaps in excess of 23,000 feet per minute (7,015 m/min) (high kinetic energy). The transfer of energy that results from this collision fractures the grain into many pieces. Sizing is a function of hammer-tip speed; hammer design and placement; screen design and hole size; and whether or not air assist is used. Because impact is the primary force used in a hammer mill to reduce particle size, anything that: increases the chance of a collision between a hammer and a target, increases the magnitude of the collision, or improves material take-away, would be advantageous to particle size reduction. The magnitude of the collisions can be escalated by increasing the speed of the hammers. Anderson, (1994) stated that when drive speed and screen size were kept constant, the increased hammer-tip speed obtained from increased rotor diameter produced particles of smaller mean geometric size. Particles produced using a hammer mill will generally be spherical in shape with a surface that appears polished. The distribution of particle sizes will vary widely around the geometric mean such that there will be some large-sized and many small-sized particles. Particulation, useful application for grain kinds sorts.

3.7.3. Particle size analysis

Few studies have reported the determination of detailed particle size distributions to evaluate and compare wheat properties. Wu et al., (1990) studied the size distributions of flours measured by sieving and air classification. They found that the mean particle size and the percentage of flour being passed through a 53 μm screen and retained by a 44 μm screen differed between hard and soft wheat. Detailed particle size distributions can be easily determined by using a laser light diffraction apparatus. The advantage is to be able to analyze the amount of small particle $< 50 \mu\text{m}$ in a rapid and simple way. Moreover, particle size distribution can be determined by laser diffraction for milling fractions such as flour or bran as well as for the whole wheat meal. Detailed distributions measured by laser diffraction can be considered as characteristic curves to study and compare powdered samples. Such an approach has been tested by Hareland, (1994), who compared flour particle size distribution among different wheat types and milling methods. He showed that the milling method affected the particle size distributions of hard wheat flours but not those of soft wheat flours.

3.8. Rheological properties of wheat and composed flour dough

Wheat flour dough is the basis of many food products such as bread, crackers, and cookies. Its rheological response is important at many stages in the manufacturing of the finished product. It is thought that the rheological properties of dough play a key role in dough piece weight and shape control, dough expansion during baking and finished product textural attributes.

3.8.1. Definition of Rheology

Rheology is defined as a study of the deformation and flow of matter (Bourne, 2002). The applications of rheology have expanded into food processing, food acceptability, structure determination and handling. Many researches have been conducted to understand the rheology of various types of food such as food powder (Grabowski et al., 2008), liquid food (Park, 2007), gels (Foegeding, 2007), emulsions (Corredig and Alexander, 2008) and pastes (Lim and Narsimhan, 2006). Vast food materials show a rheological behaviour that classifies them in between the liquid, semi solid and solid states; meaning that their characteristic varies in both viscous and elastic behaviours. This behaviour, known as viscoelasticity, is caused by the entanglement depended on specific microrheological interactants such as: the long chain molecules with other molecules. Figure (9) shows the creep and recovery test on the ideal elastic, ideal viscous and viscoelastic materials. The ideal elastic materials have the ability to recover to its original shape upon the removal of stress while the stress acted on the ideal viscous materials caused them to deform and it is non-recoverable. By combining both the ideal elastic and viscous behaviours, the viscoelastic materials exhibit behaviour in recovering some of its original shape by storing the energy. They show a permanent deformation less than the total deformation applied to the material.

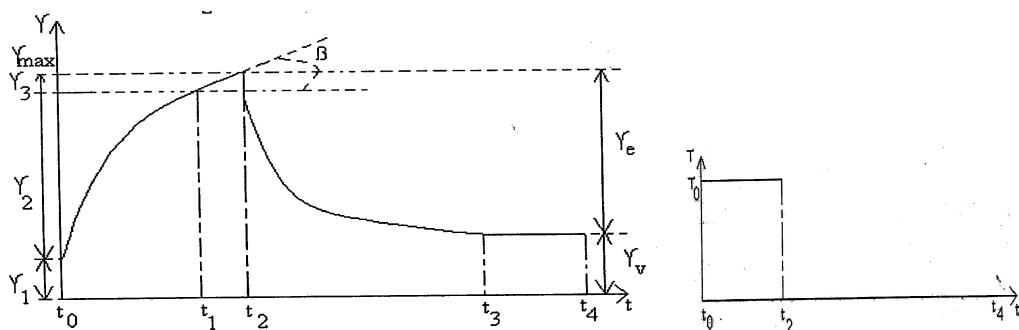


Figure 9: Creep and recovery test (Kunzek et al., 1997).

3.8.2. Factors affecting dough rheological properties

Rheological properties of dough and gluten during mixing are affected greatly by the flour composition (low or high protein content), kind of proteins, processing parameters (mixing time, energy, temperature) and ingredients (water, salt, yeast, fats and emulsifiers). Studies were conducted to investigate the effect of protein content on the gluten quality and rheological properties (Sliwinski et al., 2004a), on bread making quality (Sliwinski et al., 2004b) and also on volume expansion resulted from frying (Chiang et al., 2006). These works, conclusively suggested that the strong flour produces a better gluten and dough quality than the weak flour in terms of giving a higher response in extensibility, bread loaf volume and height and also volume expansion.

3.8.2.1. Water absorption and structure formation

Water is responsible in hydrating the protein fibrils and start the interactions between the proteins cross links with the disulphide bonds during dough mixing (mechanical agitation). Too much water addition to the flour will result in slurry and too little water results in slightly cohesive powder (Faubion and Hoseney, 1989). Hence, an optimum water level is required to develop cohesive, viscoelastic dough with optimum gluten strength depended on gluten behaviour. While the optimum water level differs from flour to flour, the strong flours require higher water level than weak flours largely due to the higher protein content and dense particles in the strong flours. Protein content is known to be an important factor in determining the water uptake of flour (Sliwinski et al., 2004a). Janssen et al., (1996) reported that the G' and G" decreased as the water content of dough increased in rheological relevant / valid ranges. Ablett et al., (1985) explained the effect of water content on gluten networks in terms of a rubber network such that its elongation reduced as water content increased as if in rubber network. However, for dough, the elongation increased as water content increased. It was suggested that the soft continuous phase of dough will swell in direct proportion of free-water which is responsible in the increase of the elongation.

3.8.2.2. Temperature

Temperatures are very important in dough systems because dough displays different rheological characteristics depending on the ambient or applied temperature. Temperature sweeps are performed in oscillatory testing by keeping the frequency and applied strain constant and running a temperature profile on the rheometer. Temperature profiles are easily programmable on

rheometers and can be set to have any number of temperature ramps and cooling periods to best measure the material (Salvador et al., 2006). This allows for a simulation of rheology changes due to temperature during baking. As would be expected, lower temperatures provide easier testing and fewer difficulties than higher temperatures.

Oscillatory testing from 20-40 °C is below the gelatinization point of wheat flour dough; therefore, dough shows a high frequency dependence and changes over this temperature range are reversible (Song and Zheng, 2007). Higher temperatures show irreversible changes as the dough approaches its gelatinization temperature, especially when temperatures near 80 °C. At high temperatures (after dough's gelatinization temperature of about 60 °C), the dough system becomes stronger and the G' becomes much larger than the G'' (Salvador et al., 2006). This transition of the dough into a more solid system could be caused by sulphhydryl/disulfide exchange (Song and Zheng, 2007), or starch granule rupture to form a gel amylase matrix coupled with protein denaturation (Salvador et al., 2006). The starch gelatinization that occurs at elevated temperature has a great effect on the viscoelastic properties of the dough, and temperature sweeps in oscillatory testing are a useful way to study these changes. The only restriction in using this method is that it cannot be used above 90 °C. Higher temperatures and cooling from higher temperatures causes extra loss of moisture and shrinkage of the sample, which has the effect of the sample pulling away from the measurement apparatus and inconsistency of measurements.

3.8.2.3. Sodium chloride

Sodium chloride or commonly known as salt is said to have a strengthening or tightening effect on the gluten during mixing of dough (Niman, 1981). Salt must be added early in the dough-mixing to give maximum dissolution time and accelerate gluten formation, tighten the dough and increase the mixing time. Salt is used to overcome the low pH of dough since the effect of pH will alter the mixing time; a low pH gives a shorter time and a high pH gives a longer time (Hoseney, 1985). Roach et al., (1992) suggested that the influences of salt on the protein solubility affect the dough properties. Salt decreases the water activity of protein in the wheat flour dough as its concentration increases. Salvador et al., (2006) found that the elastic modulus (G') falls slightly in the presence of salt. This reduction is probably due to the decrease in inter-protein hydrophobic interactions which reduce the tendency of the proteins to aggregate and thus reduce the elasticity. The amount of salt added into the dough mixing can be varied from

1.8-2.1 % on flour basis (Farahnaky and Hill, 2007). However, due to increase concern in health related issues by consumers in food intake, addition of lower amount of salt has become one of the main focus in recent studies (Farahnaky and Hill, 2007). Omission of salt entirely leads to a significant reduction in dough and bread quality and also the sensory attributes of bread, where the bread was described as sour/acidic and having yeasty flavour (Lynch et al., 2009).

3.8.2.4. Mixing process

Mixing is an important step in producing gluten with desired strength as to produce a good quality end-product. Processing factors during flour-water mixing include the mixing time, work input, mixer type and temperature. In order to achieve optimum dough development, the mixing time and work input must be above the minimum critical level (Angioloni and Rosa, 2005). Different wheat flour has different optimum mixing time. A longer mixing time is expected for mixing dough from strong flour. It is probably due to the dense particles of strong flour and slower water penetration (Hoseney, 1985). Sliwinski et al., (2004a) reported that a positive correlation was observed between dough mixing time and the percentage of glutenin protein in flour. Dobraszczyk and Morgenstern, (2003) related optimum mixing time of dough with the development of the glutens networks and monomers. Increasing mixing time and work input above the optimum level during mixing induces the changes in mechanical properties of dough (Cuq et al., 2002).

3.8.2.5. Effects of lupine flour or fiber addition on dough quality

In general, the addition of up to 10 % lupine flour improves water binding, texture, shelf-life, and aroma (Martínez-Villaluenga et al., 2006b). The presence of lupine flour in the products increased the amount of water required for the optimum bread making absorption. It was also concluded that lupine flour, at 5 % substitution level, increased the stability and tolerance index of the dough (Dervas et al., 1999), however, the mixing time and dough stability decreased as the substitution level increased (Doxastakis et al., 2002). The unique bread-making properties of wheat flour can be attributed mainly to the ability of its gluten proteins to form a viscoelastic network when mixed with water. The worsening of the viscoelastic properties of wheat flour dough, after substitution with lupine, reduces the bread-making potential. It was suggested that the weakening effect of foreign proteins (lupine) on wheat flour doughs is the result of the dilution of the gluten structure by the protein added (Mohammed, 2011).

The assessment of the suitability of high dietary fiber lupine product and its utilization as a valuable source of dietary fiber was carried out in experimental baking, where 10 %, 15 %, and 20 % additions of high dietary fiber lupine product to wheat dough were used. It was found that the contribution of high dietary fiber lupine product in the mixture with wheat flour affects the increase the water absorption capability (of water absorbability) in comparison with the control dough. Also, advantageous effects were observed of high dietary fiber lupine product on the rheological properties of dough such as its development, time stability, and index tolerance to kneading. The best organoleptic effect was obtained when a 10 % addition of high dietary fiber lupine product was used. Furthermore, a delicate structure of crumb was also observed (Ciesiołka et al., 2005; Mohammed, 2011).

3.8.3. Rheological behaviour measurements

Rheological behaviour of dough can be determined by two distinct measurements that are fundamental and empirical. Studies on the fundamental rheology of dough are usually carried out using small deformation while the empirical measurements are measured using large deformation. Nonetheless, fundamental dough rheological testing using large deformation is growing popularity with the presence of newer techniques and equipment. Thus, the rheological behaviour of dough was predicted using molecular models of gluten development during mixing by Letang et al., (1999). In these models, gluten development mainly involves glutenin proteins interactions with each other in the loop by disulphide bonds. At the early stage of mixing, the gluten fibrils are in contact with the mixer blade, the sides of the bowl and other flour particles. The hydrated gluten fibrils and starch granules are continuously dispersed throughout. Glutenins, which are the long polymeric proteins, are folded and the chains are in random orientation. As mixing proceeds, more protein becomes hydrated and the glutenins tend to align because of the shear and stretching forces imposed. At this stage, gluten networks are more developed by the cross-linking of protein with disulphide bonds. At optimum dough development, the interactions between the polymers cross-links are becoming stronger which leads to an increase in dough strength, maximum resistance to extension and restoring force after deformation. When the dough is mixed longer past its optimum development, the cross-links begin to break due to the breaking of disulphide bonds. The glutenins become depolymerised and the dough is over mixed. The presence of smaller chains in the dough makes the dough stickier. The monomeric proteins, gliadins form a matrix within the long polymer networks and contribute

to resistance to extension by forming viscous behaviour. Increasing the interactions between protein polymers increases gluten viscous resistance and resistance to extension. It was said that gliadins show dominant viscous behaviour and acted like a plasticiser, promoting viscous behaviour and extensibility of gluten (Kuktaite, 2004).

3.8.3.1. Empirical rheology

The mixograph and farinograph (Brabender 1927) are the empirical instruments and techniques that have been developed and utilized for the measurement of the empirical properties of dough (Ross et al., 2004).

During processing, the empirical tests have been used to characterize the behavior of bread doughs (Dobraszczyk and Schofield, 2002). The interactions between flour type, breadmaking process and antistaling additives in wheat dough were studied by Armero and Collar, (1998) while the mixing characteristics of flour water dough were studied by Rao et al. (2000).

Farinograph is the most frequently used equipment for empirical rheological measurements (Razmi-Rad et al., 2007) for rheological characterization. They used artificial neural network (ANN) technology for predicting the correlation between farinographic properties of wheat flour dough like water absorption, dough development time, dough stability time, degree of dough softening (Figures 10) with its chemical composition like protein content, wet gluten, sedimentation value and falling number etc. Since the approach of ANN analysis is a black box simulation, this type of study fails to reveal the physical understanding behind established correlations, even though these might be excellent. The texture and density of baked products such as bread and cakes are controlled by the way their rheology and vapor content change during the baking process. Dobraszczyk and Morgenstern, (2003) reviewed the rheological properties of gluten polymers of wheat flour which in turn affects the rheological properties of bread.

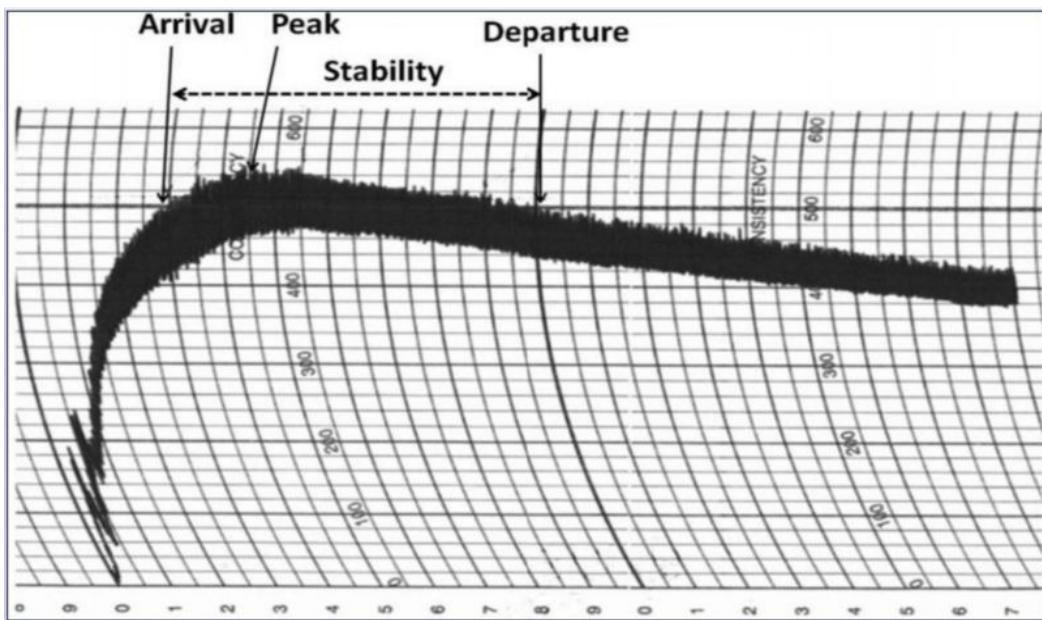


Figure 10: Farinogram of wheat flour

Farinogram parameters:

- 1. Water absorption:** is the amount of water required to center the farinograph curve on the 500 Farinograph Unit (FU) line. This relates to the amount of water needed for a flour to be optimally processed into end products. Absorption is expressed as a percentage.
- 2. Peak Time:** indicates dough development time, beginning the moment water is added until the dough reaches maximum consistency. This gives an indication of optimum mixing time under standardized conditions. Peak time is expressed in minutes.
- 3. Arrival Time** is the time when the top of the curve touches the 500-FU line. This indicates the rate of flour hydration (the rate at which the water is taken up by the flour). Arrival time is expressed in minutes.
- 4. Departure Time** is the time when the top of the curve leaves the 500-FU line this indicates the time when the dough is beginning to break down and is an indication of dough consistency during processing. Departure time is expressed in minutes.
- 5. Stability Time** is the difference in time between arrival time and departure time. This indicates the time the dough maintains maximum consistency and is a good indication of dough strength. Stability time is expressed in minutes

6. Mixing Tolerance Index (MTI) is the difference in FU value at the top of the curve at peak time and the value at the top of the curve 5 minutes after the peak. This indicates the degree of softening during mixing. Mixing tolerance index is expressed in FU

The mixograph is a rapid tool for measuring the mixing behavior of dough because of the reduced small sample size (Chung et al., 2001). The mixograph data are empirical in nature and the parameters measured by it are 15 poorly defined (Bourne, 2002). The mixograph provides the important information on the reaction of the input materials being utilized in the formulation of the dough based products and state of the mixing process. Wheat proteins are directly correlated with flour's water absorption capacity, oxidation requirements and mixing strength (Bushuk, 1998).

Scanlon et al., (2000) studied the mechanical properties of bread crumb prepared from flours of different dough strength. Because starch is damaged in hard wheat during milling, the flour consequently absorbs more water. The viscoelastic properties of weaker flours changed more markedly during storage than those of stronger flours in the sense of a significant improvement of their quality. The flour with higher water absorption may give more favorable products, because the bread may remain softer for a longer period (Hruskova and Machova, 2002).

The rheological properties of dough are dependent on both time and strain. During the empirical tests, Irreversible changes in samples occur which are major disadvantage in some empirical tests (Dobraszczyk and Morgenstern, 2003).

3.8.3.2. Fundamental rheology

Fundamental rheometry describes the physical properties of a material over a wide range of strains. The small strain rheological properties of cereal doughs are measured through the fundamental rheological techniques by the application of sinusoidally oscillating stress or strain with time and measuring the resulting response. These techniques have the advantage of a well developed theoretical background, readily available instrumentation, and simultaneous measurement of elastic and viscous moduli, while the nondestructive nature of the test enables multiple measurements to be performed as temperature, strain or frequency are varied (Steffe, 1996).

One of the advantages of dynamic rheometry is the possibility of utilizing various strains to obtain more complete information of a material's physical properties. Very low strains will allow measurements and will not disturb or destroy the inherent structure, are important in describing the time and temperature dependent changes in materials. Several researchers have demonstrated that rheometry that can give a better prediction to the quality of final products (Shiau and Yeh, 2001).

Several different fundamental rheological measurements, differing in terms of magnitude of stress or deformation, in type of deformation (shear, compression, extension, and biaxial extension), or in deformation rate or in terms of the length of time of the constant stress (creep) or deformation (relaxation) have been used to measure the rheological properties of wheat doughs. The measurement should also be sensitive to the water content of doughs because the best mechanical properties are achieved by an optimum flour-to-water ratio.

Oscillatory measurements of wheat doughs in the linear viscoelastic region with strains < 0.1 % have been used to study effects of water, ingredients, and flour type. Most rheological measurements on dough have been performed in shear because shear deformation is easier to measure. The elastic component is accounted as the storage modulus (G') and the viscous component is measured as the loss modulus (G''). The ratio of the viscous to elastic modulus (G''/G') is equal to the tangent of the phase angle ($\tan \delta$). Both storage modulus (G') and loss modulus (G'') decrease as the water content of doughs increase. Oscillatory measurements in the linear viscoelastic region have not been able to predict the baking quality of different flours (Safari-Ardi and Phan-Thien, 1998). Some studies suggest that these tests can show differences between different baking-quality wheat glutens (Kokelaar, 1994). The application of larger strains is more relevant to doughs; therefore high-amplitude oscillatory measurements have also been done (Miller and Hosney, 1999). When working within the linear viscoelastic range, data analysis can be conducted with the mathematical theory of linear viscoelasticity. This is not the case in the nonlinear region.

In the study of Tronsmo et al., (2003), wet gluten was tested with a small strain of 2 % and frequency between 0.005-10 Hz. They reported that the elastic modulus (G') was higher than the viscous modulus (G''). This result agrees with studies by Amemiya and Menjivar, (1992) who found that the storage modulus (G') for all tested doughs are higher than the loss modulus (G'').

They further described that the gluten network behaves like a cross-linked polymer at the tested frequency. Uthayakumaran et al., (2002) who conducted a study on rheological behavior of wheat gluten using dynamic oscillation testing found that both the elastic and viscous modulus of flour doughs were significantly higher than gluten doughs. This indicates that starch content in the flour dough influence the viscoelasticity of the flour dough. Other work which utilised this testing method on dough include studies on effect of different protein content, water level (Uthayakumaran et al., 2002) and mixing time on the rheological properties of dough and gluten. Tronsmo et al., (2003) found that dough with higher protein content gave lower G' and G" but higher tan δ. Janssen et al., (1996) found that the resistance to small deformation was higher and more elastic for gluten with higher protein content and as the angular frequency increased, G" increased more than G', indicating a viscous behaviour of gluten due to more bonds are involved in the response of stress or strain. Generally, it can be concluded that gluten from poor quality wheat are reologically characterised as less elastic and more viscous than glutens from good quality wheats (Khatkar et al., 2002).

Creep recovery test

Creep recovery test is performed by subjecting the material to a constant shear stress and the shear strain is monitored as a function of time. Sivaramakrishnan et al., (2004) performed creep recovery test on pure wheat flour and combinations with long/short grain rice flour found that the pure wheat flour dough showed high recovery of elastic strain after removal of load while the creep behaviour of the two composite flours with long and short grain rice flour showed considerable variation with the pure rice flours. Janssen et al., (1996) conducted creep recovery test on two different wheat flours, weak (Obelisk) and strong flour (Katepwa) found that Obelisk showed a higher recovery of elastic strain after removal of load compared to Katepwa. Janssen et al., (1996) suggested that the apparent viscosity (η_{app}) can be estimated from the slope of the creep curve and from their observation there was no clear strain hardening in creep tests since the slope of the curve was nearly independent of time and strain at the end of the load phase.

3.9. Effects of lupine flour or fiber addition on quality of bakery products

Bread and bakery products have an important role in human nutrition. Generally, wheat bread is considered to be a good source of energy and irreplaceable nutrients for the human body. This is especially true for the products made from wholegrain or high-yield flour types. Bread

prepared from refined flour is nutritionally much poorer and does not adequately meet the requirements for many macro- or micro-nutrients. It has been reported that bread made from refined flour has low micronutrient content (Isserliyska et al., 2001). Also, wheat protein lacks the balance of essential amino acids such as lysine, threonine and valine. Therefore, there have been many on-going investigations on enhancing the nutritive value of bread to fulfill the expanding demands of modern dietary habits, considering the products' protein, mineral, vitamin and/or fibre contents. Bakery products, supplemented with various nutritious, protective and ballast substances, have been gaining popularity worldwide. Mixed grain, wholegrain breads and related products are even considered as functional foods because they are convenient vehicles for important nutrients and phytochemicals.

Composite breads are made from blends of wheat and non-wheat flour. These flours are advantageous to developing countries because wheat imports can be reduced and elevate the use of locally grown grains (Hugo et al., 2003). Lupine flour can be incorporated into wheat flour to improve the nutritional value of the final products without detrimental effects on the quality (Pollard et al., 2002). Furthermore, lupine exhibits useful techno-functional properties allowing its use as an ingredient in the production of several palatable food products, such as biscuits, pasta and bread (Drakos et al., 2007) while producing satiety at the same time (Lee et al., 2006). According to literature, about 10 % of lupine replacement is the most convenient amount to improve breadmaking properties (Doxastakis et al., 2002) and the allowed upper limit by the European food authorities.

Studies have shown that lupine flour can be successfully incorporated into products (Hung et al., 1990), at up to 20 % inclusion, to produce products that rate higher in terms of color, texture, taste and overall acceptability than the control. A number of pasta products containing lupine flour are currently available on the domestic market. Lupine flour is also being tested as a base for vermicelli like product. Also, lupine can be incorporated at up to 50 % level in biscuits (Kyle, 1994).

The development of breads enriched in lupine proteins, by incorporating lupine flours, has been the subject of research conducted by Doxastakis et al., (2002). These workers demonstrated that substitution of wheat flour by full fat lupine flour, concentrated lupine flour and defatted concentrated lupine flour, at a 5 % substitution level, increased the stability and the tolerance

index of the dough, while a marked decrease was noted at higher levels (15 %) of supplementation. In addition, the bread volume decreased as the level of lupine flour increased something that was primarily attributed to the dilution of the wheat gluten structure by the added protein. However, as wheat flour fortification was conducted only with lupine flour with a protein content ranging between 30 % and 36 %, it was not possible to appreciate the real impact of the proteins of lupine flour on the dough properties since other lupine flour constituents (mainly fiber) could, also, have an effect. Furthermore, no information was reported in these studies on the effect of lupine flour addition on the Bread staling performance.

3. 10. Nutritional benefits of legumes

Legumes play an important role in human nutrition since they are rich sources of protein, calories, certain minerals and vitamins. In African diets, legumes are also the major contributors of protein and calories for economic and cultural reasons (El Maki et al., 2007). Madhusudhan and Tharanathan, (1995) found that, legumes have been shown to decrease blood glucose responses compared to other cereal based foods such as whole meal bread and are of very vital benefit in the diets of diabetes and hyperlipidemia patients. Moreover, Wolever et al., (2003) reported that the low fat, high-carbohydrate diets are known to stimulate hepatic triglyceride production in diabetic subjects.

Legumes supply protein, complex carbohydrates, fibre and essential vitamins and minerals to the diet, which are low in fat and sodium and contain no cholesterol. Legumes have been identified as low glycaemic index foods (Bornet et al., 1997). Selecting foods of low glycaemic index is very important in the dietary treatment of diabetes mellitus, increases satiety, facilitates the control of food intake and has other health benefits for healthy subjects in terms of post-prandial glucose and lipid metabolism (Rizkalla et al., 2002). Regular consumption of pulses may have important protective effects on risk for cardiovascular disease (Anderson and Major, 2002). Moreover, pulses contain a rich variety of compounds, which, if consumed in sufficient quantities, may help to reduce tumour risk (Mathers, 2002). In fact, most health organizations encourage their frequent consumption (Leterme, 2002).

These nutritional benefits are related to the reduced digestibility of legume starch and dietary fibre content of legumes, mainly located in their husk fractions. The low digestibility of legume starch has been attributed to its amylose, which is considerably branched and of high

molecular weight (Tharanathan and Mahadevamma, 2003). In the last decades, attitudes and perceptions towards legumes have been changing, bringing about a revival of interest on the part of consumers (Morrow, 1991). The annual per capita consumption of pulses in 1999 was 5.9 kg worldwide, and 2.8 kg in Europe. These consumption figures rose by 10 % from 1989 to 1999 but they could increase even further if the food industry and professional organizations take up the challenge to incorporate grain legumes in novel, convenient and healthy food products (Schneider, 2002).

Several studies about the influence of the addition of legume flours on the functional properties of bread dough and final bread quality have been reported in the last 30 years. Among the legumes tested, it is worth mentioning the addition of chickpea flour (Mohammed, 2011), germinated chickpea flour (Fernandez and Berry, 1989), germinated pea flour (Sadowska et al., 2003), lupine flour (Pollard et al., 2002), fermented lentil flour (Sadowska et al., 1999), lentil and bean flours (Lorimer et al., 1991) to wheat flour for obtaining bread. However, despite the good results obtained with bread, those studies have not been extended to other cereal baked products.

3.10.1. Lupine flour

There is growing interest in industrial exploitation of new protein sources such as plant proteins to broaden the range and variety of foods. Sweet lupine seeds (e.g. *Lupinus albus* L.) seem to be particularly promising as a source of innovative ingredients having, on average, a protein content similar to soybean (34-43 % of dry matter) and an adequate composition of essential amino acids (Yanez et al., 1983). Foods based on sweet lupine protein are gaining attention from industry and consumers because of their possible role in the prevention of cardiovascular disease as well as in reduction of blood glucose and cholesterol levels (Duranti, 2006).

The lupines (Family, Fabaceae–pea family) belong to a diverse group of plants that contain a large number of biologically active compounds in their leaves and seeds. The seeds in particular contain a significant number of alkaloids, predominantly with anticholinergic activity (Ruiz and Sotelo, 2001). The white lupine is also known to contain hypoglycemic agents [*Lupinus albus* (L. *termis* L.)] (Kubo et al., 2000). Lupine is also valuable nutritionally and is of particular interest due to the large seed size and the ability to remove the unpleasant and potentially toxic alkaloid components by soaking in water (Santana et al., 2002). The white lupine has been reportedly used

in Egypt by people having type II diabetes, although the outcome in humans of this use has not been documented (Eskander and Won Jun, 1995). Mansour et al., (2002) suggest that the white lupine would be useful for lowering blood glucose levels post-prandially and by inference from allopathic medications, would decrease glycosylated hemoglobin levels.

Lupine kernel flour is a novel food ingredient derived from the endosperm of lupine, a grain legume. It contains 40–45 % protein, 25–30 % fiber, and negligible sugar and starch (Evans et al., 1993). It can be incorporated into high carbohydrate foods, resulting in significant increases in protein and fiber, reductions in refined carbohydrate, and little change in product acceptability (Lee et al., 2006). Increasing protein at the expense of refined carbohydrate in the diet may benefit blood pressure. An inverse association between estimated protein intake and blood pressure was reported in many cross-sectional population studies (Appel, 2003). In randomized controlled trials, lower blood pressure with protein, in comparison to carbohydrate, is also a consistent finding (Hodgson et al., 2006).

A meta-analysis of randomized controlled trials comparing carbohydrate with monounsaturated fat found that a higher intake of carbohydrate of 55 g/d resulted in higher systolic and diastolic blood pressures of 1.3 and 0.9 mm Hg, respectively (Shah et al., 2007). An increased intake of dietary fiber may also lower blood pressure. Many randomized controlled trials have now investigated the effects of increasing fiber intake on blood pressure. Meta-analyses of these trials showed that an increase in fiber intake of 10–15 g/d was associated with falls in systolic and diastolic blood pressures of 1–1.5 mm Hg (Whelton et al., 2005). Soluble fiber may be more effective than insoluble fiber (Streppel et al., 2005). In addition, more substantial decreases in systolic and diastolic blood pressures were observed in trials of 8 weeks duration (3.1 and 2.6 mm Hg; systolic and diastolic blood pressures, respectively) and in hypertensive subjects (6.0 and 4.2 mm Hg; systolic and diastolic blood pressures, respectively) (Whelton et al., 2005). Increasing both protein and fiber intakes, at the expense of refined carbohydrate, may benefit blood pressure.

Lupine flours can be an excellent choice for improving the nutritional value of bread. The high-lysine, low methionine content complements that of wheat flour proteins, which are poor in lysine and relatively higher in the sulphur-containing amino acids (Bloksma and Bushuk, 1988). In lupins, the main limiting amino acids are methionine and cystine followed by valine and then

tryptophan. Since lupins are legumes, the lack of sulphur-containing amino acids is not surprising. Valine seems to be adequate in *L. albus* (Aguilera and Trier, 1978). Lupine protein isolates, prepared on a bench scale, have been shown to have good nutritional properties when supplemented with methionine or mixed with cereals. Functional properties of proteins, such as solubility, water absorption and binding, viscosity, gelation, cohesion-adhesion, elasticity, emulsification, fat absorption, flavour binding, foaming and color control are influenced by agronomic factors, storage, composition and processing (Cherry et al., 1979). Although carbohydrate is the major component of legumes, the protein component has received considerably more attention (McWatters, 1990). The use of vegetable proteins as functional ingredients in foods depends mainly on the benefits that they can produce (Kyle, 1994).

3.10.2. Lupine fiber

The interest in foods rich in dietary fibre increased in the recent decades and this led to the development of a large market for fibre-rich products and ingredients (Drzikova et al., 2005). The specific properties of dietary fibre has been reported to play an important role in the prevention and treatment of various gastrointestinal disorders (hernia, duodenal ulcer, gall stones, appendicitis, constipation, hemorrhoids, colon carcinoma), obesity, atherosclerosis, coronary heart diseases, colorectal cancer and diabetes (De Escalada Pla et al., 2007). Addition of fibre to foods is an alternative way to compensate for the existent deficiency in the diet. Apart from the nutritional application, fibre can be used for technological purposes such as bulking agent or fat substitute in foods (Guillon and Champ, 2000).

The World Health Organization (WHO, 2003) currently recommends consumption of foods containing > 25 grams (30-45 g) of total dietary fibre/day. In fact, WHO has identified dietary fibre as the only dietary ingredient with “Convincing Evidence” showing a protective effect against weight gain and obesity. Bread can be enriched with dietary fibre such as wheat bran, gums such as guar gum and modified celluloses and beta-glucans. Wheat flour contains 1.5–2.5 % total arabinylans, non-starch polysaccharides of cereal which is an important source of dietary fibre where one-third to half is water-extractable and the other is water-unextractable (Su et al., 2005). According to Peng, (2002), the pumpkin polysaccharide had the function of reducing the blood sugar and showed that it had very important value in auxiliary cure for diabetes.

The dietary fibre is composed of total dietary fibre (TDF), which includes both soluble (SDF) and insoluble dietary fibre (IDF). In terms of health benefits, both kinds of fibre complement with each other. A well balanced proportion is considered when there is 70-50 % insoluble and 30-50 % soluble dietary fibre F (Grigelmo-Miguel et al., 1999).

Soluble fibre is found in fresh and dried fruit, vegetables, oats, legumes and seeds. Examples of the soluble fibre are pectins, gums, mucillages and some hemicellulose. Some soluble fibres increase the viscosity of the intestinal contents and assist in reducing cholesterol absorption. Other soluble fibres are fermented by the bacteria within the large intestine and can assist in maintaining colon health and increasing the mineral absorption. Soluble fibre fermentation results in the production of short-chain fatty acids, principally acetate, propionate and butyrate. Butyrate has been found to act as a protective agent against experimental tumor genesis of these cells. Propionate could be related to hypocholesterolemic effects (Redondo-Cuenca et al., 2007).

Insoluble fibre is found in the plant cell walls of whole grain bread, whole grain cereals, fruits, vegetables, unprocessed bran and wheat germ. Examples of the insoluble fibre are cellulose, lignan and hemicellulose. Many insoluble fibres, including cellulose and psyllium, are not fermentable. Insoluble dietary fibre has a high water-holding capacity, increases the fecal bulk and reduces the gastro intestinal transit time. This effect may be related to the prevention and treatment of different intestinal disorders, such as constipation, diverticulitis, haemorrhoids and other bowel conditions (Goñi and Martin-Carrón, 1998).

Lupine kernel fiber is a novel food ingredient that can be isolated from the endosperm of Australia's major animal feed legume crop, the Australian sweet lupine (*Lupinus angustifolius*). This legume has already gained legislative approval for use as human food in some countries, including Australia. Demonstration that lupine kernel fiber can be used to formulate food products with acceptable sensory properties is required to introduce this novel ingredient into the food supply system. This fiber is predominantly nonstarch polysaccharide in the form of thickened cell walls of the lupine seed endosperm, with some residual protein. Although it is primarily insoluble in nature, the nonstarch polysaccharide component has paradoxically been described as a "pectin-like" rhamnogalacturonan, pectin generally being considered a soluble fiber. Lupine kernel fiber has been described as a powder that is pale in color, low in odor and

flavor, and suitable for use as a ‘nonintrusive’ fiber ingredient in foods such as baked goods and meat products (Johnson and Gray, 1993).

Nevertheless, there is a lack of published data on the sensory acceptability of foods containing lupine kernel fiber. Comparisons may be tentatively drawn, however, from published studies on flour derived from other lupine species, since nonstarch polysaccharide (chemically similar to that found in Australian sweet lupine kernel fiber) is a major component of the cotyledons of many lupine species (Brillouet and Riochet, 1983). Accordingly, the properties of the nonstarch polysaccharide would be predicted to influence the sensory performance of the flour in food products. Ballester et al., (1984) added to bread full-fat sweet lupine flour derived from *Lupinus albus* cv Multolupa, which resulted in increased water absorption, loaf volume, and loaf weights. Crust color and bread texture were reportedly not affected by lupine flour addition, though the method of determining these parameters was not clarified. Villarroel et al., (1996) evaluated the sensory acceptability of marmalade that incorporated lupine flour as a replacement for fructose by using a facial hedonic test, and found no statistically significant difference between the control and lupine marmalade.

3.11. Review conclusion

To summarize the literature review, legumes represent, together with cereals, the main plant source of proteins in human diet. They are also generally rich in dietary fibre and carbohydrates. Minor compounds of legumes are lipids, polyphenols, and bioactive peptides. It turns out that the protein potential of wheat and legumes (lupine flour) approach, from a nutritional, nutritional view almost perfect complements (cysteine and methionine are sulfur-containing essential amino acid as enriched). The implementations of the concept of enrichment of wheat flour proteins with proteins leguminous crops are facing back technical concerns. When necessary processes deliver dough the protein / starch together an implementation of the wheat to be relatively ideal processing dough as the primary protein-water interaction. The result is a viscoelastic system with known stable processing properties. The admixture of a primarily non-compliant egg white prevents compounds the formation of a known viscoelastic system based on gluten interactions in the matrix. Further, changes in the sensory acceptance, depending on the mixing ratio to be expected, with crumb pore structure, etc. The critical mixing ratio of the lupine flour or fibers with wheat flour is approximately 20 : 80 %. This mixture has acceptable technical and sensory characteristics. All conventional and modern dough rheological measurement

techniques can be a specific control or comparison methods were used. Since the results are not modern rheological measurement techniques in the literature extensively be described, is a particular focus on the application / results oscillation and its generalizable measurement guidelines.

4. Materials and Methods

4.1. Materials

Local Egyptian breeds of lupine (*Lupinus albus L.* variety Giza) were obtained from the Agricultural Research Centre, Giza, Egypt. Lupine flours and hulls were obtained after grinding lupine grains in a laboratory hammer mill (Retsch - Germany) until they could pass through a 250 µm screen. Commercial wheat flour type 405 was obtained from Lidl Market (Berlin-Germany). All other chemical reagents used in the experimental analysis were of analytical grade.

4.2. Chemical analysis

4.2.1. Proximate composition

Proximate composition was carried out according to ICC Standard Methods (ICC, 2001). Moisture content was determined by drying the samples at 105 °C to constant weight (ICC 109/01). Ash content was determined by calcinations at 900 °C (ICC 104/1). Nitrogen content was determined by using Kieldahl method with factor of 5.7 to determine protein content (ICC 105/2). The total lipid content was determined by defeating in the Soxhelt apparatus with hexane (ICC 136). The determination of starch content was assessed using a polarimetric method according to Ewers, modified by (Davidek et al., 1981). All the measurements of analyzed samples were made in triplicate.

4.2.2. Amino acid analysis

Amino acid content was determined as described by Moore et al., (1958). The analysis was performed in Central Service Unit, National Research Centre, Egypt using LC3000 amino-acid analyzer (Eppendorf-Biotronik, Germany). The technique was based on the separation of the amino acids using strong cation exchange chromatography followed by the ninhydrin colour reaction and photometric detection at 570 nm. Standard amino acids were used for comparison of resulting profiles, allowing quantitation of amino-acid residues. The defatted powdered seeds were hydrolyzed with 6 N HCl at 110 °C in teflon capped vials for 24 h. After vacuum removal of HCl, the residues were dissolved in a lithium citrate buffer, pH 2.2. Twenty µL of the solution were loaded into the cation exchange column (pre-equilibrated with the same buffer), then four lithium citrate buffers with pH values of 2.2, 2.8, 3.3 and 3.7, respectively, were successively applied to the column at a flow rate of 20 mL/min. The ninhydrin flow rate was 10 mL/h under these conditions and a typical analysis required 160 min.

Methionine was determined as methionine sulfone, after oxidation with performic acid. An amino acid standards containing cysteine were treated parallel with the samples and used to quantify the methionine content. The amino acid content of the reference protein was taken from (FAO/WHO, 2007).

4.2.3. Determination of total phenolics

Total phenolic content was determined by the Folin–Ciocalteu micro-method (Arabshahi-Delouee and Urooj, 2007). A 20 µL aliquot of extract solution was mixed with 1.16 mL of distilled water and 100 µL of Folin–Ciocalteu's reagent followed by 300 µL of 200 g L⁻¹ Na₂CO₃ solution. The mixture was incubated in a shaking incubator at 40 °C for 30 min and its absorbance at 760 nm was measured. Gallic acid was used as standard for the calibration curve. Total phenolic content expressed as gallic acid equivalent (GAE) was calculated using the following linear equation based on the calibration curve:

$$Ab = 0.98C + 9.925 * 10^{-3} \quad (R^2 = 0.9996) \quad (1)$$

where Ab is the absorbance and C is the concentration (mg GAE g⁻¹ dry weight (DW)).

4.2.4. Determination of total flavonoids

Total flavonoid content was determined by the method of Ordoñez et al., (2006). A 0.5 mL aliquot of 20 g L⁻¹ AlCl₃ ethanolic solution was added to 0.5 ml of extract solution. After 1 h at room temperature the absorbance at 420 nm was measured. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg ml⁻¹. Total flavonoid content expressed as quercetin equivalent (QE) was calculated using the following equation based on the calibration curve:

$$C = 0.0255 * Ab \quad (R^2 = 0.9812) \quad (2)$$

where Ab is the absorbance and C is the concentration (mg QE g⁻¹ DW).

4.2.5. Determination of total flavonols

Total flavonol content was determined by the method of Kumaran and Joel Karunakaran, (2007). To 2 mL of extract solution, 2 mL of 20 g L⁻¹ AlCl₃ ethanolic solution and 3 mL of 50 g L⁻¹ sodium acetate solution were added. The absorption at 440 nm was read after 2.5 h at 20 °C. Extract samples were evaluated at a final concentration of 0.1mg mL⁻¹. Total flavonol content expressed as QE was calculated using the same equation of flavonoids.

4.2.6. Antioxidant activity of extracts

Because of the differences among the various test systems available, the results of a single method can provide only a limited assessment of the antioxidant properties of a substance (Sacchetti et

al., 2005). For that reason, in this study the antioxidant capacity of each extract was determined through two complementary assay procedures.

4.2.6.1. DPPH· radical-scavenging activity

The DPPH assay according to Lee et al., (2003) was utilised with some modifications. The stock reagent solution (1×10^{-3} mol L $^{-1}$) was prepared by dissolving 22 mg of DPPH in 50 mL of methanol and stored at -20°C until use. The working solution (6×10^{-5} mol L $^{-1}$) was prepared by mixing 6 mL of stock solution with 100 mL of methanol to obtain an absorbance value of 0.8 ± 0.02 at 515 nm, as measured using a spectrophotometer. Extract solutions of different concentrations (0.1 mL of each) were vortexed for 30 s with 3.9 mL of DPPH solution and left to react for 30 min, after which the absorbance at 515 nm was recorded. A control with no added extract was also analysed. Scavenging activity was calculated as follows:

$$\text{DPPH radical-scavenging activity (\%)} = [(Ab_{\text{control}} - Ab_{\text{sample}})/Ab_{\text{control}}] * 100 \quad (3)$$

where Ab is the absorbance at 515 nm.

4.2.6.2. ABTS radical-scavenging activity

For the ABTS assay the method of Re et al., (1999) was adopted. The stock solutions were 7 mmol L $^{-1}$ ABTS solution and 2.4 mmol L $^{-1}$ potassium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12–16 h at room temperature in the dark. Then 1 mL of the resulting ABTS $^{+}$ solution was diluted with 60 mL of methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm, as measured using a spectrophotometer. ABTS $^{+}$ solution was freshly prepared for each assay. Extract solutions of different concentrations (1 mL of each) were allowed to react with 1 mL of ABTS $^{+}$ solution for 7 min, after which the absorbance at 734 nm was recorded. A control with no added extract was also analysed. Scavenging activity was calculated as follows:

$$\text{ABTS radical-scavenging activity (\%)} = [(Ab_{\text{control}} - Ab_{\text{sample}})/Ab_{\text{control}}] * 100 \quad (4)$$

where Ab_{control} is the absorbance of ABTS radical + methanol

Ab_{sample} is the absorbance of ABTS radical + extract

4.2.7. HPLC analysis

A total of 20 mg grounded dried samples were extracted for 15 min using 750 μL 70 % methanol (v/v, pH 4, phosphoric acid) in an ultrasonic water bath (Sonorex digital 10p ,

Bandelin) on ice. Samples were centrifuged for 5 min at 6000 rpm. The supernatants were collected and the pellets were re-extracted twice more with 500 µL 70 % methanol. The combined supernatants from each sample were reduced to near dryness in a centrifugation evaporator (Speed Vac, SC 110) at 25 °C. Samples were then made up to 1 ml with 40 % acetonitrile. The samples were filtrated using 0.22 µm filter, and then analyzed with HPLC (Dionex Summit P680A HPLC-System), equipped with P680 pump, ASI-100 automated sample injector, a Narrow-Bore Acclaim PA C16-column (3 µm, 2.1 * 150 mm, Dionex) and PSA-100 photodiode array detector (Dionex) and software Chromeleon 6.8 (Dionex, USA). The column was operated at a temperature of 35 °C.

The mobile phase consisted of 0.1 % (v/v) phosphoric acid in ultrapure water (eluent A) and of 40 % (v/v) acetonitrile in ultrapure water (eluent B). A multistep gradient was used for all separations with an initial injection volume of 40 µL and a flow rate of 0.4 mL/min. The multistep gradient was as follows: 1 min: 0.5 % (v/v) B; 1-10 min: 0.5-40 % B; 10-12 min: 40 % B; 12-18 min: 40-80 % B; 18-20 min: 80 % B; 20-24 min: 80-99 % B; 24-30 min: 99- 100 % B; 30-34 min: 100-0.5 % B; 34-39 min: 0.5 % B. Simultaneous monitoring was performed at 290, 330 and 254 nm at a flow rate of 0.4 mL/min. Phenolic acid quantity was calculated from HPLC peak areas at 290 nm against the internal standard and external standards. Identification and quantification of phenolic acids present was done by comparing retention time and area of the peaks in the extracts with that of the standard phenolic acids (chlorogenic acid, caffeic acid, cinnamic acid, coumaric acid, rosmarinic acid and sinapic acid).

4.3. Particle size analysis by laser particle analysis

To determine the grain size distribution of the flour and the flour fractions, a counting or scattered light measuring procedures was used. The measurement of particle size distribution was carried out in the laser-light scattering spectrometer "HORIBA LA-950" manufactured by Retsch (Figure 11), on the basis of static laser light scattering, according to DIN / ISO 13320. Formed by the diffraction of the laser beam through the solid diffraction pattern which projected through a lens onto a screen and scanned.



Figure 11: Particle size analyzer HORIBA LA-950 Retsch

The resulting diffraction rings whose intensity and scattering angle for particle size proportionally. The statistical analysis of grain size distribution, the volume fractions and the specific surface of the sample was carried out by the computerized data processing. The main parameters for characterizing the grain size distribution are: d_{10} , d_{50} , d_{90} and the specific surface of the powder. Module operating air pressure 0.3 MPa was used in the measurement.

4.4. Doughs preparation

Blends were prepared according to Mohammed, (2011) by mixing the wheat flour with lupine flours or lupine fibre in the proportions of 5, 10 and 15 % substitutions of wheat flour using a mixer with a spiral blade, which is usually used for dough mixing. The doughs were prepared by mixing different blends with 58 % water for 5 min in a Kitchene Aid Professional mixer (KPM5) at 25 °C. The concentration (5, 10 and 15 % from wheat flour) of lupine fibre but with different amount of water (62, 64 and 68 % respectively) was done.

4.5. Empirical rheological properties of dough

The dough mixing of the different wheat /lupine flour or fiber blends were studied using farinograph instrument Farinograph Brabender, (Brabender, Duisburg, Germany) (Figure 12) according to Mohammed, (2011). To determine the absorption of water and kneading flour, 50 g of flour input to the farinograph and kneaded with a supply of water. The resistance of the forming dough against the mechanical stress is a farinogram torque measurement recorded over time. The measurements were conducted according to the constant flour weight procedure of ICC method 115/1 and all measurements were made at room temperature (25 °C). From the farinograph curves, water absorption (percentage of water required to yield dough consistency of

500 FU), dough development time (DDT, time to reach maximum consistency), stability (time during dough consistency is at 500 FU) and degree of softening (difference in FU) between the line of the consistency and the medium line of the torque curve 12 min after development time), were determined.



Figure 12: Brabender Farinograph

4.6. Fundamental rheological properties of bread dough and cake batter

A rheometer UDS 200 from Paar Physica (GmbH measurement technique Stuttgart) with temperature control with a plate-plate system (measurement system MP 31) (Figure 13) was used for measuring the rheological properties of dough samples and cylinder (measurement system Z3-DIN) (Figure 14) was used for measuring the rheological properties of paster samples according to Mohammed, (2011). Operation, including temperature control and data handling, was conducted using PC-based software. Each time, a sample was taken of a given blend (wheat flour + lupine flour), containing 100 g of flour and combined with a specific amount of water, equivalent to the water absorption of the blend at the level of 58 % (at 14 % moisture basis). The consistency of the sample obtained at that level of dough moisture permitted its placement by hand within the measurement system of the rheometer. The dough was kneaded for 5 min using a mixer with a spiral blade. Next, 10 g dough was transferred onto the lower plate of the rheometer and pressed down with the upper plate, 25 mm in diameter, until a gap of 2 mm was obtained. The excess of the sample, protruding beyond the edge of the upper plate, was trimmed off, while drops of fluid silicon oil were placed around the uncovered surface of the sample to protect the sample from loss of moisture during the test. In this condition the sample was left to rest for 1 min. That period permitted the relaxation of normal stresses generated in the course of compression of the sample. All rheological tests were made at a constant temperature of the lower plate (25 °C), controlled by means of an external thermostatic bath.



Figure 13: Laboratory instrument universal dynamic spectrometer UDS 200 plate–plate system (measure system MP 31 right side)

4.6.1. Oscillation test

The characteristic values of storage modulus, loss modulus and loss factor were recorded during the measurement with air bring measurement system.

4.6.1.1. Amplitude sweep

The amplitude of relative strain was $10^{-4} \leq \gamma \leq 1$ and fell within the linear viscoelastic region for all samples. The limits of the region were determined based on an experiment in which increasing stress was applied, at constant oscillation frequency of 1 Hz.

4.6.1.2. Frequency sweep

Applying oscillation frequencies within the range from 0.1 to 20 Hz at constant strain $\gamma = 10^{-3}$. Each logarithmic frequency decade corresponded to 10 measurement points nearly, all about 30 points.

4.6.1.3. Temperature sweep

Temperature dependence of storage (G') and loss (G'') modulus as well as loss tangent ($\tan \delta$) were measured by heating the systems from 15 to 90 °C. The temperature gradient was 1 K/min on heating scan, while the strain was fixed to $\gamma = 10^{-3}$ with a constant frequency of 1 Hz. This heating rate was chosen in order to secure gradient in the sample and to detect the occurring processes such as denaturizing of proteins, gelatinization of starch and the immobilization of the water. The baking properties of wheat lupine flour or fiber dough's with temperature sweep are comparatively simulated.



Figure 14: Laboratory instrument universal dynamic spectrometer 200 cylinder measure system

4.6.2. Creep test

The cycle of conservative tests was followed by a 10 min period of relaxation. Then, the dough sample was subjected to the creep test, applying a constant shear stress of 50 Pa for 60 s on the sample and allowing the sample to recover the strain in 180 s after removal of load. The dynamic tests and the creep tests were made in three replications, each on a freshly prepared sample of dough. The figures present the measurement results after their averaging.

4.7. Technological properties methods

4.7.1. Bread formulation and baking

Breads were prepared according to ICC-Standard Nr.131 as follows: 500 g (wheat flour or wheat flour substituted with 10, 20 or 30 % lupine flour or fiber), were first dry-mixed in the mixer bowl for 1 min. Next, 1 % sugar, 1.2 % salt, 3 % fresh compressed yeast, previously dissolved in water, were added followed by the addition of water up to 500 FU consistency and the dough kneading process was continued for a total of 5 min and placed in baking pans then a proofing cabinet at 30 °C and 75-80 % relative humidity.

After 45 min fermentation, the dough was punched down to remove gases, proofed for further 45 min and baked at 240 °C for 30 min. During baking, some water was vaporized in the oven to avoid any extreme dryness of the bread crust. Each baking test was conducted in triplicate.

4.7.2. Cake formulation and baking

The recipes of butter cake used: 100 g flour, 80 g egg, 60 g sugar, 50 ml Milk (1.5 % fat), 50 g margarine and 4 g baking powder. A creaming mixing procedure was used. All ingredients, except for the flour and baking powder, were mixed for 2 min at speed 6 using a Kitchene Aid

Professional mixer (KPM5). After the addition of the flour and baking powder, the mixing process continued for 3 min at speed 8. 200 g of cake batter were placed into 120 mm diameter and 45 mm height, metallic, lard coated pan, and were baked in an electric oven for 25 min at 200 °C. Wheat flour was substituted by lupine flour or fibre, based on the same way in bread making.

4.7.3. Loaf volume determination

Bread or cake mass was weighted after 3 hours at room temperature. The volume (cm³) was measured by rapeseed replacement method described in the (AACC, 1983). The specific volume was obtained by dividing the volume of loaves by their weights.

4.7.4. Color measurements

Crumb and crust color of fresh bread was measured with a Minolta Calorimeter (CR 200 Japan). Color readings were expressed by Hunter values for L*, a* and b*.

4.7.5. Sensory evaluation

Evaluation of the baked loaves quality characteristics was carried out following cooling to room temperature for 2 h. Sensory evaluation was performed by ten panelists who were graduate students and staff members of the Department of Rheology, Institute of Food Technology and Food Chemistry, Technical University, Berlin. Loaves were randomly assigned to each panelist. The panelists were asked to evaluate each loaf for appearance, crumb texture, crumb grain, crust color, taste, odor and overall acceptability. A 10 point scale was used where 10 "excellent" and 1 "extremely unsatisfactory".

4.8. Diet and experimental animals design:

Forty eight male albino rats (Sprague dawley strain) ranged weight 110-120 g were obtained from National Research Center, Giza, Dokki, Egypt. They were housed in individual cages at room temperature. The animals were left to acclimatize for ten days before the start of experiment. They were fed with standard laboratory diet according to (AOAC, 2002) and randomly divided into two main groups. First group (6 rats) was fed on a basal diet without lupine flour or fiber for 35 days and considered as control rats (control A). The second main group (42 rats) was fasted overnight and injected with alloxan solution (120 mg/kg rat weight) to induce hyperglycemia (Arbeeny and Bergquist, 1991). After 48 h of injection, the second main group was divided into seven subgroups (6 rats each). The first subgroup represents the diabetic

rats (control B) was fed on basal diet without lupine flour or fiber. The rats of 2nd, 3ed and 4th subgroups were fed separately on basal diet plus different levels (5, 10 and 15 %) of lupine flour respectively. The rats of 5nd, 6ed and 7th subgroup were fed separately on basal diet plus different levels (5, 10 and 15 %) of lupine fiber respectively. Animals were weighted weekly and sacrificed at the end of the experiment. Blood samples were collected from hepatic portal vein; serum was separated by centrifugation at 3000 rpm, for 15 minutes and kept at -5 °c tell analysis. Serum cholesterol, total lipids and glucose levels were determined according to Hewitt and pardue, (1973); Frings and Dunn, (1970); Trinder, (1969) respectively.

4.9. Statistical analysis

Analysis of variance (ANOVA) was carried out using SAS program (Statistical Analysis System version. 9.1) SAS Institute Inc. (SAS, 2004). The rheological properties and bread characteristics of wheat dough with or without lupine flour or fiber were analysed using ANOVA. When the treatment factor effect was found significant, indicated by a significant F-test ($p < 0.05$), differences between the respective means were determined using least significant difference (LSD) and considered significant when $p < 0.05$. Mean ± standard deviation of mean was used.

5. Chemical composition

The objectives of this study were to investigate chemical composition (proteins, lipids, starch, sugars, fiber, ash, amino acids), phytochemicals compounds (total phenolic, flavonoids, flavonols contents) and antioxidant capacity (DPPH and ABTS). The potential use of different sweet lupine seed derivatives (flour and fiber) at different concentration (5, 10 and 15 %) for baking applications (bread and cake) and the influence of lupine addition on the rheological properties (empirical rheology and fundamental) of dough and quality of final products were also described. Effects of different sweet lupine seed derivatives (flour and fiber) at different concentration (5, 10 and 15 %) on diabetic rats were also studied.

5.1. Wheat flour, lupine flour and their blends

Lupine is a good source of nutrients, not only proteins but also lipids, dietary fibre, minerals, and vitamins (Martínez-Villaluenga et al., 2009). The results for the chemical composition of wheat flour (WF), lupine flour (LF), and their blends are shown in Table (1). The lupine flour showed higher levels of moisture, crude protein, ash, crude fat and dietary fiber than the wheat flour. Conversely, wheat flour showed higher levels of starch. These results confirmed by statistical analysis, which highly significant differences ($P < 0.05$) were observed between the two types of flours. Mean protein and dietary fiber increased with increasing amount of lupine flour added to be 13.73 ± 0.24 , 14.75 ± 0.27 , 16.28 ± 0.31 and 4.66 ± 0.27 , 6.61 ± 0.43 8.57 ± 0.60 g/100 g for substituting wheat flour with lupine flour at 5, 10 and 15 %, respectively on dry weight basis. There was no significant difference between wheat flour and supplemented flour with different concentration of lupine for moisture, ash and fat content.

The chemical properties of wheat flours have been studied previously by several researchers and they found that moisture content ranged between 12.5 to 14.6 % crude protein content 8.23 to 12.71 % and ash content 0.42 to 0.66 (Ahmad et al., 2001).

Table 1: Chemical composition of wheat flour (WF), lupine flour (LF) and their blends

Analysis	WF	LF	LF level (%)		
			5	10	15
Moisture	11.27 ± 0.09	12.37 ± 0.46	11.35 ± 0.19	11.42 ± 0.17	11.54 ± 1.16
Protein	12.1 ± 0.20	38.6 ± 0.87	13.73 ± 0.24	14.75 ± 0.27	16.28 ± 0.31
Ash	0.40 ± 0.02	3.41 ± 0.03	0.55 ± 0.01	0.70 ± 0.04	0.85 ± 0.03
Fat	1.62 ± 0.19	9.94 ± 0.16	2.04 ± 0.19	2.45 ± 0.17	2.87 ± 0.16
Starch	69.8 ± 1.96	0.98 ± 0.04	66.36 ± 3.94	62.92 ± 1.88	59.48 ± 2.84
S. D. F	1.1 ± 0.09	11.0 ± 1.36	1.60 ± 0.14	2.09 ± 0.27	2.59 ± 0.31
I. D. F	1.6 ± 0.23	30.8 ± 2.45	3.06 ± 0.31	4.52 ± 0.42	5.98 ± 0.53
T. D. F	2.7 ± 0.15	41.8 ± 3.08	4.66 ± 0.27	6.61 ± 0.43	8.57 ± 0.60

Mean ± standard deviation of mean

Protein content of lupine (38.6 %) was higher than that of a lot of legumes. Favier et al., (1995) reported that haricot bean, lentil and soy bean contain 28.8 %, 26.7 % and 40.5 % protein, respectively. Because of the high protein content, lupine flour could be used in the human diet. Also, temperature of denaturation of these proteins is higher than animal protein, so they are technologically easier to handle (Chapleau and de Lamballerie-Anton, 2003). Lupine flour had a high amount of crude fibre (16.2 %). These fibres have many desirable properties, including white color, high water-holding capacity (7.1 g H₂O/g) and beneficial effects on human health (Huyghe, 1997). Therefore, lupine flour can be incorporated into a wide range of foods to make dietary products.

5.2. Wheat flour, lupine fiber and their blends

The proximate compositions of wheat flour (WF), lupine fiber (L-fiber) and wheat flour substituted with different levels of lupine fiber are given in Table (2). The lupine fiber showed higher levels ash, crude fat and dietary fiber than the wheat flour. Conversely, wheat flour showed higher levels of moisture, crude protein and starch. These results confirmed by statistical analysis, which highly significant differences ($P < 0.05$) were observed between wheat flour and lupine fiber. Mean dietary fiber increased with increasing amount of lupine fiber added to be 6.82 ± 0.32 , 10.95 ± 0.53 and 15.07 ± 0.75 for substituting wheat flour with lupine flour at 5, 10 and 15 %, respectively on dry weight basis. There was no significant difference between wheat flour and supplemented flour with different concentration of lupine fiber for moisture, ash and fat content.

Table 2: Chemical composition of wheat flour (WF), Lupine fiber (L-fiber) and their blends

Analysis	WF	L-fiber	L-fiber level (%)		
			5	10	15
Moisture	11.27 ± 0.09	8.76 ± 0.06	11.14 ± 0.08	11.02 ± 0.07	10.89 ± 0.16
Protein	12.1 ± 0.20	4.8 ± 0.26	11.74 ± 0.23	11.37 ± 0.25	11.01 ± 0.29
Ash	0.40 ± 0.02	2.57 ± 0.18	0.51 ± 0.12	0.62 ± 0.06	0.73 ± 0.44
Fat	1.62 ± 0.19	2.1 ± 0.11	1.64 ± 0.17	1.67 ± 0.14	1.69 ± 0.18
Starch	69.8 ± 1.96	0.20 ± 0.04	66.32 ± 1.91	62.84 ± 1.86	59.36 ± 3.82
S. D. F	1.1 ± 0.09	43.7 ± 2.19	3.23 ± 0.15	5.36 ± 0.32	7.49 ± 0.45
I. D. F	1.6 ± 0.23	41.5 ± 1.12	3.60 ± 0.25	5.59 ± 0.27	7.59 ± 0.34
T. D. F	2.7 ± 0.15	85.19 ± 4.38	6.82 ± 0.32	10.95 ± 0.53	15.07 ± 0.75

Mean ± standard deviation of mean

5.3. Amino acids content

Lupine seeds represent a good balance of essential amino acids (Drakos et al., 2007). They are considered to be a good source of lysine, and are generally poor in the sulfur-containing amino acids (methionine and cysteín) (Gulewicz et al., 2008) and threonine (Pisariková et al., 2008).

The results for the amino acid content of wheat flour (WF) and lupine flour (LF) are shown in Table (3). In contrast to plants, humans and animals are able to synthesize only 9 amino acids used in protein synthesis (non-essential amino acids). The biosynthesis of the remaining (essential) amino acids, thereby the protein synthesis, is not possible without their continuous supply through food consumption. In the case of low-protein diets, symptoms, such as delay in growth, negative nitrogen uptake or disturbances in protein synthesis, can take place. Therefore foods rich in exogenous amino acids are desirable.

Table 3: The total amino acids % dry matter, for wheat (WF) and lupine flour (LF).

Amino Acid g/kg	WF	LF	FAO/WHO**	Amino Acid g/kg	FAO/WHO	WF	LF
Lysin*	3,0 ± 0.13	16.35 ± 0.56	5.8	Leucin	6.6	6,7 ± 0.12	26.13 ± 1.08
Threonin*	2,7 ± 0.08	11.48 ± 0.47	3.4	Phenylalanin*	6.3	4,1 ± 0.16	14.20 ± 0.40
Valin	4,8 ± 0.28	16.65 ± 0.76	3.5	Tyrosin	6.1	2,6 ± 0.10	13.90 ± 0.53
Methionin*	1,7 ± 0.15	1.59 ± 0.16	2.5	Tryptophan*	1.1	1,4 ± 0.07	10.94 ± 0.70
Serine	2,3 ± 0.50	1.52 ± 0.60	-	Arginin	-	4,3 ± 0.18	36.13 ± 3.78
Isoleucin*	3,4 ± 0.20	14.85 ± 0.36	2.8	Histidin	1.9	2,5 ± 0.19	5.89 ± 0.33

*Essential Amino Acids **FAO/WHO, (2007)

The results showed that the essential amino acids (lysine, threonine, isoleucine, phenylalanine and tryptophane) in lupine flour were higher than those in wheat flour except methionine content which was higher in wheat flour (1.7 g/kg). This result was confirmed by Lubowicki et al., (2000). Sujak et al., (2006) reported that lupine seeds of different species representing diverse varieties of sweet lupine grown in Poland manifest a large deficiency of sulphur containing amino acids, for which the recommended level is 3.5 g/16 g N (Molvig et al., 1997). Methionine levels of 1.59 g/kg, found for the lupine flour was low but comparable to results reported previously for other lupins (El-Adawy et al., 2001). The recommended level of methionine is 2.5 g/kg (Tabe and Higgins, 1998). Of great importance is the presence of sulphur containing amino acids, mainly methionine, which is necessary for the synthesis of cysteine, as well as phenylalanine needed for the synthesis of tyrosine (Molvig et al., 1997).

The protein demand of different organisms depends on their physiological state stipulated mainly by age. For example, young and growing mammals (up to approximately two years in humans) need proteins rich in amino acids, such as arginine and histidine, as such amino acids are the source of the active centers of many enzymes. In contrast, adults show almost no physiological demand for these amino acids.

From the results we can noticed that lupine flour is rich with arginine and histidine (36.13 and 5.89 g/kg respectively). Protein quantity, as well as composition, is the limitation of protein quality (Tabe and Higgins, 1998). For humans, adequate quantities of lysine, methionine and tryptophan are considered necessary in food of high nutritive value (Molvig et al., 1997). A number

of approaches, based on the analysis of amino acids, have been considered for the estimation of protein quality in human and fodder foods. According to Alsmeyer et al., (1974), the nutritional value of food should be expressed in terms of leucine and tyrosine contents, while other classifications are based on the chemical scores for 9–11 amino acids considered essential. Lupine flour showed high content of lysine (16.35 g/kg) more than wheat flour (3.0 g/kg).

5.4. Phenolic compounds and antioxidants capacity

Phenolic compounds ubiquitous in plants are key phytochemical drivers of the health and functional foods and nutraceutical industry. Research with polyphenol compounds from various crops has created a growing market for polyphenol-rich ingredients, estimated to be worth around \$ 99 million in Europe in 2003 (Nutraingredients, 2005).

5.4.1. Wheat flour, lupine flour and their blends

Conventional solvent extraction has been reported in a laboratory scale using acetone, hexane, methanol and ethanol (Kosar et al., 2004). In this study, methanol was used for the extraction of antioxidant compounds from wheat, lupine flour and their blends (Table 4). The extraction yield 13.7 and 36.2 g/100g dry weight for wheat and lupine flour respectively.

Table 4: Extract yield, total polyphenols content and antioxidant capacity of wheat flour (WF), lupine flour (LF) and their blends.

Analysis	WF	LF	LF level (%)		
			5	10	15
Yield extract (%)	13.7 ± 3.99	36.2 ± 1.73	11.9 ± 0.35	13.6 ± 1.35	14.0 ± 1.21
Total phenolic (µg GAE/g DW)	126.63 ± 3.52	138.17 ± 8.35	132.17 ± 0.58	142.5 ± 7.10	156.53 ± 3.88
Total flavonoids (µg QE/g DW)	6.33 ± 0.15	8.93 ± 0.06	7.67 ± 1.27	7.93 ± 0.06	8.4 ± 0.52
Total flavonols (µg QE/g DW)	32.03 ± 6.13	31.60 ± 4.70	29.10 ± 2.48 [*]	28.27 ± 2.96 [*]	27.00 ± 1.08 [*]
DPPH (%)	3.31 ± 0.35	20.62 ± 1.22	5.1 ± 0.10 [†]	6.04 ± 0.77 [†]	7.16 ± 0.26 [†]
ABTS (%)	26.7 ± 0.21	43.42 ± 0.37	29.41 ± 0.37 [†]	31.09 ± 0.00 [†]	32.35 ± 0.37 [†]

Mean ± standard deviation of mean, * 3replicates, Deviation of mean correctly calculated

The lupine flour showed higher levels of total phenolic and total flavonoids than the wheat flour. Conversely, wheat flour showed higher levels of total flavonols. These results confirmed by statistical analysis, which highly significant differences ($P<0.05$) were observed between the two type of flours. Total phenolic and total flavonoids increased with increasing amount of lupine flour added to be 132.17 ± 0.58 , 142.5 ± 7.10 , 156.53 ± 3.88 ($\mu\text{g GAE/g DW}$) and 7.67 ± 1.27 , 7.93 ± 0.06 , 8.4 ± 0.52 ($\mu\text{g QE/g DW}$) for substituting wheat flour with lupine flour at 5, 10 and 15 %, respectively on dry weight basis. The contents of phenolic acids in lupine used in this study are comparable to levels reported previously (Ricardo-da-Silva et al., 1993), especially in cultivars of *L. albus* grown in Portugal. Phenolic content of lupins were higher than those of bean cultivars grown in Manitoba (Oomah et al., 2005) probably as a result of relatively high flavonoid content. The methanolic extracts of lupine seed were analysed by high performance liquid chromatography to see the phenolic profiles Figure (15).

As shown in Figure (15), nine phenolic acids were separated and identified. This method is well reproducible and provides good separation in terms of migration time and resolution.

The antioxidant effects of extracts of various wheat flour (WF), lupine flour (LF) and their blends at different concentration (5, 10 and 15 %) were measured. Since the active substances of flour extracts tested are different, the antioxidant activities of these extracts cannot be evaluated by only a single method. Therefore, two different models were used in this study (Huang et al., 2005).

Free radicals which are involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathologies, such as cancer and cardiovascular diseases among others (Dorman et al., 2003). The DPPH radical has been widely used to evaluate the free radicals' scavenging ability of various natural products and has been accepted as a model compound for free radicals originating in lipids (Da Porto et al., 2000). The effect of antioxidants on diphenyl-p-picryl hydrazyl (DPPH) radical scavenging was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The assay is based on the reduction of DPPH. Because of its odd electron, DPPH gives strong absorption maxima at 515 nm (purple color) by visible spectroscopy. As the odd electron of the radical becomes paired off in the presence of a hydrogen donor, i.e., a free radical scavenging antioxidant, the absorption intensity is decreased, and the resulting decolorization is stoichiometric with respect to the number of electrons captured (Yamaguchi et al., 2000).

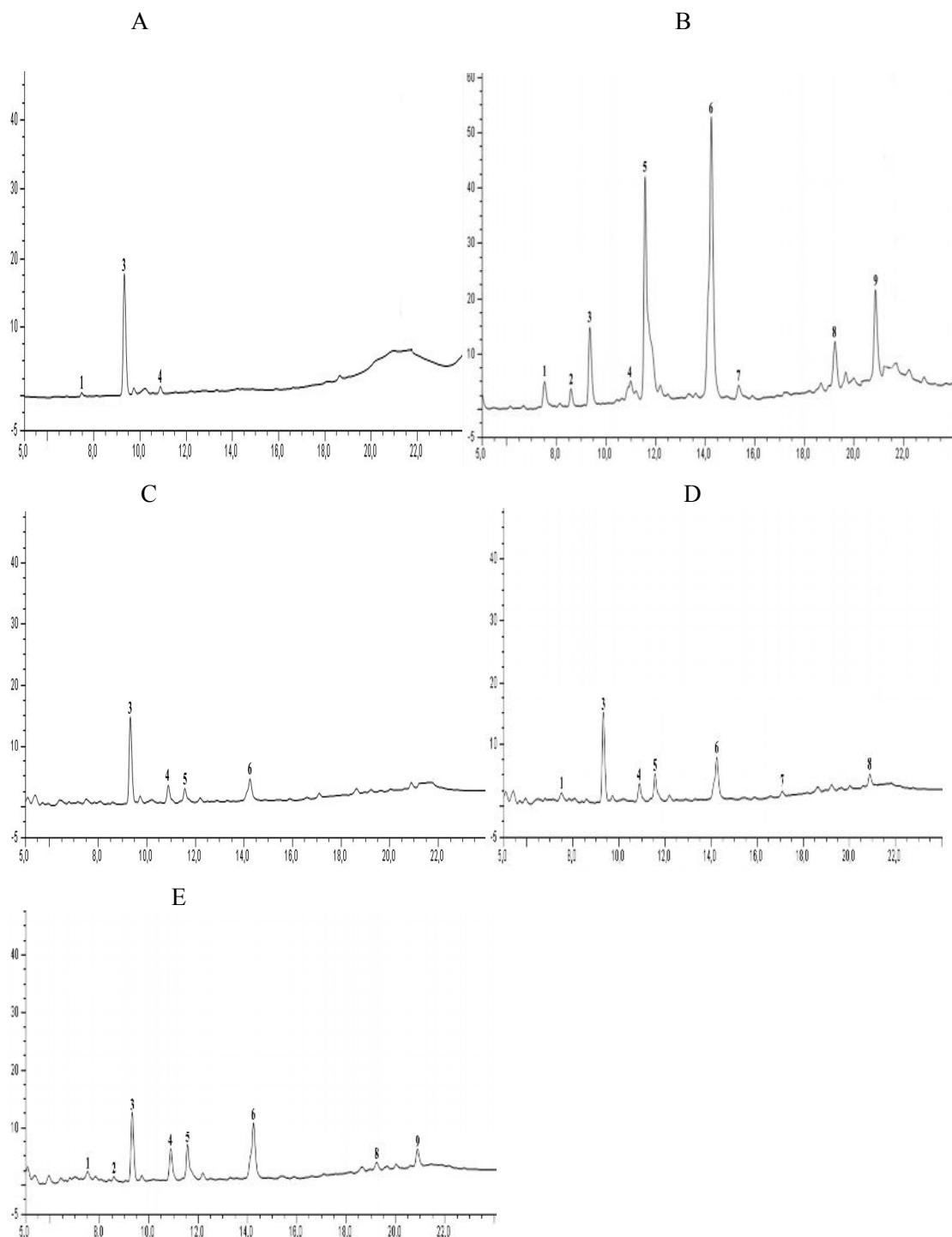


Figure 15: HPLC chromatogram of methanol extract of: wheat flour (A), lupine flour (B) and wheat flour supplemented with lupine flour at different concentration, 5 % (C). 10 % (D) and 15 % (E). 1. gallic, 2. procatechuic, 3. p-hydroxybenzoic, 4. vanillin, 5. P coumaric, 6. chlorogenic, 7. cinnamic 8. sinapine and 9. ferulic acid.

Table (4) showed that the scavenging activity of methanolic extracts against DPPH[•] for wheat flour (WF), lupine flour (LF) and their blends. Significant ($p < 0.05$) differences between wheat and lupine flour extracts were observed. Results clearly indicate that lupine flour exhibited higher antioxidant activity with DPPH and ABTS than the wheat flour. The antioxidant activity increased with increasing amount of lupine flour added to be 5.1 ± 0.10 , 6.04 ± 0.77 , 7.16 ± 0.26 in DPPH and 29.41 ± 0.37 , 31.09 ± 0.00 , 32.35 ± 0.37 in ABTS respectively, for substituting wheat flour with lupine flour at 5, 10 and 15 %, respectively on dry weight basis.

Wang et al., (1998) found that some compounds which have ABTS⁺ scavenging activity did not show DPPH scavenging activity. In this study, there was not the case. The ABTS⁺ scavenging data suggests that the components within the extracts are capable of scavenging free radicals via a mechanism of electron/hydrogen donation and should be able to protect susceptible matrices from free radical-mediated oxidative degradation.

5.4.2. Wheat flour, lupine fiber and their blends

The hull constitutes a considerable part of the lupine seeds (ca. 20 %) with a high content of dietary fibre (50–54 %) of good functionality (Gorecka et al., 2000). Compared to other leguminous crops, lupine seeds have a large proportion of hulls, which can be a source of valuable health promoting ingredients, including those with antioxidant properties. Therefore lupine hulls were also estimated.

Table 5: Extract yield, total polyphenols content and antioxidant capacity of wheat flour (WF), lupine fiber (L-fiber) and their blends.

Analysis	WF	L-fiber	L-fiber level (%)		
			5	10	15
Yield Extract (%)	13.7 ± 3.99	16.1 ± 0.46	14.2 ± 0.52	15.1 ± 0.96	15.4 ± 0.46
Total Phenolic ($\mu\text{g GAE/g DW}$)	126.63 ± 3.52	43.63 ± 2.57	126.27 ± 2.08	114.37 ± 2.05	105.17 ± 1.79
Total Flavonoids ($\mu\text{g QE/g DW}$)	6.33 ± 0.15	7.63 ± 0.06	6.63 ± 1.47	7.00 ± 0.95	7.30 ± 0.72
Total Flavonols ($\mu\text{g QE/g DW}$)	32.03 ± 6.13	30.53 ± 3.97	30.57 ± 3.38	29.5 ± 6.94	28.50 ± 7.18

DPPH (%)	3.31 ± 0.35	3.75 ± 1.64 *	3.98 ± 0.25 *	4.15 ± 0.54 *	4.76 ± 0.68 *
ABTS (%)	26.7 ± 0.21	27.54 ± 0.40 *	27.45 ± 1.09 *	28.90 ± 0.29 *	29.83 ± 0.00 *

Mean ± standard deviation of mean, * 3replicates, Deviation of mean correctly calculated

Table (5) showed that extract yield, total polyphenols content and antioxidant capacity of wheat flour (WF), lupine fiber (L-fiber) and their blends. It was noticed that lupine fiber had total phenolic, and flavonols lower than wheat flour or lupine flour. No significant difference between wheat flour and lupine fiber in flavonoids content. The same trend was observed with the antioxidant activity for lupine fiber in DPPH and ABTS tests. These results were similar with the results of Lampart-Szczapa et al., (2003a) who studied the antioxidant properties of lupine flours and hulls using the rancimat and oxidograph tests and he found that lupine tannins contents in the flours were a few times higher than in the hulls. Antioxidant activity was found both in the flours and in the hulls.

6. Physical investigation

6.1. Milling and particle size distribution

The grinding of the respective cereal raw material produced the dough as an intermediate of the bakery products after the addition of water and mechanical agitation. The degree of grinding of the grain may be determined by a sieve or laser particle analysis and is also an indicator of the mechanical disruption of the raw material and its baking characteristics. Before grinding lupine was wetted to 14 % moisture to a optimum moisture content to ensure a good separation of the shells from the endosperm and flour to finer particles to obtain. Addition of water leads to improve the fracture properties and brittle behavior. The crushing of the lupine in a hammer mill, resulting in a nearly complete dissolution of the grain structure in the cell exposed starch granules and protein particles exposed. It is assumed that the composition of the flour content of the crushing does not change. Due to the different methods of starting flour milling (wheat and lupine flour) is on the determination of particle size fractions and different mass fractions example of single-frequency determined.

The lupine was crushed by the hammer mill and preliminary tests of the total lupine flour fractionated by sieving and others. A criterion of the fractionation is $\leq 250 \mu\text{m}$.



Figure 16: Laser diffractionetry of wheat flour (WF), lupine flour (LF) and lupine fiber (L-fiber).

Table 6: Laser particle analysis for wheat flour (WF), lupine flour (LF) and lupine fiber (L-fiber)

Sample	d_{10} µm	d_{50} µm	d_{90} µm	Arithmetic average µm	Geometric average µm	Specific surface cm ² /cm ³
WF	18.7	56.9	163.4	76	55	1507.5
LF	23.6	81.8	257.7	117	82	1118.9
L-fiber	803.9	1286.7	1986.7	1344.1	1214.8	91.619

Figure (16) shows that the distribution functions of the lupine flour have bimodal distribution similar with wheat flour, which is typical of a hammer mill to milled flour but the lupine fiber is so coarse. Differentiated descriptions of the experimental data in Table (6) contain the grain size characterization.

6.2. Dough physical tests (farinograph test)

The dough is a criterion for the quality of flour. The addition of a component having different flour particle size influences not only the structure of flour, but also the functional properties of the mixed flours.

6.2.1. Wheat flour

Figure (17) shows farinogram of wheat flour. The wheat flour with adding 56.1 % according to the ICC method, and the resulting dough has a development time of 2.5 min, a stability of 5.5 min and softening of 58 FU. A slight drop in dough consistency with 491 FU was registered with the wheat dough. The experimental data are in Table (7) obtained from a duplicate.

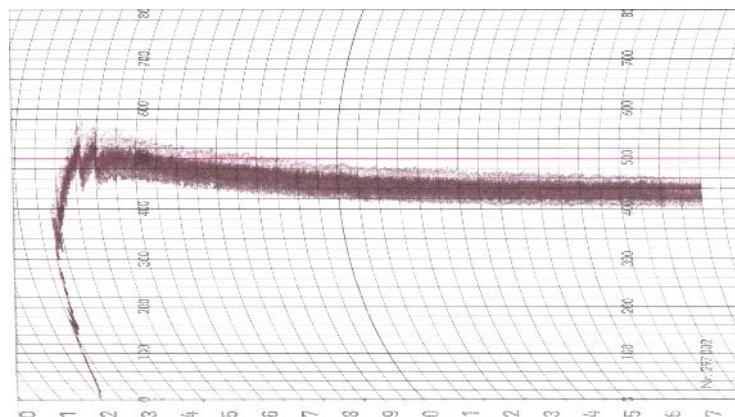


Figure 17: Farinogram for wheat flour (WF).

Table 7: Farinogram data of doughs made from wheat flour (WF), lupine flour (LF) and lupine fiber (L-fiber).

Sample	Water absorption %	Development time min	Dough stability min	Dough softening FU
WF	56,1	2,5	5,5	58
LF 5 %	57,6	3,5	9,8	18
LF 10 %	57,5	4,5	6,5	50
LF 15 %	57,2	5,5	3,5	120
L. Fibre 5 %	60,5	6	8,5	-
L. Fibre 10 %	64,8	7,5	6,5	-
L. Fibre 15 %	68,5	6,5	8,0	-

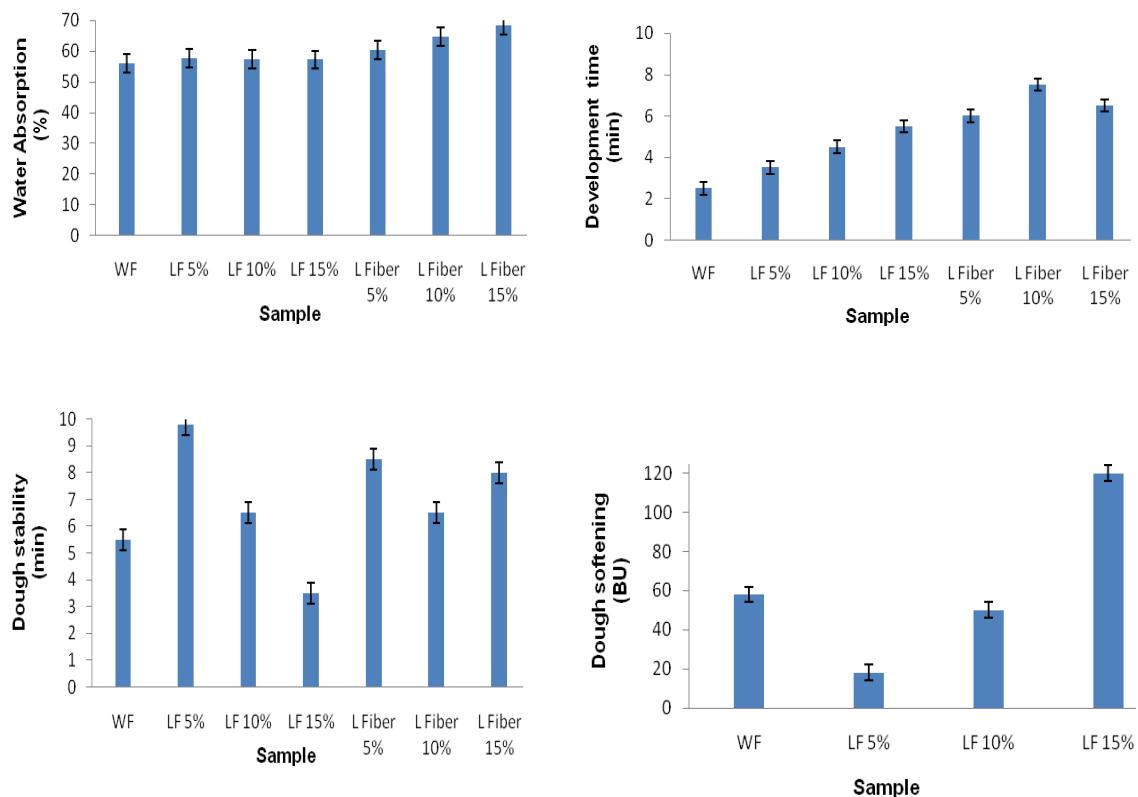


Figure 18: Farinograph data of doughs made from wheat flour (WF), lupine flour (LF) and lupine fiber (L-fiber) (3 replicates)

6.2.2. Flour mixtures (5, 10 and 15 % lupine flour or lupine fiber)

The addition of either lupine flour or lupine fiber to wheat flour brought about some significant changes in its dough mixing behavior as measured by the farinograph. Farinograph data of wheat flour (control) and those of the supplemented with lupine flour or lupine fiber, at a 5 %, 10 % or 15 % level, are shown in Table (7).

Supplementation of wheat flour with lupine flour (Figure 19) or lupine fiber (Figure 20) increased the water required for optimum bread making absorption ($p < 0.05$) (from 56.1 % for wheat flour to 57.2 % and 68.5 % for the 30 % lupine flour or lupine fiber respectively). An increase in water absorption, following incorporation of various vegetable protein concentrates or isolates to wheat flour, has also been reported by other researchers who attributed the water absorbing capacity of these protein preparations to their ability to compete for water with other constituents in the dough system.

According to these authors the ability of these proteins to absorb high quantities of water results in doughs which exhibit increased farinograph water absorption values (Doxastakis et al., 2002). The quantity of added water is considered to be very important for the distribution of the dough materials, their hydration and the gluten protein network development.

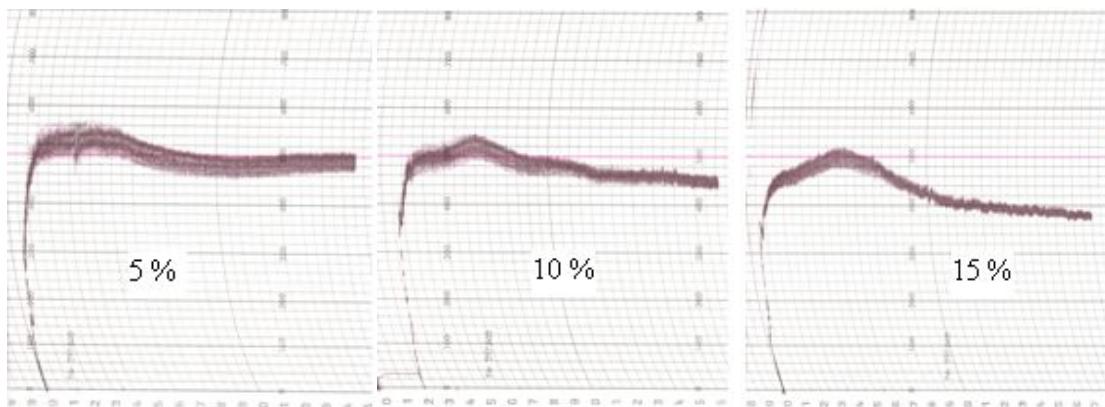


Figure 19: Farinogram data of doughs made from flour mixtures (5, 10 and 15 % lupine flour).

These results confirmed by Sudha et al., (2011) who studied the effects of wheat bran and oat bran as sources rich in insoluble dietary fiber and soluble dietary fiber in the formulation of instant vermicelli and study its influence on the rheological characteristics and product quality. The incorporation of wheat bran and oat bran from (0 to 20 %) in the blends increased the water absorption significantly from 58.3 to 64.1 %.

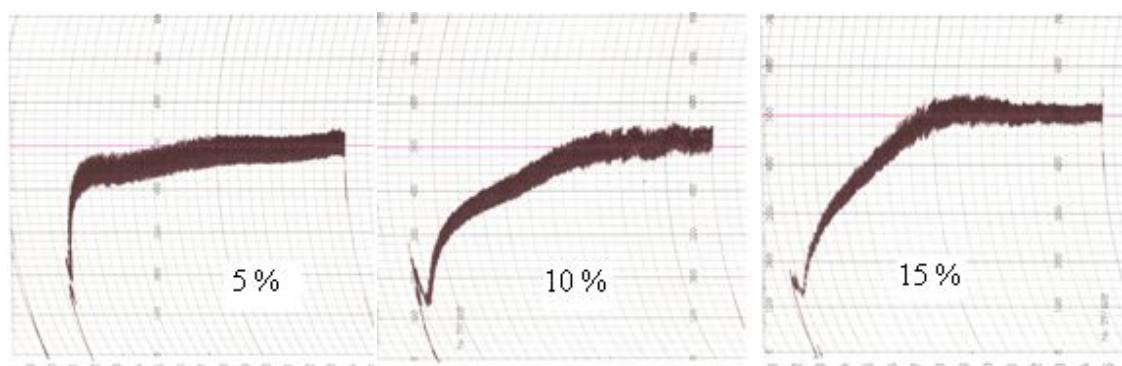


Figure 20: Farinogram data of doughs made from flour mixtures (5, 10 and 15 % lupine fiber).

Rosell et al., (2001) reported that the differences in water absorption is mainly caused by the greater number of hydroxyl group that exist in the fiber structure and allow more water interaction through hydrogen bonding. It could be noticed that water absorption increased with

increasing amount of lupine fiber. The observed effect agrees with the increased water absorption found by Sosulski and Wu, (1988) when they added field pea hulls, wheat, corn and wild oat brans to the bread dough.

The time required for the control dough to reach 500 FU consistency was also modified by lupine flour addition. During this phase of mixing, the water hydrates the flour components and the dough is developed. Dough development time (DDT) was significantly higher ($p < 0.05$) for all wheat-lupine flour or fiber blends than control (2.5 min), also between lupine samples significant difference was observed at different concentration ($p > 0.05$) (Table 7). The increase in dough development time resulting from lupine flour or fiber addition could have been due to the differences in the physicochemical properties between the constituents of the lupine and those of the wheat flour, as has been previously reported by Paraskevopoulou et al., (2010) who studied the incorporation of lupine protein in wheat flour.

The time required for the dough development or time necessary to reach 500 FU of dough consistency was modified in a different by each cereal bran. Highest development time values were obtained in doughs with lupine fiber (5, 10 and 15 %) (Figure 20). Similar results were expressed by Daglioglu and Gundogdu, (1999) who studied with stabilized rice bran in bread making.

Regarding dough stability, it appears that the dough sample containing 5 % lupine exhibited higher stability and resistance to mechanical mixing values than the control, while it decreased as the substitute level increases from 10 % to 15 %. In general, the stability value is an index of the dough strength, with higher values indicating stronger dough. The increase in the stability time was related to the amount of substitution. Thus, stability times of 6.5 and 3.5 min are observed for the dough supplemented with 10 and 15 % lupine, respectively.

Dough softening degree increased significantly with increasing amount of lupine flour in blends. Similar dominant viscoelastic behavior in dough characteristics on blending with cowpea flour and chickpea flour were observed by Sharma et al., (1999). The changes in dough characteristics upon addition of lupine flour may be attributed to dilution of gluten-forming proteins causing weakening of dough's. Variation in hydration behavior of two proteins may be another reason for differences in dough characteristics.

In general, the increasing of the dough development time from 2.5 min for wheat flour dough to 5.5 min for 15 % lupine flour and the reduction of dough stability to 3.5 min demonstrated to weakening of the gluten network configuration during the kneading. This is attributed to an intense incompatibility between the protein spectrum of lupine and wheat gluten protein. It is assumed that the increasing of lupine in blend-flours, the requirements energy for the optimal development of dough consistency increased, which lead to increased mechanical agitation requirement of non-gluten proteins in the dough system through the lupine proportion. This conclusion is consistent with the results of studies by Roccia et al., (2009) who found that the substitution of wheat protein by soy protein decreased mixture elasticity, indicating dough network weakening. One other reason for the weakening of dough strength resulting from vegetable protein addition could stem from the fact that the substitution of gluten proteins by the non-gluten-forming vegetable proteins causes a dilution effect and consequently weakens the dough. This confirms the data from literature that the both protein fractions (gliadin and glutenin) must be present for optimal gluten network development in a specific ratio. Trend to viscoelastic behavior is given.

6.3. Oscillation measurements

Fundamental rheometry is capable of describing the physical properties of a material over a wide range of strains and strain rates. The mechanical tests conducted within the linear viscoelastic region are useful for understanding the dough properties in terms of physical and chemical structure. The rheological properties of the dough reflect its machine properties during processing and the quality of the end product (Mani et al., 1992).

Following a strain sweep at 1 Hz within the linear viscoelastic region (LVR) was selected for additional testing.

6.3.1. Amplitude sweep measurements

The amplitude sweep is used to determine the linear viscoelastic region of the matrix.

6.3.1.1. Wheat and lupine flour dough

Below are the results of the fundamental rheological studies of wheat and lupine flour dough listed.

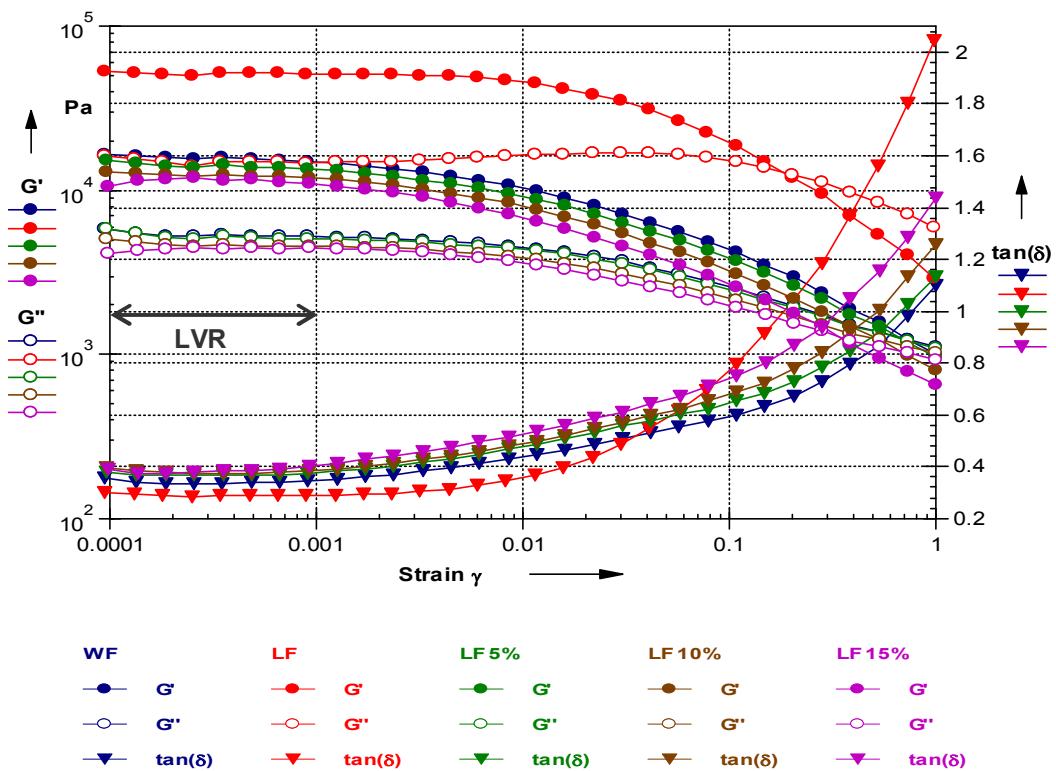


Figure 21: Amplitude sweep the flour mixture in dough (5, 10 and 15 % lupine flour)

Table 8: Amplitude sweep data for the flour mixture in dough (5, 10 and 15 % lupine flour).

Strain	Sample	G' 10^4 Pa	G'' 10^4 Pa	$\tan \delta$	$ \eta^* $ 10^4 Pa · s	$ G^* $ 10^4 Pa
$\gamma = 10^{-3}$	WF	1.51	0.52	0.35	0.25	1.59
	LF 5 %	1.35	0.51	0.37	0.23	1.44
	LF 10 %	1.19	0.46	0.38	0.20	1.28
	LF 15 %	1.11	0.44	0.40	0.19	1.19
	LF	5.11	1.48	0.29	0.85	5.32

Figure (21) is a collection of storage and loss modulus and loss factor for lupine dough compared with wheat flour dough. In the double logarithmic representation of the deformation-dependent behavior of the studied dough is pronounced with a dominant LVR solid behavior ($G' > G''$ or detect loss factor < 1), followed by a decrease of both moduli and an increase in the loss factor with structural break ($\tan \delta > 1$). The overall structure and macro-structure is experiencing a "break down", will be completely destroyed. A substructure is not available. The destruction of deformation γ_z with $G' = G''$ is located at wheat flour dough at 0.6, with 0.5 lupine flour dough. The shape of the curves shows a linear viscoelastic behavior in the area of $10^{-4} \leq \gamma \leq 10^{-3}$.

Made for the implementation of the frequency sweep defining the boundary of the LVR with $\gamma = 10^{-3}$. Compared to the wheat flour dough should be noted that despite the higher protein content, the higher level structure and the relatively larger particulation at lupine dough quickly leads to a structural instability of the dough, recognizable by the curve of $\tan \delta$ (Table 8).

This higher strength / stiffness mainly results from the present at lupine flour-dough non-cross linked starch particles, as dispersion point of contact and there are no interacting network. Of interest is the $\tan \delta$ clearly lies with wheat flour dough with 0.35 ratio in a well-known viscoelastic compared with 0.29 lupine flour dough (more viscoelastic, stronger, stiffer and more brittle) as the material properties before.

Subsequently, the influence of the lupine-additive in three different mixing ratios to wheat flour, the rheological properties of dough were examined. In Figure (21) is a compilation of storage modulus and $\tan \delta$ of the three dough preparations compared to the wheat and lupine flour dough. The storage and loss modulus level of the flour mixture has reduced differences among themselves in compared to the wheat flour-dough. LVR for the mixtures with deformation was of $10^{-4} \leq \gamma \leq 10^{-3}$.

Table (8) showed that the moduli (G' , G'' and G^*) with increasing lupine flour content in the mixtures in comparison to the wheat flour with a deformation of $\gamma = 10^{-3}$ decreased slightly. A stable structure in hibernation is determined based on the dominance of the wheat flour in the blends.

6.3.1.2. Wheat and lupine fiber dough

Below are the results of the fundamental rheological studies of dough of wheat flour supplemented different levels of lupine fiber.

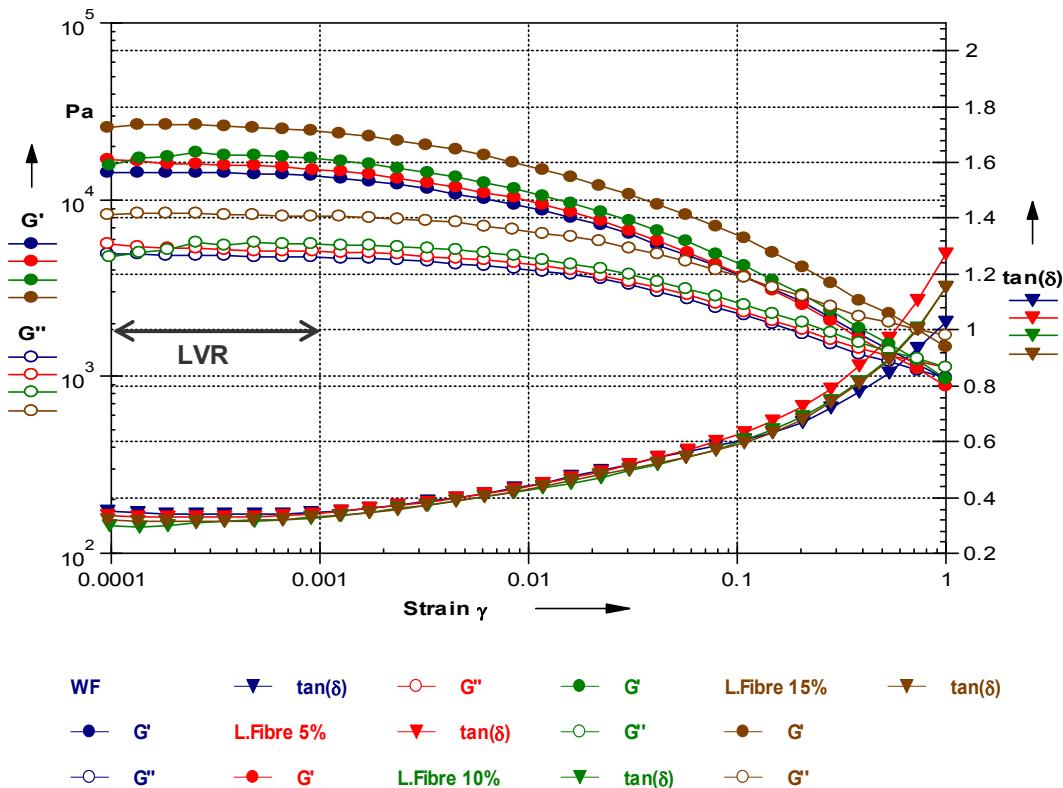


Figure 22: Amplitude sweep the flour mixture in dough (5, 10 and 15 % lupine fiber)

Table 9: Amplitude sweep data for the flour mixture in dough (5, 10 and 15 % lupine fiber).

Strain	Sampel	G'	G''	$\tan \delta$	$ \eta^* $	$ G^* $
$\gamma = 10^{-3}$	WF	1.51	0.52	0.35	0.25	1.59
	L-Fibre 5 %	1.49	0.51	0.34	0.25	1.58
	L-Fibre 10 %	1.72	0.56	0.33	0.29	1.81
	L- Fibre 15 %	2.47	0.81	0.33	0.41	2.59

The addition of lupine fiber caused a shift of curves G' and G" towards higher values, while curve $\tan \delta$ moved towards lower values. The data indicate that the additions applied caused an increase in wheat flour dough elasticity (G') and viscosity (G"), the increase in elasticity dominating over that in viscosity, as a result of which $\tan \delta$ decreased. Likewise, Lamacchia et al., (2010) studying doughs with a constant addition of water (30 %), recorded significantly higher values of G' and G" for oat whole meal dough than for wheat (semolina) dough. Also, oat whole meal dough, compared to wheat dough, was characterized by significantly higher values of $\tan \delta$.

Subsequently, the influence of the lupine fiber-additive in three different mixing ratios to wheat flour, the rheological properties of dough were examined. In Figure (22) is a compilation of storage modulus and $\tan \delta$ of the three dough preparations compared to the wheat dough. The storage and loss modulus level of the flour mixture had increased differences among themselves in compared to the wheat flour-dough. LVR for the mixtures with deformation was of $10^{-4} \leq \gamma \leq 10^{-3}$.

Table (9) showed that the moduli (G' , G'' and G^*) with increasing lupine fiber content in the mixtures in comparison to the wheat flour with a deformation of $\gamma = 10^{-3}$ increased slightly. A stable structure in hibernation is determined based on the dominance of the wheat flour in the blends.

6.3.1.3. Wheat and lupine flour batter

Below are the results of the fundamental rheological studies of wheat and lupine flour dough listed.

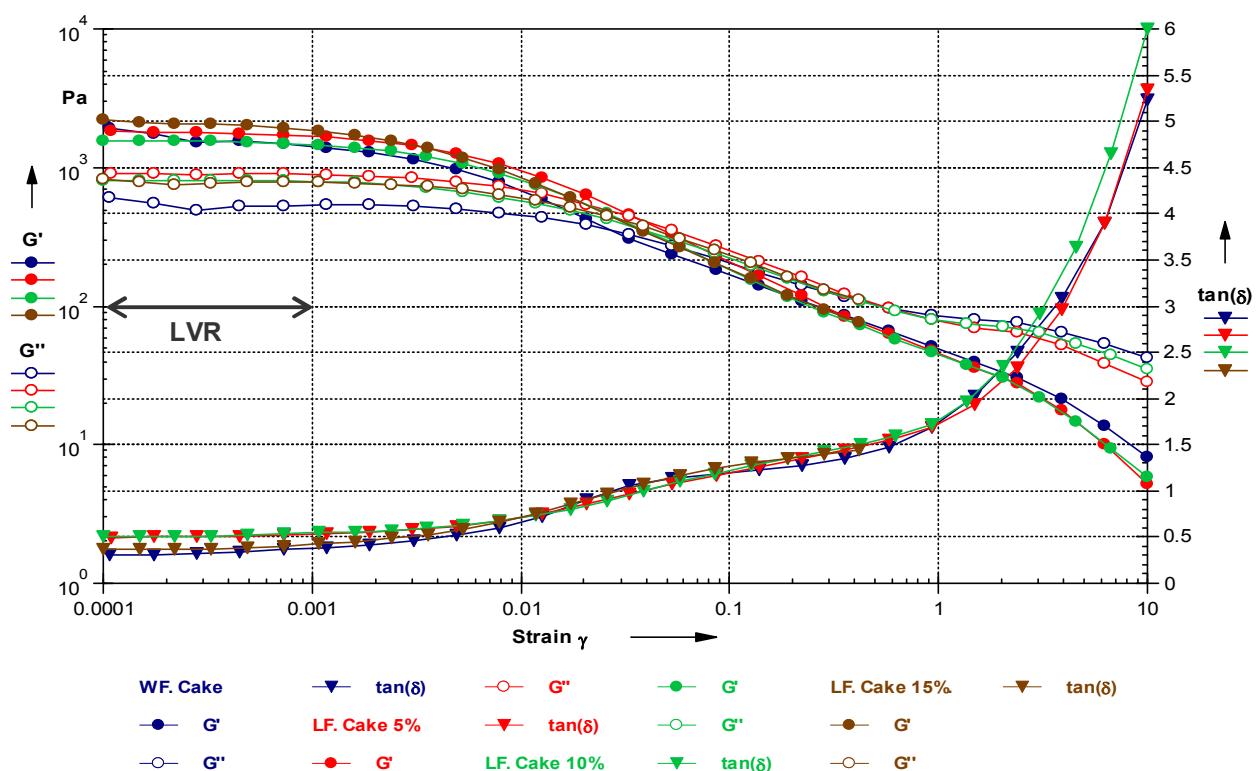


Figure 23: Amplitude sweep the flour mixture in batter cake (5, 10 and 15 % lupine flour).

Table 10: Amplitude sweep data for the flour mixture in batter (5, 10 and 15 % lupine flour).

Strain	Sample	G' 10 ³ Pa	G" 10 ³ Pa	tan δ	η* 10 ³ Pa · s	G* 10 ³ Pa
$\gamma = 10^{-3}$	WF	1.39	0.54	0.39	0.24	1.49
	LF. 5 %	1.66	0.89	0.54	0.20	1.89
	LF. 10 %	1.06	0.67	0.63	0.20	1.25
	LF. 15 %	1.18	0.69	0.58	0.22	1.37

Figure (23) is a collection of storage and loss modulus and loss factor for wheat flour batter supplemented with different concentration of lupine flour. In the double logarithmic representation of the deformation-dependent behavior of the studied batter is pronounced with a dominant LVR behavior ($G' > G''$ or detect loss factor <1), followed by a decrease of both moduli and an increase in the loss factor with structural break ($\tan \delta = 1$). The overall structure and macro-structure is experiencing a "break down", will be completely destroyed. A sub-structure is not available. The shape of the curves shows a linear viscoelastic behavior in the area of $10^{-4} \leq \gamma \leq 10^{-3}$. Made for the implementation of the frequency sweep defining the boundary of the LVR with $\gamma = 10^{-3}$. Compared to the wheat flour batter should be noted that despite the higher protein content, the higher level structure and the relatively larger particulation at lupine-batter quickly leads to a structural instability of the batter, recognizable by the curve of $\tan \delta$ (Table 10).

Table (10) showed that the moduli (G' , G'' and G^*) with increasing lupine flour content more than 5 % in the mixtures in comparison to the wheat flour with a deformation of $\gamma = 10^{-3}$ decreased slightly. A stable structure in hibernation is determined based on the dominance of the wheat flour in the blends.

6.3.1.4. Wheat and lupine fiber batter

Below are the results of the fundamental rheological studies of wheat and lupine flour dough listed.

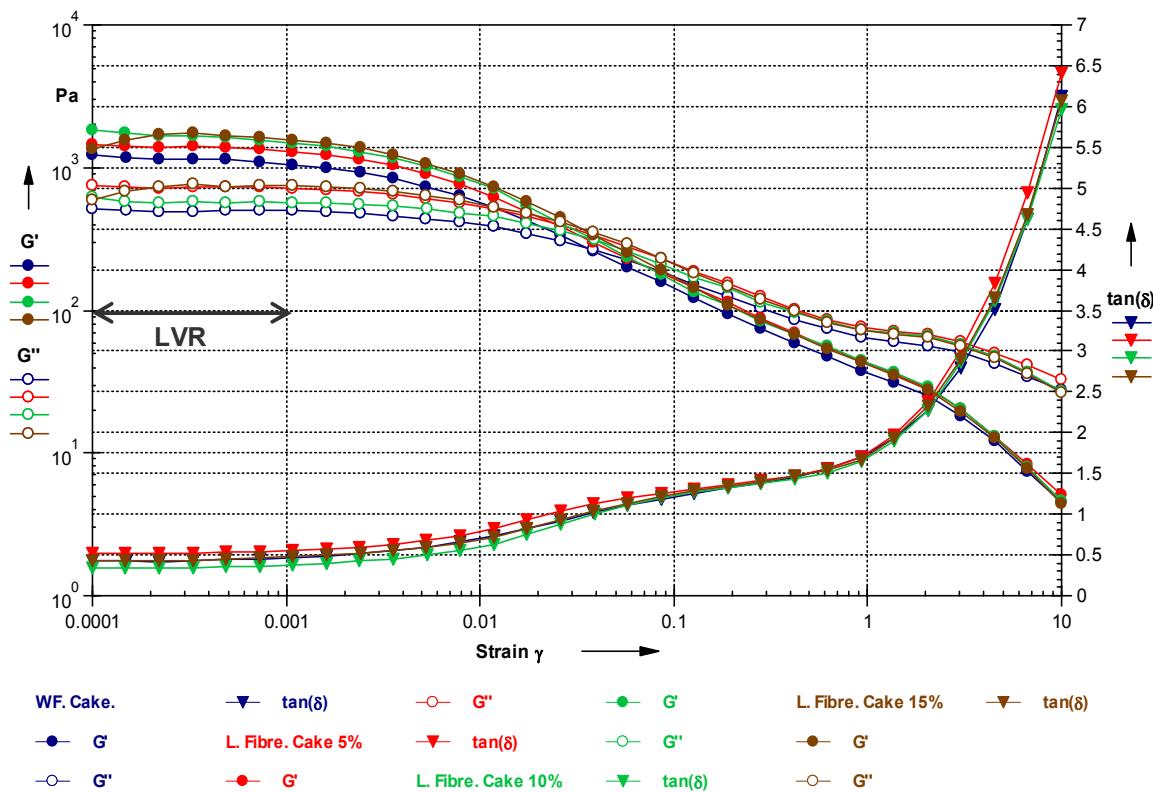


Figure 24: Amplitude sweep the flour mixture in batter cake (5, 10 and 15 % lupine fiber).

Table 11: Amplitude sweep data for the flour mixture in batter (5, 10 and 15 % lupine fiber).

Strain	Sample	G' 10^3 Pa	G'' 10^3 Pa	$\tan \delta$	$ \eta^* $ 10^3 Pa · s	$ G^* $ 10^3 Pa
$\gamma = 10^{-3}$	WF	1.05	0.49	0.47	0.19	1.17
	L. Fibre 5 %	1.29	0.72	0.55	0.24	1.48
	L. Fibre 10 %	1.49	0.57	0.38	0.25	1.59
	L. Fibre 15 %	1.57	0.74	0.48	0.28	1.74

The addition of lupine fiber caused a shift of curves G' and G'' towards higher values, while curve $\tan \delta$ moved towards lower values. The data indicate that the additions applied caused an increase in wheat flour dough elasticity (G') and viscosity (G''), the increase in elasticity dominating over that in viscosity, as a result of which $\tan \delta$ decreased. Subsequently, the influence of the lupine fiber-additive in three different mixing ratios to wheat flour, the rheological properties of dough were examined. In Figure (24) is a compilation of storage modulus and $\tan \delta$ of the three dough preparations compared to the wheat dough. The storage and

loss modulus level of the flour mixture had increased differences among themselves in compared to the wheat flour-dough. LVR for the mixtures with deformation was of $10^{-4} \leq \gamma \leq 10^{-3}$.

Table (11) showed that the moduli (G' , G'' and G^*) with increasing lupine fiber content in the mixtures in comparison to the wheat flour with a deformation of $\gamma = 10^{-3}$ increased slightly. A stable structure in hibernation is determined based on the dominance of the wheat flour in the blends.

6.3.2. Frequency sweep measurements

To characterize the dough as the dispersed material systems, the frequency-dependent behavior of the wheat flour and lupine flour or fiber depending on the subsequent mixing ratios (5 %, 10 % and 15 % lupine flour or finer) was examined by oscillatory measurements.

6.3.2.1. Wheat and lupine flour dough

In Figure (25), there is a comparison of the measured data of lupine and wheat flour dough shown the storage and loss modulus and loss factor.

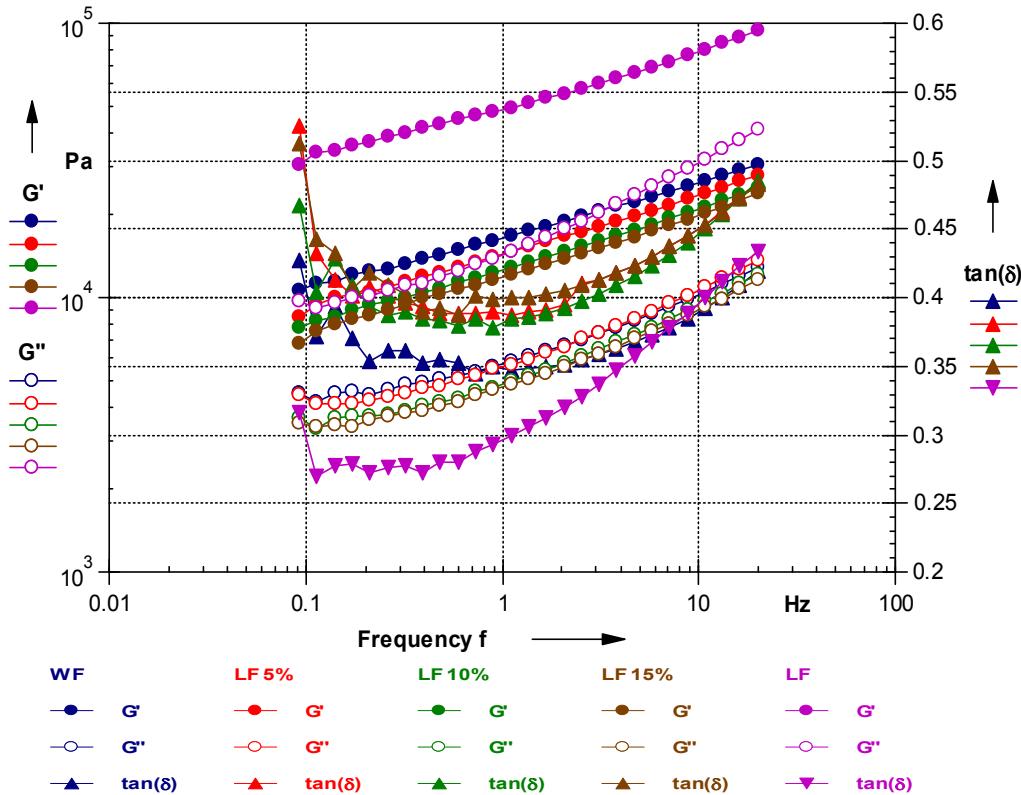


Figure 25: Frequency sweep the flour mixture in dough (5, 10 and 15 % lupine flour).

Table 12: Frequency sweep data for the flour mixture in dough (5, 10 and 15 % lupine flour).

Sample	Dynamic parameters (Moduls)						
	coefficient $G'_1 \text{ Hz}$	x	coefficient $G''_1 \text{ Hz}$	y	$\tan \delta$	$ \eta^* $	$ G^* $
	10^4 Pa		10^4 Pa		$10^4 \text{ Pa} \cdot \text{s}$	10^4 Pa	
WF	1.69	0.195	0.59	0.206	0.35	0.26	1.78
LF 5 %	1.47	0.210	0.57	0.228	0.39	0.23	1.58
LF 10 %	1.29	0.213	0.49	0.236	0.38	0.20	1.38
LF 15 %	1.21	0.220	0.48	0.234	0.40	0.19	1.30
LF	4.93	0.196	1.48	0.283	0.30	0.74	5.15

The relations G' , G'' and $\tan \delta$ with frequency sweep for pure wheat flour dough, composite flour dough's and pure lupine flour dough are presented in Figure (25). Particle system (dispersion), no see like system because measurements curves have slop.

The presented data indicate that increase of oscillation frequency within the range from 0.1 to 20 Hz caused an increase in the values of the dynamic moduli – the storage modulus and the loss modulus for pure wheat- and lupine flour dough as well as for composite flour dough. Whereas, the values of the tangent of the phase angle, being the ratio of G''/ G' , decreased gently while the oscillation frequency increased from 0.1 to approximately 1 Hz, while higher frequencies caused an increase of those values.

Table (12) showed that the additions of lupine at different concentration (5, 10 and 15 %) had a similar effect on the run of the mechanical spectra of wheat dough. Increase in the percentage share of the additions caused a shift of curves G' and G'' towards lower values. The data indicate that the additions applied caused a decrease in tested dough elasticity (G') and viscosity (G''), the increase in viscosity dominating over elasticity as a result of $\tan \delta$ increased. Frequency sweep experiments showed that for all tested dough formulations the elastic (or storage) modulus, G' , was greater than the viscous (or loss) modulus, G'' , in the whole range of frequencies and both moduli slightly increased with frequency which suggests a solid elastic-like behavior of the lupine doughs. Therefore, $\tan \delta$ values for all dough formulations were lower than 1. Similar observations on dynamic rheological studies have been reported previously for wheat

flour doughs (Edwards et al., 2003), as well as for rice flour dough (Sivaramakrishnan et al., 2004).

6.3.2.2. Wheat and lupine fiber dough

In Figure (26), there is a comparison of the measured data of wheat flour dough with different concentration of lupine fiber shown the storage and loss modulus and loss factor.

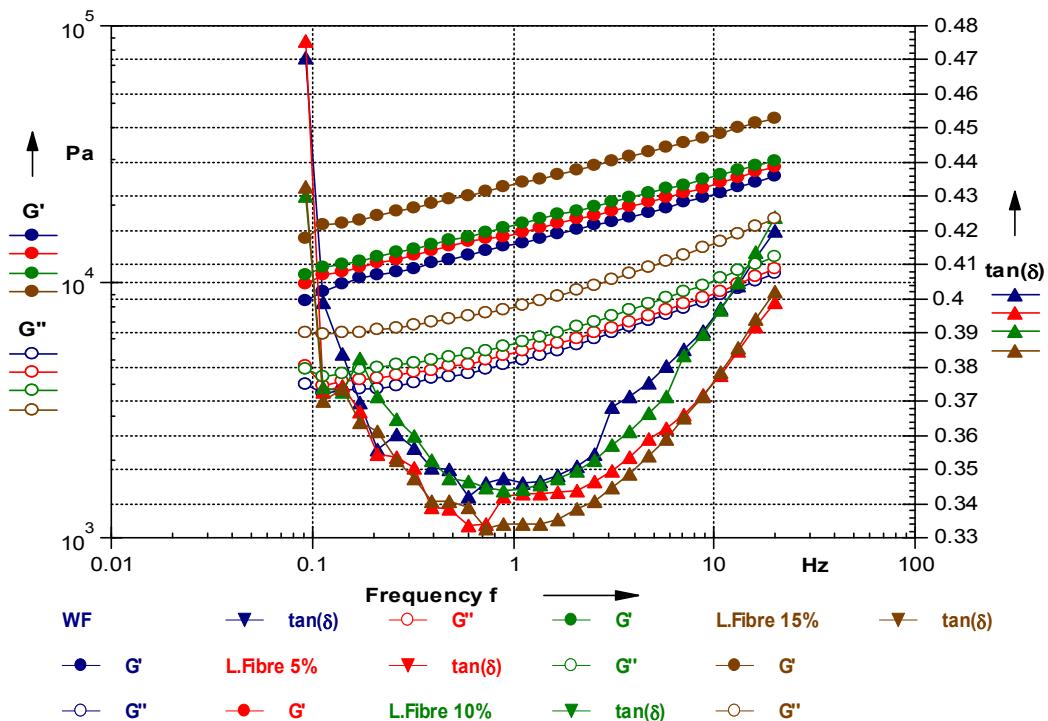


Figure 26: Frequency sweep the flour mixture in dough (5, 10 and 15 % lupine fiber).

Table 13: Frequency sweep data for the flour mixture in dough (5, 10 and 15 % lupine fiber).

Sample	Dynamic parameters (Modulus)						
	coefficient $G'_{1\text{Hz}}$	x	coefficient $G''_{1\text{Hz}}$	y	$\tan \delta$	$ \eta^* $	
	10^4 Pa		10^4 Pa		$10^4 \text{ Pa} \cdot \text{s}$	10^4 Pa	
WF	1.42	0.194	0.49	0.203	0.35	0.22	1.51
L.Fibre 5 %	1.56	0.186	0.54	0.188	0.34	0.24	1.65
L.Fibre 10 %	1.69	0.188	0.58	0.199	0.34	0.26	1.79
L.Fibre 15 %	2.43	0.189	0.81	0.195	0.33	0.37	2.57

The presented data indicate that increase of oscillation frequency within the range from 0.1 to 20 Hz caused an increase in the values of the dynamic moduli – the storage modulus and the

loss modulus for pure wheat- and lupine fiber dough as well as for composite flour dough. Whereas, the values of the tangent of the phase angle, being the ratio of G''/ G' , decreased gently while the oscillation frequency increased from 0.1 to approximately 1 Hz, the higher frequencies caused an increase of those values.

Table (13) showed that the additions of lupine fiber at different concentration (5, 10 and 15 %) had a similar effect on the run of the mechanical spectra of wheat dough. Increase in the percentage share of the additions caused a shift of curves G' and G'' towards higher values. The data indicate that the additions applied caused an increase in tested dough elasticity (G') and viscosity (G''), the increase in elasticity dominating over viscosity as a result of $\tan \delta$ decreased. Frequency sweep experiments showed that for all tested dough formulations the elastic (or storage) modulus, G' , was greater than the viscous (or loss) modulus, G'' , in the whole range of frequencies and both moduli slightly increased with frequency which suggests a solid elastic-like behavior of the lupine doughs. Therefore, $\tan \delta$ values for all dough formulations were lower than 1.

6.3.2.3. Wheat and lupine flour batter

In Figure (27) and Table (14), there is a comparison of the measured data of lupine and wheat flour batter shown the storage and loss modulus and loss factor.

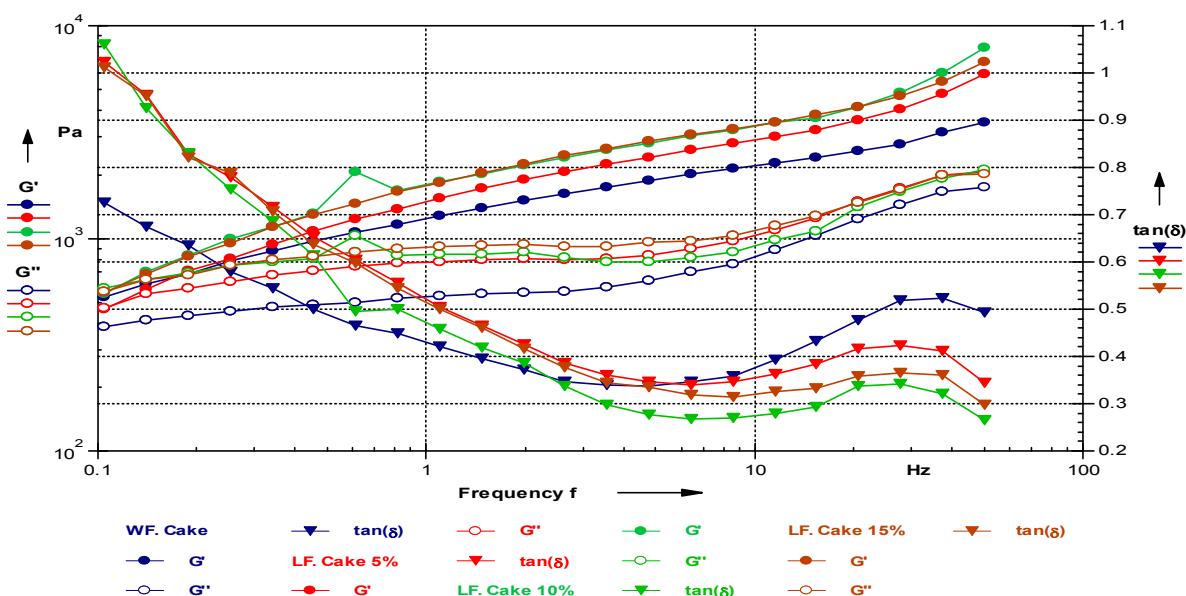


Figure 27: Frequency sweep the flour mixture in batter cake (5, 10 and 15 % lupine flour).

Table 14: Frequency sweep data for the flour mixture in batter cake (5, 10 and 15 % lupine flour).

Sample	Dynamic parameters (Moduli)						
	coefficient $G'_1 \text{ Hz}$ 10^3 Pa	x	coefficient $G''_1 \text{ Hz}$ 10^3 Pa	y	$\tan \delta$	$ \eta^* $	$ G^* $
WF. Cake	1.27	0.284	0.53	0.225	0.42	0.19	1.38
LF. 5 %	1.54	0.363	0.78	0.199	0.51	0.25	1.72
LF. 10 %	1.84	0.358	0.85	0.147	0.46	0.29	2.03
LF. 15 %	1.83	0.357	0.91	0.167	0.49	0.30	2.05

The presented data indicate that increase of oscillation frequency within the range from 0.1 to 50 Hz caused an increase in the values of the dynamic moduli – the storage modulus and the loss modulus. Whereas, the values of the tangent of the phase angle, being the ratio of G''/ G' , decreased gently while the oscillation frequency increased from 0.1 to approximately 10 Hz , while higher frequencies caused an increase of those values then decrease again at 30 Hz. These results mean that the capacity of wheat dough for dissipation (whose measure is the value of G'') and storage (whose measure is the value of G') of the energy used for its deformation increases with increase in the oscillation frequency (f). Energy is dissipated through friction that takes place during the slippage of one dough structural element past another, e.g., chains of gliadin proteins which are synonymous of the viscous component of gluten. Whereas, the storage of energy takes place through reversible rearrangements within the particular elements building the structure of dough, as glutenin subunits which represent the elastic component of gluten (Song and Zheng, 2007).

In the dynamic tests, increase in frequency is equivalent to increase in the shear rate of dough sample and to a shortening of the duration of a single measurement cycle. It is a known fact that with increase in shear rate there is an increase in friction during viscous flow of dough, as a result of which the loss modulus (G'') increases. In turn, shortening of the length of the period in which dough is subject to deformation causes a reduction of the degree of relaxation of stress, and that is the immediate reason for increase in the amount of stored energy (G') in the

function of frequency. The increase in the value of G' is the lower the more the dough structure resembles that of a highly cross-linked material.

6.3.2.4. Wheat and lupine fiber batter

In Figure (28) and Table (15), there is a comparison of the measured data of wheat flour batter with different concentration of lupine fiber shown the storage and loss modulus and loss factor.

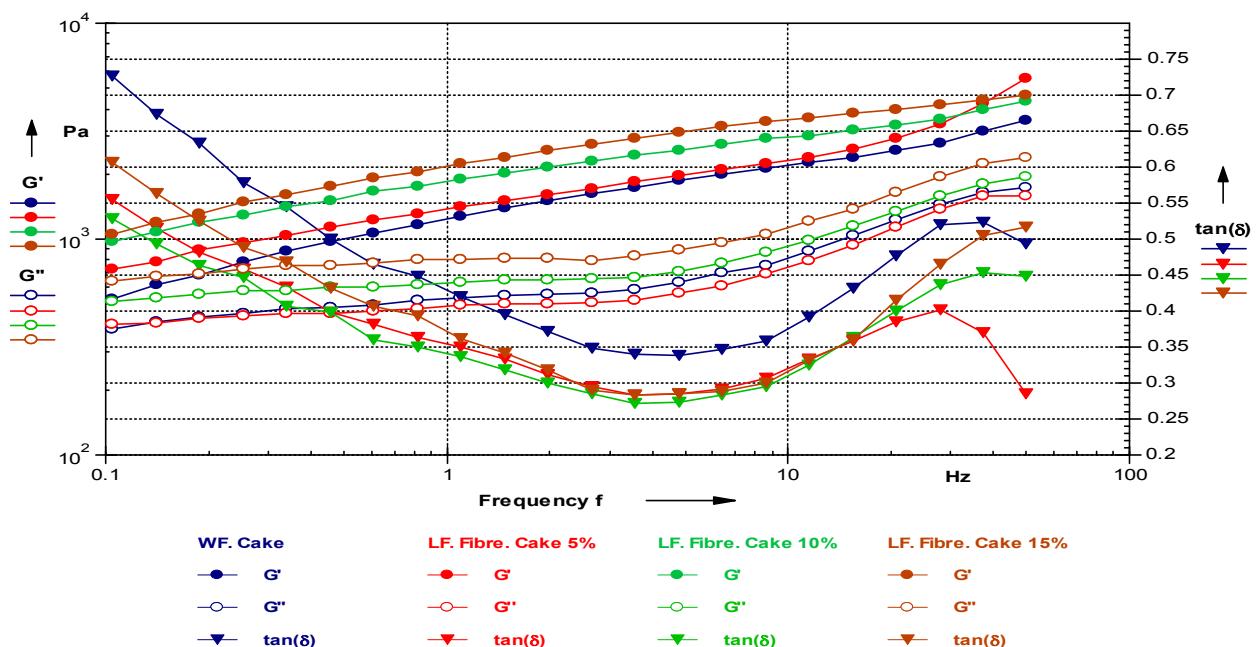


Figure 28: Frequency sweep the flour mixture in batter cake (5, 10 and 15 % lupine fiber)

Table 15: Frequency sweep data for the flour mixture in batter cake (5, 10 and 15 % lupine fiber).

Sample	Dynamic parameters (Modulus)						
	coefficient $G'_{1\text{ Hz}}$	x	coefficient $G''_{1\text{ Hz}}$	y	$\tan \delta$	$ \eta^* $	$ G^* $
	10^3 Pa		10^3 Pa		$10^3 \text{ Pa} \cdot \text{s}$		10^3 Pa
WF	1.27	0.284	0.53	0.225	0.42	0.19	1.38
L. Fibre 5 %	1.41	0.280	0.49	0.212	0.35	0.22	1.49
L. Fibre 10 %	1.89	0.226	0.63	0.195	0.34	0.29	1.99
L. Fibre 15 %	2.23	0.233	0.81	0.189	0.36	0.34	2.37

The presented data indicate that increase of oscillation frequency within the range from 0.1 to 50 Hz caused an increase in the values of the dynamic moduli – the storage modulus and the loss modulus. Whereas, the values of the tangent of the phase angle, being the ratio of G''/ G' , decreased gently while the oscillation frequency increased from 0.1 to approximately 5 Hz , while higher frequencies caused an increase of those values then decrease again at 30 Hz. These results mean that the capacity of wheat dough for dissipation (whose measure is the value of G'') and storage (whose measure is the value of G') of the energy used for its deformation increases with increase in the oscillation frequency (f).

Comparing the values of parameter a in the models of G' and G'' (Figure 28) one can see that at the lowest oscillation frequency ($f = 0.1$ Hz) the storage module for wheat flour dough with no additions (0 %) were almost three fold higher than the loss module.

Such a notable domination of the storage modulus over the loss modulus indicates that wheat dough is a highly structured material and behaves like a viscoelastic body in which the elastic properties (G') clearly dominate over the viscous properties (G''), which is in agreement with earlier studies (Letang et al., 1999).

6.3.3. Temperature sweep measurements (TSM)

At constant amplitude of 10^{-3} , as well as constant frequency of 1 Hz, the temperature was heating rates of 1 K/min changed. This heating rate was chosen to free gradient in the sample and secures processes taking place such as denaturation of proteins, pre-and gelatinization of starch and to be able to detect the mobilization of the water clear. Should sweep through the temperature the baking properties of wheat flour, lupine (flour or fiber) dough or batter and preparations are comparatively simulated.

The evaluation of each TSM measurements were made using the criteria:

1. Measurement of G' and G'' curves and $\tan \delta$ as a function of temperature.
2. Analysis of the $\tan \delta$ curve shape as a phase transition indicator.
3. Identification of the hysteresis of the $\tan \delta$ curve when heated.

6.3.3.1. Wheat and lupine flour dough

Figure (29) presents the changes of G' and G'' with temperature for wheat flour and lupine flour dough between 15 and 90 °C.

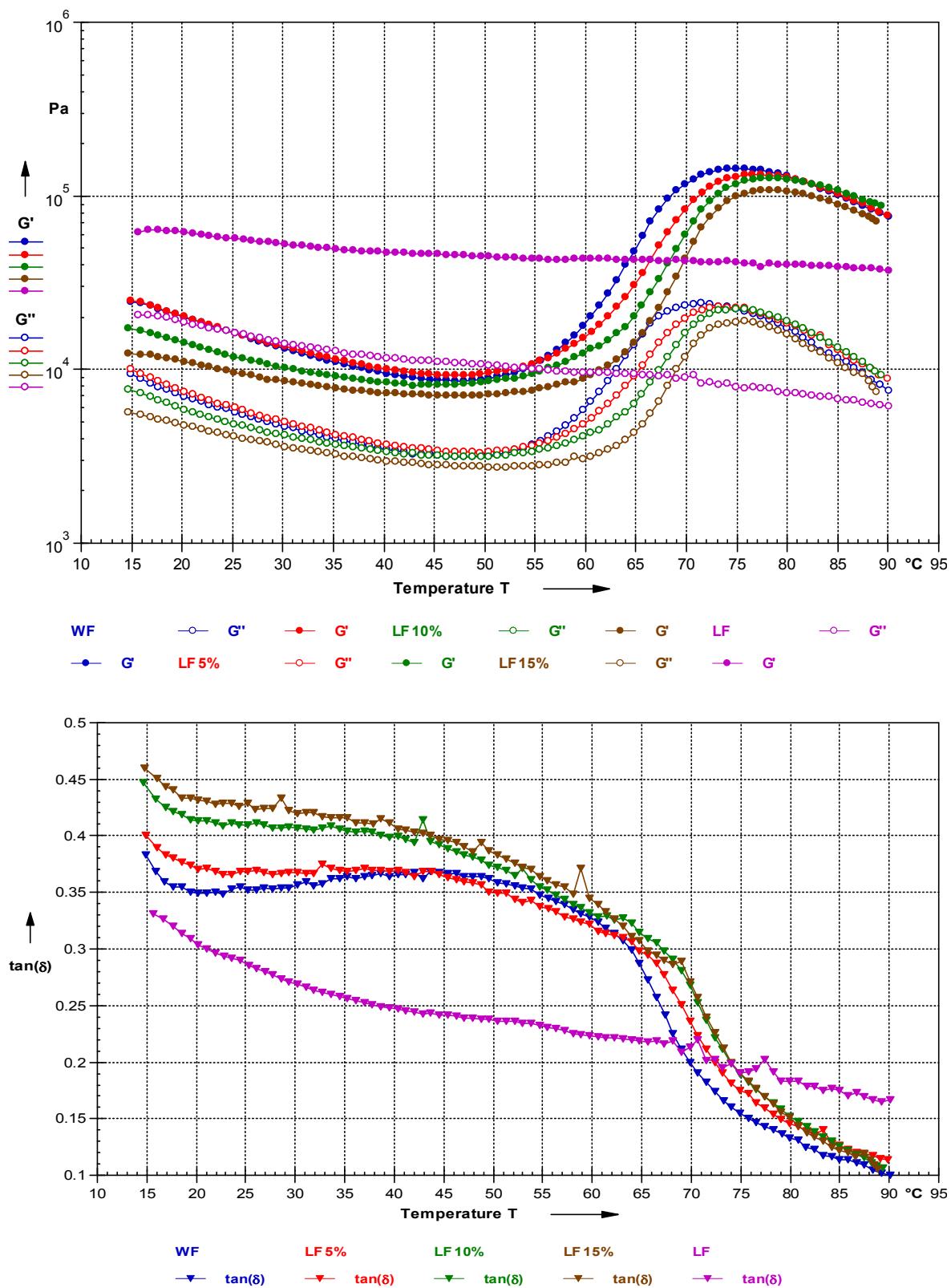


Figure 29: Temperature sweep the flour mixture in dough (5, 10 and 15 % lupine flour).

Results and discussion

Table 16: Temperature sweep the flour mixture in dough (5, 10 and 15 % lupine flour).

Sample	Dynamical Parameters	Temperatur °C	I			II			III			
			15	25	35	45	55	60	65	75	85	
WF	G'	10 ⁴ Pa	2.45	1.61	1.20	0.87	1.06	2.02	4.81	14.4	9.69	7.58
	G''	10 ⁴ Pa	0.94	0.57	0.40	0.32	0.37	0.65	1.38	2.23	1.09	0.76
	tan δ		0.38	0.35	0.36	0.37	0.35	0.32	0.29	0.16	0.11	0.10
	η*	10 ⁴ Pa·s	0.42	0.27	0.19	0.15	0.18	0.34	0.79	2.32	1.55	1.21
	G*	10 ⁴ Pa	2.63	1.71	1.17	0.93	1.12	2.12	5.01	14.6	9.75	7.62
LF 5 %	G'	10 ⁴ Pa	2.50	1.63	1.15	0.94	1.08	1.66	3.05	12.9	9.79	7.73
	G''	10 ⁴ Pa	1.01	0.59	0.42	0.34	0.36	0.52	0.91	2.26	1.21	0.88
	tan δ		0.40	0.37	0.37	0.37	0.34	0.32	0.30	0.18	0.12	0.11
	η*	10 ⁴ Pa·s	0.43	0.28	0.19	0.16	0.18	0.28	0.51	2.09	1.57	1.24
	G*	10 ⁴ Pa	2.69	1.74	1.22	0.99	1.14	1.74	3.19	13.1	9.87	7.78
LF 10 %	G'	10 ⁴ Pa	1.72	1.19	0.91	0.82	0.95	1.32	2.02	11.7	10.3	8.75
	G''	10 ⁴ Pa	0.76	0.49	0.37	0.32	0.34	0.43	0.63	2.23	1.26	0.93
	tan δ		0.45	0.41	0.40	0.39	0.35	0.33	0.31	0.19	0.12	0.11
	η*	10 ⁴ Pa·s	0.29	0.21	0.16	0.14	0.16	0.22	0.34	1.90	1.66	1.40
	G*	10 ⁴ Pa	1.88	1.28	0.98	0.88	1.01	1.39	2.11	11.9	10.4	8.79
LF 15 %	G'	10 ⁴ Pa	1.24	0.96	0.78	0.71	0.76	0.93	1.42	9.87	8.57	7.10
	G''	10 ⁴ Pa	0.57	0.41	0.32	0.28	0.28	0.32	0.44	1.88	1.03	0.75
	tan δ		0.46	0.43	0.42	0.40	0.36	0.34	0.31	0.19	0.12	0.10
	η*	10 ⁴ Pa·s	0.22	0.17	0.13	0.12	0.13	0.16	0.23	1.60	1.37	1.13
	G*	10 ⁴ Pa	1.36	1.05	0.84	0.77	0.81	0.98	1.48	10.1	8.63	7.14
LF	G'	10 ⁴ Pa	5.50	5.67	4.94	4.60	4.34	4.32	4.31	4.14	3.90	3.68
	G''	10 ⁴ Pa	1.84	1.62	1.27	1.11	1.01	0.96	0.94	0.79	0.67	0.61
	tan δ		0.33	0.29	0.26	0.24	0.23	0.22	0.22	0.19	0.17	0.17
	η*	10 ⁴ Pa·s	0.92	0.94	0.81	0.75	0.71	0.71	0.70	0.67	0.63	0.59
	G*	10 ⁴ Pa	5.80	5.89	5.10	4.73	4.45	4.43	4.41	4.22	3.96	3.74

The results in Figure (29) and Table (16) demonstrated that the blend-dough's curves showed similar tendency behaviour of wheat flour dough (return to dominant wheat flour proportion). The reason was the great difference of lupine dough curves which exhibited another behaviour (singularity behaviour). There were significant difference between composite flour dough themselves, in range below 55 °C and above 75 °C the storage and loss modulus decreased with increase lupine flour concentration. But in between, these two ranges the module increased which was attributed to the difference in the gelatinization temperature of the starch during heating and the variation in hydration behaviour of two proteins, wheat and lupine. The interaction between the different compounds (protein and starch) of wheat and lupine flour play important role in the differentiation of the level of dynamical modules.

6.3.3.2. Wheat and lupine fiber dough

Figure (30) presents the changes of G' and G'' depended on temperature for wheat flour and lupine fiber dough in the range 15 and 90 °C.

The results in figure (30) and Table (17) demonstrated that the blend-dough's curves showed similar tendency behaviour of wheat flour dough (return to dominant wheat flour proportion).

Despite similar behavior of the dough blends due to the dominante of the wheat flour component, the measured curves of the dynamic module run at a higher level compared to wheat flour dough and show clear distinct solid state properties. The curves of mixtures with 15 % located on the highest level and have double more of G'' module than the wheat flour dough. A comparison of material scientific parameters is given in table 17.

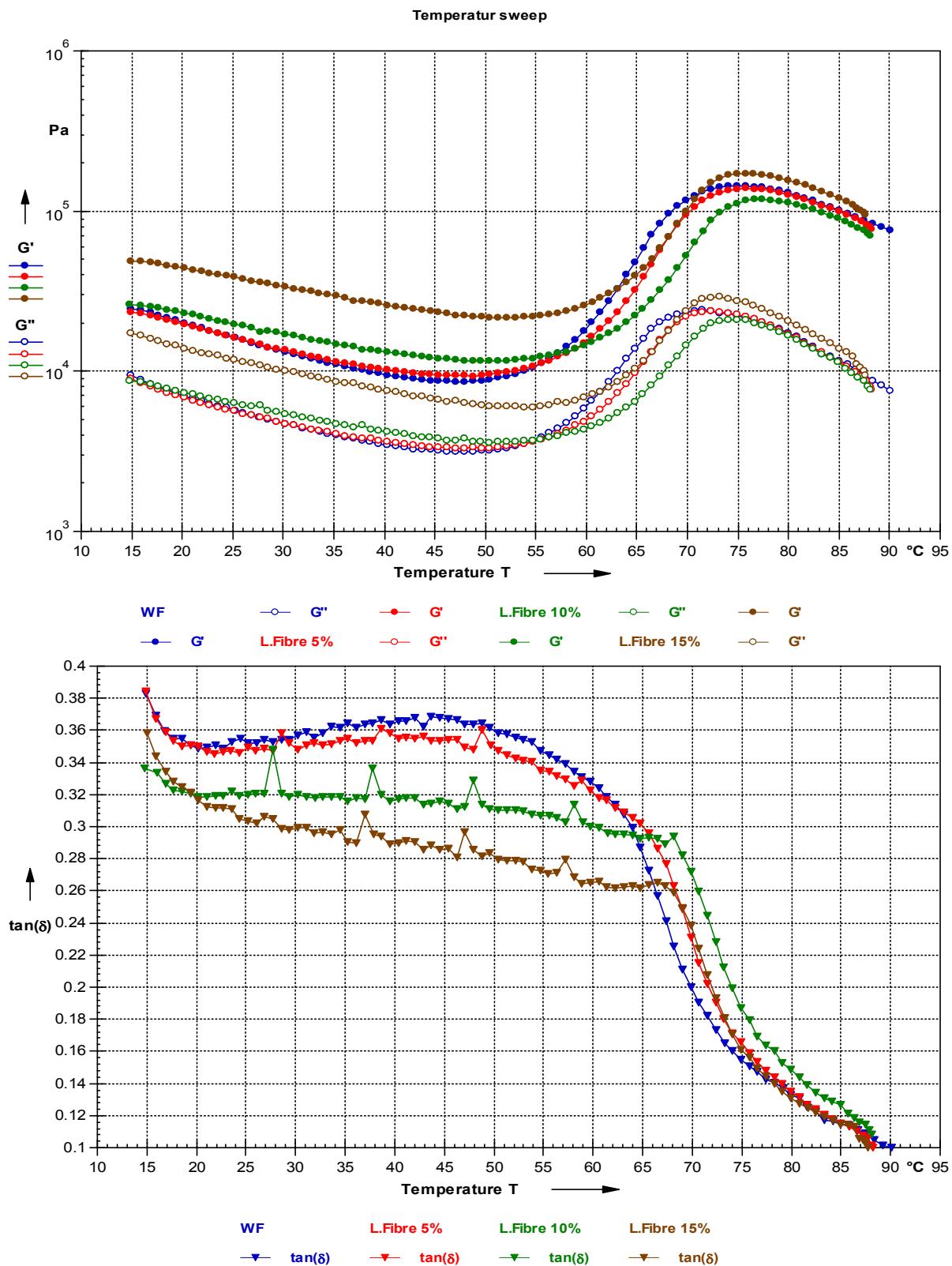


Figure 30: Temperature sweep the flour mixture in dough (5, 10 and 15 % lupine fiber).

Table 17: Temperature sweep for flour mixture in dough (5, 10 and 15 % lupine fiber).

Sample	Dynamic Parameters	Temperatur °C	I			II			III			
			15	25	35	45	55	60	65	75	85	
WF	G'	10 ⁴ Pa	2.45	1.61	1.20	0.87	1.06	2.02	4.81	14.4	9.69	7.58
	G''	10 ⁴ Pa	0.94	0.57	0.40	0.32	0.37	0.65	1.38	2.23	1.09	0.76
	$\tan \delta$		0.38	0.35	0.36	0.37	0.35	0.32	0.29	0.16	0.11	0.10
	$ \eta^* $	10 ⁴ Pa · s	0.42	0.27	0.19	0.15	0.18	0.34	0.79	2.32	1.55	1.21
	$ G^* $	10 ⁴ Pa	2.63	1.71	1.17	0.93	1.12	2.12	5.01	14.6	9.75	7.62
L-Fiber 5 %	G'	10 ⁴ Pa	2.33	1.61	1.15	0.95	1.08	1.65	3.22	13.7	9.41	7.71
	G''	10 ⁴ Pa	0.89	0.56	0.41	0.34	0.36	0.52	0.97	2.27	1.06	0.77
	$\tan \delta$		0.38	0.35	0.36	0.36	0.34	0.32	0.30	0.17	0.11	0.10
	$ \eta^* $	10 ⁴ Pa · s	0.39	0.27	0.19	0.16	0.18	0.28	0.54	2.21	1.51	1.23
	$ G^* $	10 ⁴ Pa	2.49	1.71	1.22	1.00	1.14	1.73	3.36	13.9	9.47	7.74
L-Fiber 10 %	G'	10 ⁴ Pa	2.61	1.97	1.48	1.20	1.21	1.52	2.21	11.1	8.61	7.06
	G''	10 ⁴ Pa	0.88	0.63	0.47	0.38	0.37	0.46	0.65	2.09	1.04	0.76
	$\tan \delta$		0.34	0.32	0.32	0.31	0.31	0.29	0.29	0.19	0.12	0.11
	$ \eta^* $	10 ⁴ Pa · s	0.44	0.33	0.25	0.20	0.20	0.25	0.37	1.81	1.38	1.18
	$ G^* $	10 ⁴ Pa	2.75	2.07	1.55	1.26	1.26	1.59	2.30	11.4	8.67	7.09
L-Fiber 15 %	G'	10 ⁴ Pa	4.86	3.87	2.95	2.32	2.20	2.69	3.95	17.1	11.5	9.46
	G''	10 ⁴ Pa	1.74	1.17	0.86	0.66	0.59	0.72	1.03	2.76	1.32	0.95
	$\tan \delta$		0.36	0.31	0.29	0.29	0.27	0.27	0.26	0.16	0.12	0.10
	$ \eta^* $	10 ⁴ Pa · s	0.82	0.64	0.49	0.38	0.36	0.44	0.65	2.76	1.84	1.51
	$ G^* $	10 ⁴ Pa	5.16	4.04	3.07	2.41	2.28	2.77	4.08	17.3	11.6	9.51

6.3.3.3. Wheat and lupine flour or fiber batter

Figures (31 and 32) present the changes of G' and G'' with temperature for wheat flour and lupine flour or fiber in batter between 15 and 90 °C.

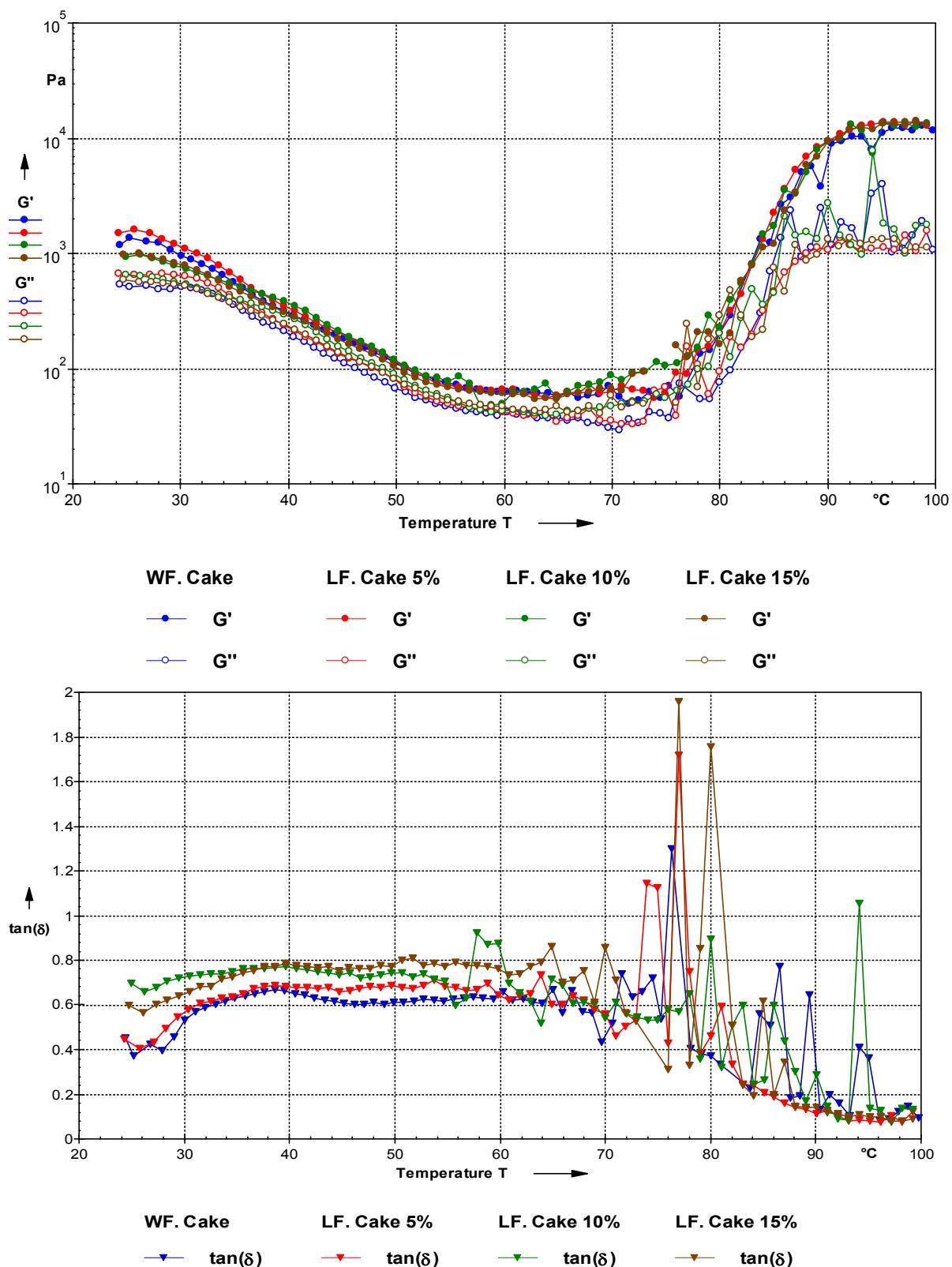


Figure 31: Temperature sweep for flour mixture in dough (5, 10 and 15 % lupine flour).

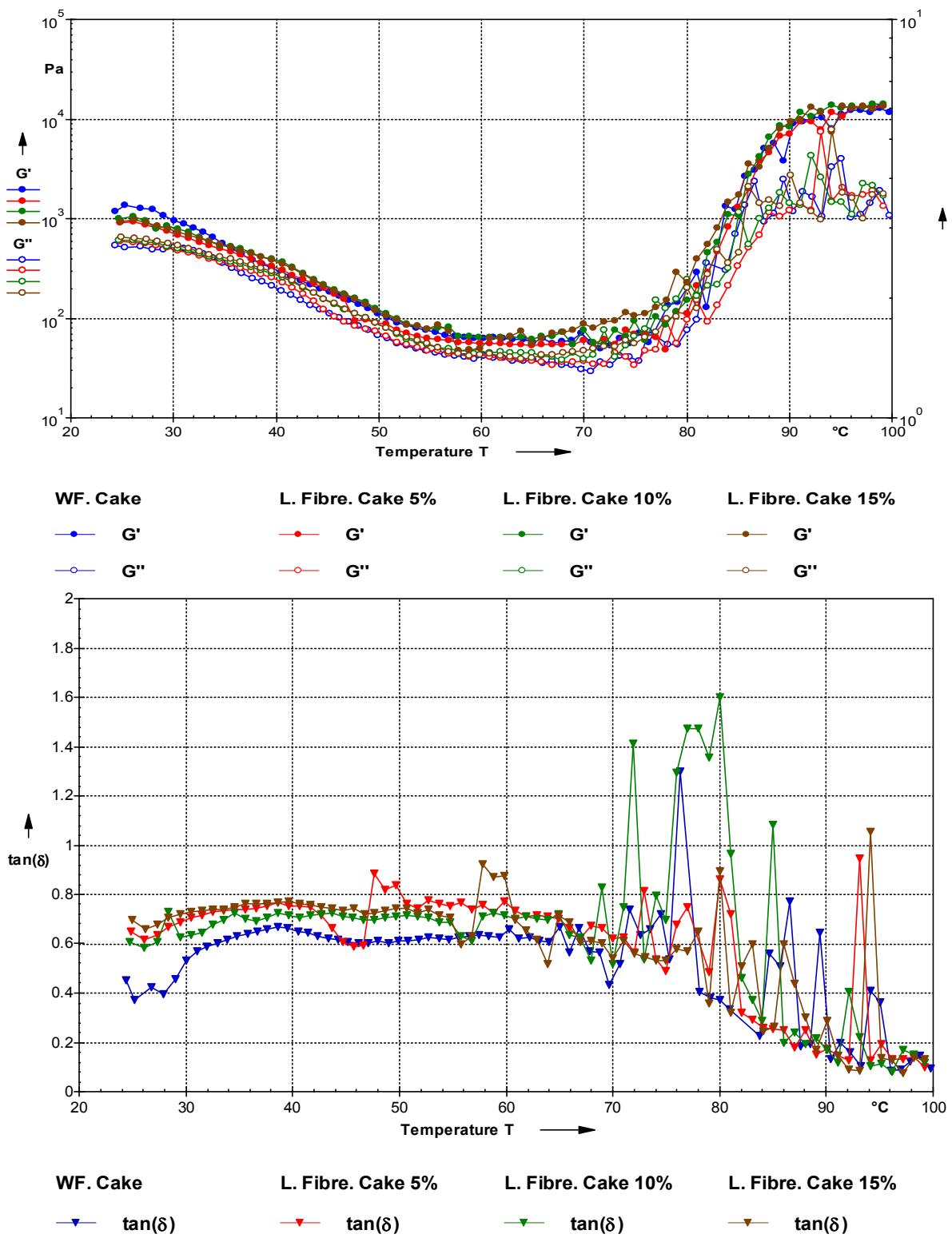


Figure 32: Temperature sweep the flour mixture in dough (5, 10 and 15 % lupine fiber).

Results and discussion

The results in Figures (31 and 32) and Tables (19 and 20) demonstrated that the blend-dough's curves showed similar tendency behaviour of wheat flour dough (return to dominant wheat flour proportion) as the same trend of the behaviour of dough. This result return to the starch content of lupine is very low, so no changes happened in starch gelatinization. The seed contains minute amounts of starch and higher levels of soluble non-starch polysaccharides (30–40 %) (Erbaş et al., 2005).

Table 18: Temperature sweep for flour mixture in batter (5, 10 and 15 % lupine flour).

Sample	Dynamical Parameters	Temperatur °C									
		25	35	45	55	65	75	80	85	90	95
WF	G' 10^3 Pa	1.38	0.57	0.18	0.07	0.06	0.07	0.21	2.67	8.98	11.1
	G'' 10^3 Pa	0.51	0.36	0.11	0.05	0.04	0.04	0.08	1.36	1.18	4.02
	$\tan \delta$	0.37	0.63	0.61	0.62	0.61	0.54	0.37	0.51	0.13	0.36
	$ \eta^* $ 10^3 Pa·s	0.23	0.11	0.04	0.01	0.02	0.01	0.04	0.48	1.44	1.88
	$ G^* $ 10^3 Pa	1.47	0.67	0.21	0.09	0.07	0.08	0.02	3.00	9.06	11.8
LF 5 %	G' 10^3 Pa	1.49	0.69	0.21	0.08	0.06	0.06	0.21	3.64	9.51	13.8
	G'' 10^3 Pa	0.66	0.44	0.14	0.05	0.04	0.07	0.09	0.69	1.08	1.13
	$\tan \delta$	0.45	0.64	0.66	0.68	0.60	1.13	0.46	0.19	0.11	0.09
	$ \eta^* $ 10^3 Pa·s	0.26	0.13	0.04	0.01	0.01	0.01	0.04	0.59	1.52	2.21
	$ G^* $ 10^3 Pa	1.63	0.82	0.25	0.09	0.07	0.09	0.23	3.70	9.52	13.9
LF 10 %	G' 10^3 Pa	1.01	0.52	0.23	0.08	0.06	0.10	0.16	1.08	8.44	12.9
	G'' 10^3 Pa	0.63	0.37	0.02	0.05	0.05	0.07	0.25	1.17	1.43	1.44
	$\tan \delta$	0.60	0.70	0.71	0.69	0.72	0.70	1.60	1.08	0.17	0.12
	$ \eta^* $ 10^3 Pa·s	0.19	0.10	0.04	0.02	0.01	0.02	0.05	0.25	1.36	2.09
	$ G^* $ 10^3 Pa	1.18	0.63	0.27	0.09	0.08	0.12	0.29	1.55	8.54	12.9
LF 15 %	G' 10^3 Pa	0.98	0.46	0.18	0.07	0.06	0.16	0.58	2.35	9.42	13.5
	G'' 10^3 Pa	0.59	0.35	0.14	0.05	0.05	0.05	0.29	0.47	1.32	1.33
	$\tan \delta$	0.60	0.74	0.75	0.77	0.86	0.31	0.51	0.20	0.14	0.09
	$ \eta^* $ 10^3 Pa·s	0.18	0.09	0.04	0.01	0.01	0.03	0.10	0.38	1.51	2.16
	$ G^* $ 10^3 Pa	1.14	0.58	0.23	0.09	0.07	0.17	0.65	2.39	9.51	13.6

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This fiber is predominantly nonstarch polysaccharide in the form of thickened cell walls of the lupine seed endosperm, with some residual protein. Although it is primarily insoluble in nature, the nonstarch polysaccharide component has paradoxically been described as a “pectin-like” rhamnogalacturonan (Evans et al., 1993). Lupine kernel fiber has been described as a powder that is pale in color, low in odor and flavor, and suitable for use as a ‘nonintrusive’ fiber ingredient in foods such as baked goods and meat products (Johnson and Gray, 1993).

Table 19: Temperature sweep an flour mixture in batter (5, 10 and 15 % lupine fiber).

Sample	Dynamical Parameters		Temperatur °C									
			25	35	45	55	65	75	80	85	90	95
WF	G'	10^3 Pa	1.38	0.57	0.18	0.07	0.06	0.07	0.21	2.67	8.98	11.1
	G''	10^3 Pa	0.51	0.36	0.11	0.05	0.04	0.04	0.08	1.36	1.18	4.02
	$\tan \delta$		0.37	0.63	0.61	0.62	0.61	0.54	0.37	0.51	0.13	0.36
	$ \eta^* $	10^3 Pa·s	0.23	0.11	0.04	0.01	0.02	0.01	0.04	0.48	1.44	1.88
	$ G^* $	10^3 Pa	1.47	0.67	0.21	0.09	0.07	0.08	0.02	3.00	9.06	11.8
L. Fibre 5 %	G'	10^3 Pa	0.91	0.47	0.18	0.06	0.05	0.07	0.11	1.29	7.02	10.7
	G''	10^3 Pa	0.59	0.35	0.10	0.05	0.04	0.03	0.10	0.33	1.21	2.06
	$\tan \delta$		0.65	0.74	0.59	0.75	0.70	0.49	0.86	0.26	0.17	0.19
	$ \eta^* $	10^3 Pa·s	0.17	0.09	0.03	0.01	0.01	0.01	0.02	0.21	1.13	1.73
	$ G^* $	10^3 Pa	1.09	0.58	0.20	0.08	0.07	0.08	0.15	1.33	7.12	10.9
L. Fibre 10 %	G'	10^3 Pa	1.00	0.53	0.22	0.08	0.06	0.09	0.15	1.07	8.41	12.9
	G''	10^3 Pa	0.61	0.37	0.02	0.05	0.05	0.07	0.24	1.16	1.42	1.48
	$\tan \delta$		0.61	0.70	0.71	0.69	0.72	0.70	1.60	1.08	0.17	0.12
	$ \eta^* $	10^3 Pa·s	0.19	0.10	0.04	0.02	0.01	0.02	0.05	0.25	1.36	2.06
	$ G^* $	10^3 Pa	1.17	0.64	0.27	0.09	0.08	0.12	0.29	1.58	8.53	13.0
L. Fibre 15 %	G'	10^3 Pa	0.97	0.52	0.21	0.08	0.06	0.11	0.23	1.73	9.42	13.4
	G''	10^3 Pa	0.65	0.39	0.16	0.06	0.04	0.06	0.20	0.46	2.72	1.80
	$\tan \delta$		0.70	0.76	0.73	0.70	0.71	0.53	0.89	0.26	0.29	0.14
	$ \eta^* $	10^3 Pa·s	0.18	0.10	0.04	0.02	0.01	0.02	0.05	0.28	1.56	2.15
	$ G^* $	10^3 Pa	1.14	0.65	0.27	0.09	0.07	0.12	0.30	1.78	9.80	13.5

6.4. Creep tests for wheat and lupine flour or fiber dough

The creep test is to compare the properties (1-point – measurements) of the materials science different flours and their mixtures at constant measuring conditions. This measured approach within the framework of classical dough investigation evaluated only partially.

Figures (32) present the general characterization of the creep tests of wheat and lupine flour or fiber curves.

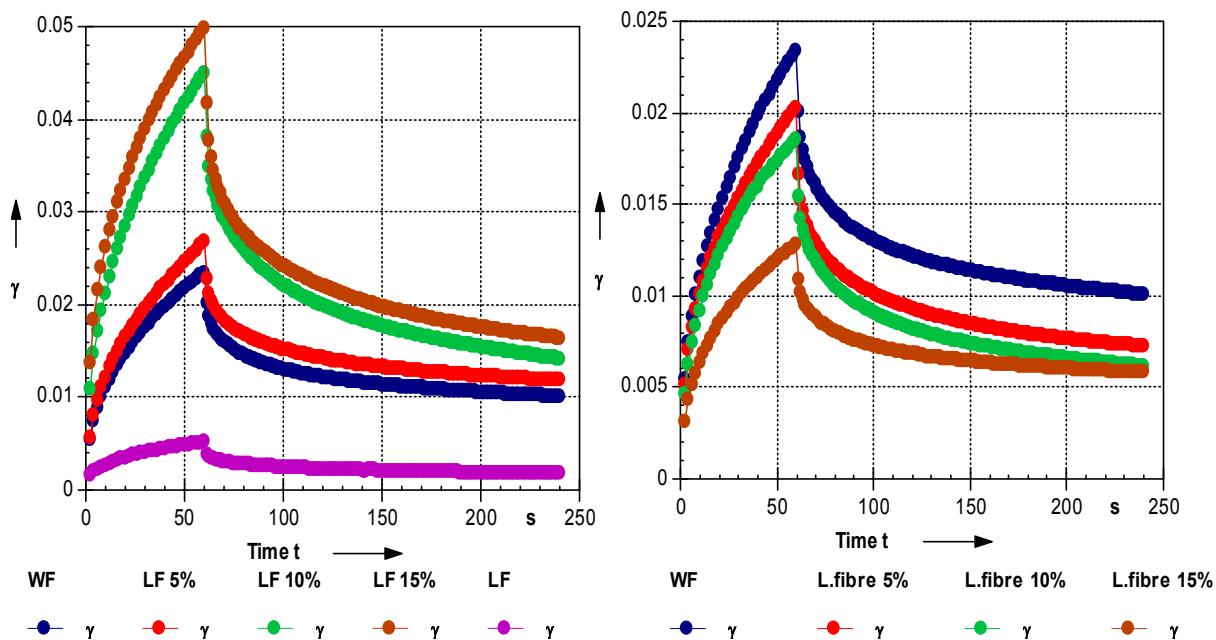


Figure 33: Creep comparison for wheat and lupine flour or fiber dough.

There are proven higher viscoelastic properties of the wheat dough. Is clearly an example this (old) conventional method has the great difference in the structural properties seen between wheat flour and lupine dough. Compared to the lupine dough, the dough with wheat flour had optimal viscoelastic material see table (20). The evaluated results in Table (20) show the measured data from Figure (33). From Table (20) is evident that the wheat flour compared to the lupine flour dough, the higher values of maximum deformation and creep compliance as well as having equal weight of the restoring force.

Table 20: Creep comparison for wheat and lupine flour or fiber dough.

Probe	Rheological parameters								
	J_e	$\dot{\gamma}_3$	γ_e	γ_v	γ_{max}	γ_e / γ_{max}	η_0	G_0	λ
	10^{-5} Pa^{-1}	10^{-4} s^{-1}	10^{-2}	10^{-2}	10^{-2}	%	$\text{Pa} \cdot \text{s}$	Pa	s
WF	2.65	2.07	2.24	1.01	3.25	68.99	242170	3779	64
LF 5 %	2.97	2.43	1.49	1.18	2.67	55.74	205540	3373	61
LF 10 %	6.19	3.81	3.08	1.42	4.49	68.46	131290	1616	81
LF 15 %	6.72	9.97	3.35	1.64	4.98	67.14	130740	1489	89
LF	0.68	1.03	0.34	0.17	0.52	66.25	141900	14140	97
L. Fibre 5 %	2.61	4.06	1.31	0.73	2.03	64.29	292880	3831	76
L. Fibre 10 %	2.47	3.71	1.24	0.62	1.85	66.83	362600	4044	89
L. Fibre 15 %	1.38	2.56	0.69	0.58	1.28	54.55	510390	7229	71

Zero shear viscosity and shear modulus show minima, which for better flow properties of the dough suggesting. The greater of zero shear viscosity and the shear modulus, the lower of the fluidity of the dough (tendency for stiffness) was found. The elastic deformation units of wheat flour dough almost twice as large compared to the viscous friction. With increasing concentration of lupine flour in the dough system (flour mixtures) increases the maximum deformation and elastic recovery (from 1.01 to 1.64) when lupine flour was add at 15 %. While, with increasing of lupine fiber concentration in the dough system (flour mixtures), decreases the maximum deformation and elastic recovery (from 1.01 to 0.58) when lupine fiber was add at 15 %. Although, deterioration of the structural development has been demonstrated in the dough system after the addition of lupine flour to wheat flour, showed the blends by the dominance of wheat flour allowable processing properties.

7. Baking properties

In the subsequent baking tests, the suitability of the flour mixtures for the preparation of a lupine flour or fiber enriched bread or cake made.

7.1. Influence of lupine flour or fiber incorporation on bread properties

Dough handling was not affected at low levels up to 10 % supplementation, but beyond 10 % level of lupine flour supplementation, the dough became sticky and was difficult to process. The dough surface of the wheat dough and the blend with 5 % and 10 % were classified as "normal" and "still normal" respectively. The blend with 15 % was described as "sticky" (Figure 34).



Figure 34: Dough properties compared between wheat flour dough and flour mixtures with lupine flour.

The wheat flour dough (standard) and dough from flour mixture 5 and 10 % lupine flour were characterized by a good (typical wheat) dough stability after resting times, so that the work-up no problem prepared. In contrast, the doughs were mixed with ratios 15 % lupine flour partially weakening and flowing properties with a moist and sticky surface to be evaluated.



Figure 35: Dough properties compared between wheat flour dough and flour mixtures with lupine fiber.

Figure (35) showed that dough handling was not affected at any levels of supplementation with lupine fiber and the dough surface of the wheat dough and the blend with 5, 10 and 15 % lupine fiber were classified as "normal".

The effect of the lupine flour or fiber incorporation on the fresh bread characteristics is summarized in tables (22,23). The volume of the control bread sample was significantly higher than that of samples incorporating lupine flour or fiber ($p < 0.05$). This effect is probably related to the decreased visco-elasticity of dough resulting from lupine addition (Table 20). As the level of lupine or fiber supplementation increased (5–15 %), the loaf volume of the corresponding fortified breads gradually decreased.

Table 21: Loaf characteristics of wheat flour and lupine flour or fiber composite flours.

Sample	Loaf height cm	Loaf weight g	Loaf volume cm ³	Specific volume cm ³ /g
WF	9.5 ± 0.73	470.8 ± 23	1900 ± 54	4.04 ± 0.4
LF 5 %	9.0 ± 0.23	486.7 ± 25	1860 ± 65	3.82 ± 0.52
LF 10 %	8.5 ± 0.13	493.9 ± 15	1800 ± 71	3.64 ± 0.32
LF 15 %	8.0 ± 0.95	496.5 ± 6	1775 ± 62	3.58 ± 0.21
L-fiber 5 %	9.3 ± 0.54	496.9 ± 12	1755 ± 26	3.53 ± 0.12
L-fiber 10 %	9.0 ± 0.56	503.6 ± 15	1700 ± 48	3.38 ± 0.13
L-fiber 15 %	8.5 ± 0.34	508.5 ± 18	1680 ± 38	3.3 ± 0.18

Mean ± standard deviation of mean.

Doxastakis et al., (2002) reported a decrease in bread volume with increasing levels of lupine or soy flour and attributed this decrease to the dilution of the wheat gluten by the legume protein.

It appears, therefore, that the decrease in bread volume resulting from lupine flour or fiber addition is most likely due to the combined effects of gluten dilution and mechanical disruption of the gluten network structure by the lupine particles. In addition, examination of the loaf internal structure revealed that the crumb of the lupine flour or fiber containing bread contained a small number of gas cells compared to the control (Figure 36).

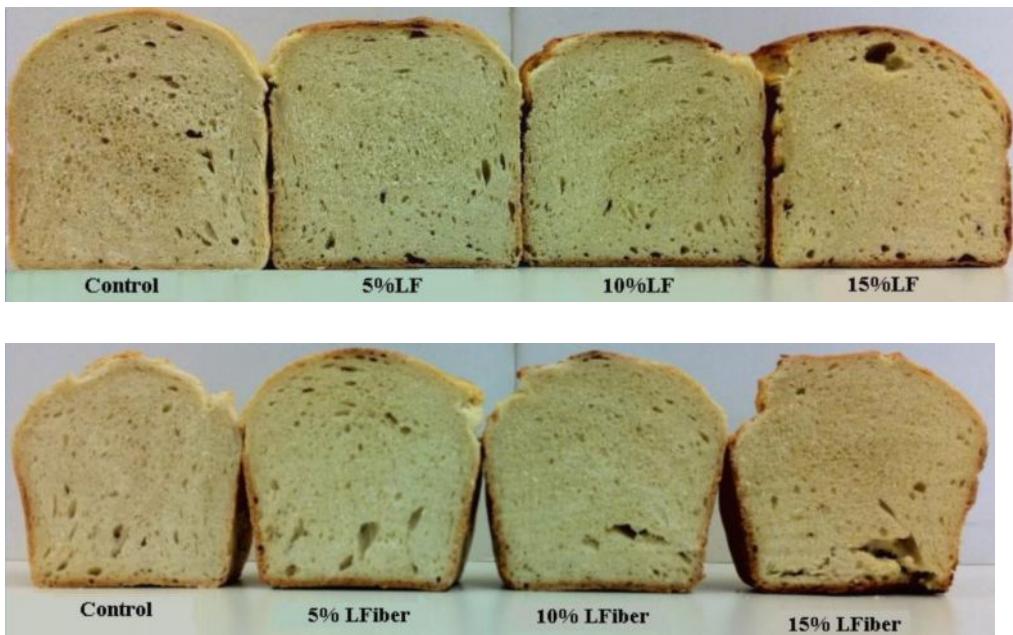


Figure 36: Comparison of the baking properties of wheat flour and mixes with lupine flour or fiber bread.

Concerning baking losses, as can be observed in Table (21) the water retention capacity was to some extent enhanced by the incorporation of lupine flour or fiber in wheat flour bread formulations. Additionally lupine-enriched breads had slightly higher moisture content than the control due to a higher water addition during bread making (higher farinographic absorption) and capacity of lupine protein to retain more water than gluten. The control bread exhibited good crumb structure than the lupine enriched breads, indicating that lupine addition exhibited a more resistant to deformation crumb (Figure 36). This behavior is reasonable considering that the control sample was prepared with wheat flour only that resulted in a stronger and more organized gluten network, due to its higher content of the gluten proteins. Lupine flour or fiber addition brought a marked increase in crumb hardness probably as a result of the thickening of the crumb walls surrounding the air cells and the strengthening of the crumb structure by the protein or fiber particles.

All color data were expressed by Hunter L*, a*, and b* values corresponding to lightness, redness, and yellowness, respectively. The crust color of samples was affected by the replacement of wheat flour with lupine flour or fiber (Table 22). In general, as lupine flour or fiber level increased, the crust color became darker as measured by the colorimeter. The crust of the control was lighter and less yellow than any of the other sample. For crumb color, as the level

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of lupine flour or fiber increased, the a^* and b^* values increased, indicating that a redder and more yellow crumb was obtained as a result of lupine flour or fiber substitution.

Table 22: Color measurements of bread from wheat flour and lupine flour or fiber composite flours.

Color parameters	Crust			Crumb		
	L*	a*	b*	L*	a*	b*
WF	97.53 ± 3.79	1.24 ± 0.69	4.92 ± 2.74	100.62 ± 3.74	0.90 ± 0.60	2.31 ± 1.58
LF 5 %	83.48 ± 8.24	4.19 ± 1.85	11.88 ± 6.20	99.39 ± 1.61	1.32 ± 0.41	2.55 ± 1.59
LF 10 %	81.38 ± 10.43	4.71 ± 2.09	14.22 ± 3.13	99.15 ± 2.31	3.00 ± 0.50	7.78 ± 1.82
LF 15 %	80.44 ± 4.50	4.94 ± 4.05	15.95 ± 1.76	98.48 ± 1.55	4.89 ± 30	11.99 ± 1.73
L-fiber 5 %	90.11 ± 6.66	1.69 ± 2.02	9.36 ± 5.15	99.77 ± 1.93	1.79 ± 0.70	5.50 ± 3.10
L-fiber 10 %	88.16 ± 8.29	2.57 ± 2.12	9.59 ± 1.74	96.9 ± 3.36	4.33 ± 0.46	13.47 ± 1.25
L-fiber 15 %	82.98 ± 7.58	3.61 ± 2.22	14.12 ± 3.22	96.8 ± 3.71	4.19 ± 0.59	13.60 ± 1.74

Mean ± standard deviation of 10 different points on Crust and Crumb.

The darkening of bread containing lupine might have been attributed to an increased Maillard reaction taking place during baking due to higher lysine content. In the Maillard reaction reducing carbohydrates react with free amino acid side chain of protein mainly lysine and lead to amino acid–sugar reaction products (polymerized protein and brown pigments). This reaction may compromise the nutritional value of foods through the blocking and destruction of essential amino nutrients (Hurrell, 1990). Color of wheat flour bread was light brown which increased significantly upon increasing the level of substitution. Typical loaves are obtained with substitution of wheat flour by lupine flour at 5 and 10 % levels (Figure 36).

Most people who have tried bread from lupine-wheat flour mixes have found the texture, taste and frequently the color to be appealing (Table 23). Substitution of lupine flour or fiber at 5, 10 or 15 % leads to reduced bread making potential degree of reduction depends on the substituent level. However, substitution at 5 and 10 % lupine flour or fiber gives parameter values at least as good as the control sample and produces acceptable bread in terms of weight,

volume, crumb structure and color. The blend with <10 % shows a substantial decrease in all values measured. There appears to be a potential market for lupine flour or fiber in bread making.

Table 23: Sensory evaluation of bread from wheat flour and lupine flour or fiber composite flours.

Sample	Appearance	Crumb texture	Crumb grain	Crust color	Taste	Odor	Overall acceptability
WF	8.3 ± 1.42	8.4 ± 1.65	8.2 ± 1.03	8.3 ± 1.49	8.3 ± 0.95	8.7 ± 1.16	8.4 ± 1.35
LF 5 %	8.5 ± 1.08	8.7 ± 1.57	8.0 ± 1.15	8.3 ± 1.42	8.5 ± 1.18	8.7 ± 1.16	8.3 ± 1.06
LF 10 %	8.0 ± 0.94	7.6 ± 1.84	7.9 ± 1.29	8.2 ± 1.14	8.0 ± 1.15	8.6 ± 1.26	8.1 ± 1.29
LF 15 %	7.1 ± 2.08	7.2 ± 1.48	6.6 ± 2.32	7.8 ± 1.81	7.8 ± 1.48	8.2 ± 1.48	7.9 ± 1.45
L-fiber 5 %	8.2 ± 1.14	8.1 ± 1.29	7.2 ± 1.75	8.5 ± 1.51	7.7 ± 1.49	8.2 ± 0.92	7.7 ± 1.70
L-fiber 10 %	7.8 ± 1.23	7.8 ± 1.40	6.4 ± 1.84	7.9 ± 1.73	7.6 ± 1.43	7.8 ± 1.40	7.6 ± 1.17
L-fiber 15 %	7.1 ± 1.91	7.1 ± 1.97	6.0 ± 2.31	7.8 ± 1.75	7.6 ± 1.26	7.8 ± 1.03	7.1 ± 1.52

Mean ± standard deviation of ten panelists

7.2. Influence of lupine flour or fiber incorporation on cake properties

According to Table (24), cake volume diminished as the lupine flour or fiber percentage increased. During the baking process, baking powder generates gases, which should be retained in order to guarantee good cake volume, and in that respect flour quality has an important role to play. Another important factor is the gelatinization temperature of the flour, as Howard, (1972), pointed out for layer cakes, whereas Mizukoshi et al., (1980) reached the same conclusion for sponge cakes. The starch gelatinization at low temperatures would prevent the correct expansion of doughs.

Table 24: Cake characteristics of wheat flour and lupine flour or fiber composite flours.

Sample	Cake height cm	Cake weight g	Cake volume cm ³	Specific volume cm ³ /g
WF	7.5 ± 0.53	265.1 ± 13	660 ± 56	2.49 ± 0.6
LF 5 %	7.0 ± 0.33	265.2 ± 38	670 ± 46	2.53 ± 0.45
LF 10 %	6.5 ± 0.23	264.5 ± 11	690 ± 68	2.61 ± 0.27
LF 15 %	6.0 ± 0.65	269.2 ± 8	700 ± 57	2.60 ± 0.30
L-fiber 5 %	7.3 ± 0.44	281.7 ± 10	665 ± 32	2.36 ± 0.16
L-fiber 10 %	7.0 ± 0.64	272.8 ± 17	700 ± 51	2.56 ± 0.19
L-fiber 15 %	6.5 ± 0.48	264.3 ± 13	740 ± 24	2.80 ± 0.14

Mean ± standard deviation of mean.

Lupine flour also presented higher protein content, and different amino acid composition than wheat flours which could affect cake characteristics, especially volume (Mohamed and Hamid, 1998). As far as lupine derivatives are concerned, flour and fiber gave cakes with higher volume. Cake elaborated with lupine fiber had higher volume than those elaborated with lupine flours. This fact can be explained by the higher fibre content of lupine fiber and its effect on pasting behavior of starches (Sasaki et al., 2000). There is no effect of the low starch content.

The volume index is an indicator of cake volume and, as expected, followed a similar tendency as volume. Symmetry indicates the differences in height between the central zone and the lateral zone. Thus, a high symmetry suggests that cakes mainly rise in their central part, while a negative symmetry indicates that cake volume falls down at the end of the baking process. Hence, symmetry gives an idea about gas retention in the final baking phase. In both cases the incorporation of lupine flour or fiber at different levels had no effect on cake symmetry (Figure 37).



Figure 37: Comparison of the baking properties of wheat flour and mixes with lupine flour or fiber cake.

This result is not confirmed with Gómez et al., (2008) who studied the influence of the total or partial replacement of wheat flour by chickpea flour on the quality characteristics of two kinds of cake. They reported that in both cases the incorporation of chickpea flour reduced their symmetry, and in the case of layer cakes with 100 % of chickpea flour it became negative. As for cake weight, no significant differences were found. Therefore, the water retention capacity was not affected by the substitution of wheat flour by lupine flour. Amongst lupine fiber, small differences were also detected, but they could not be attributed to their different chemical composition.

Crust color in cakes varied with the quantity and the kind of lupine addition (flour or fiber). This influence was more important in cakes. The crust color data of cakes are shown in Table (25). Cakes became darker (lower L^*) as the lupine flour quantity increased. Lupine fiber also resulted in dark cakes. With regard to the wheat flour, it produced the brightest cakes. No considerable differences in crust yellowness were found among the different lupine flour

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samples, while lupine fiber samples exhibited lower b values than the respective lupine flour samples and all lupine flour or fiber gave higher b values than the control (wheat flour). The crust color of cakes was generated in the baking process due to the Maillard reactions between sugars and amino acids, and the caramelization process of sugars. Therefore, the differences observed when the quantity of lupine flour increased could be attributed to the high protein content and the different amino acid composition of the composite flours compared to the wheat flour.

Table 25: Color measurements of cake from wheat flour and lupine flour or fiber composite flours.

Color parameters	Crust			Crumb		
	L*	a*	b*	L*	a*	b*
WF	97.53 ± 3.79	1.24 ± 0.69	4.92 ± 2.74	100.62 ± 3.74	0.90 ± 0.60	2.31 ± 1.58
LF 5 %	83.48 ± 8.24	4.19 ± 1.85	11.88 ± 6.20	99.39 ± 1.61	1.32 ± 0.41	2.55 ± 1.59
LF 10 %	81.38 ± 10.43	4.71 ± 2.09	14.22 ± 3.13	99.15 ± 2.31	3.00 ± 0.50	7.78 ± 1.82
LF 15 %	80.44 ± 4.50	4.94 ± 4.05	15.95 ± 1.76	98.48 ± 1.55	4.89 ± 30	11.99 ± 1.73
L-fiber 5 %	90.11 ± 6.66	1.69 ± 2.02	9.36 ± 5.15	99.77 ± 1.93	1.79 ± 0.70	5.50 ± 3.10
L-fiber 10 %	88.16 ± 8.29	2.57 ± 2.12	9.59 ± 1.74	96.9 ± 3.36	4.33 ± 0.46	13.47 ± 1.25
L-fiber 15 %	82.98 ± 7.58	3.61 ± 2.22	14.12 ± 3.22	96.8 ± 3.71	4.19 ± 0.59	13.60 ± 1.74

Mean ± standard deviation of 10 different points on Crust and Crumb.

Cake crumb does not reach temperatures above 100 °C, so the Maillard or caramelization reactions by sugars fail to take place. Therefore, crumb color must be the result of the raw materials colors and their interactions. The crumb color data of cakes are shown in Table (25) and Figure (38). Cake crumb of lupine flour samples were lightly darker than control, and the lupine flour addition reduced its luminosity, but this effect was not significant. Differences between lupine flour and lupine fiber were observed. This effect was expected because of the more intense yellow color of lupine flour. These results agree with those obtained by Dodok et al., (1993), who observed that chickpea flour triggered the change in the crumb color when they studied the addition of chickpea flour to bread doughs.

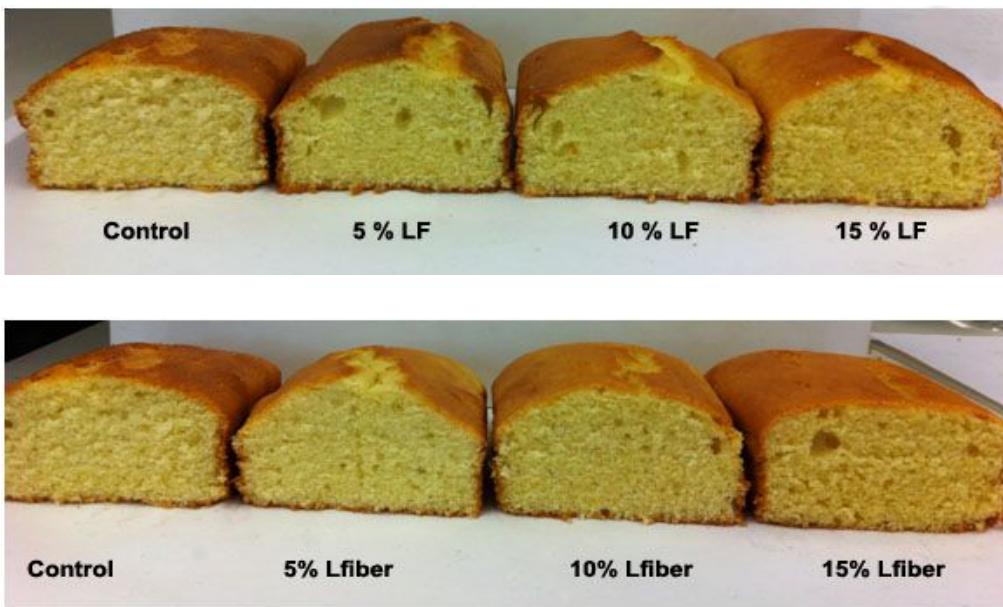


Figure 38: Crumb color of wheat flour and mixes with lupine flour or fiber cake.

Sensory evaluation results of fortified cake with lupine flour or fiber are presented in Table (26). It was found that cake which was fortified with different levels of lupine flour had non significant difference in taste, odour and overall acceptability as compared to unfortified cake (control).

Table 26: Sensory evaluation of cake from wheat flour and lupine flour or fiber composite flours.

Sample	Appearance	Crumb texture	Crumb grain	Crust color	Taste	Odor	Overall acceptability
WF	7.9 ± 1.5	7.9 ± 1.5	7.1 ± 1.6	7.6 ± 1.8	7.7 ± 2.1	8.1 ± 1.9	8.2 ± 1.3
LF 5 %	7.7 ± 2.0	8.1 ± 1.1	7.9 ± 0.9	8.3 ± 1.6	7.8 ± 1.8	8.2 ± 1.6	8.1 ± 1.5
LF 10 %	8.0 ± 1.4	8.0 ± 1.5	8.3 ± 0.9	8.1 ± 1.5	7.4 ± 2.2	7.9 ± 1.8	8.1 ± 1.2
LF 15 %	8.6 ± 1.3	7.7 ± 2.1	8.9 ± 1.5	8.7 ± 1.3	8.2 ± 2.0	8.2 ± 1.1	8.7 ± 1.3
L-fiber 5 %	7.6 ± 1.9	8.3 ± 0.9	7.4 ± 1.7	7.7 ± 1.8	8.0 ± 2.2	8.4 ± 0.9	8.4 ± 1.4
L-fiber 10 %	6.8 ± 2.1	8.3 ± 1.6	7.9 ± 1.4	7.7 ± 1.8	8.1 ± 2.3	8.2 ± 0.7	8.1 ± 0.9
L-fiber 15 %	6.4 ± 3.1	7.0 ± 1.5	8.3 ± 1.2	8.0 ± 2.1	7.2 ± 2.6	7.8 ± 1.6	7.7 ± 1.0

Mean ± standard deviation of ten panelists

Lupine fiber cakes at 15 % had lower scores for appearance and crumb texture. In addition, the quality characteristics were acceptable at 5, 10 and 15 % for lupine flour and 5, 10 % lupine fiber. It is evident from this data, that addition of lupine fiber more than 10 % caused gradually decreased in sensory characteristics scores. From the above results, it can be concluded that cake can be fortified with lupine flour (5, 10 and 15 %) and lupine fiber (5 and 10 %). The present results came in agreement with Sabanis et al., (2006) who reported that, organoleptic properties (colour, flavour and overall acceptability) improved with a low proportion of chickpea flour, especially for 5 % w/w substitution. Alabi and Anuonye, (2007) indicated that up to 50 % of some legume products could be added without significant loss in palatability.

8. Biological evaluation of lupine flour (LF) and lupine fiber (L-fiber)

8.1. Body weight and Food intake

As shown in Table (27) it was found that gain in body weight was 63.9 g for negative control, while it was decreased for the positive diabetic one to be 34.3 g and the reduction in body weight was 46.3 %. Diabetic rats fed on neither lupine flour nor fiber showed similar results as normal control, and there were significant difference.

Abdel-Salam and Abdel-Megeid, (1998), reported that alloxan injection caused a significant decrease in average body weight in rats and there was a decrease in body weight in groups treated with raw and blanched lupine. These results were in agreement with the present results. But Newairy et al., (2002) showed that diabetic rats which treated with lupine showed an increase of their body weight as compared with the diabetic group.

Food intake\day decrease in alloxan diabetic fed on 5, 10 and 15 % lupine fiber (12.3, 11.5 and 10.4 g/day respectively, compared to negative control 13.2 g/day. Slightly decrease was found in diabetic group fed on 15 % lupine flour and 5 % lupine fiber.

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Table 27: Gain in body weight, feed intake and feed efficiency ratio (FER) of healthy and diabetic rats fed on basal diet supplemented with different levels of lupine flour (LF) and lupine fiber (L-fiber).

Diets	Initial body weight g	Final body weight g	Feed intake g	Gain body weight g	Daily feed intake g	FER %
Control A*	111.6 \pm 2.2	175.5 \pm 3.4	462.0 \pm 6.3	63.90 \pm 2.1	13.20 \pm 2.5	13.8 \pm 1.6
Control B*	113.8 \pm 1.6	148.1 \pm 2.8	318.5 \pm 5.7	34.30 \pm 2.0	9.10 \pm 2.5	10.8 \pm 1.1
Group 1	115.7 \pm 1.7	185.4 \pm 4.5	500.5 \pm 5.3	69.70 \pm 3.9	14.30 \pm 1.5	13.9 \pm 1.7
Group 2	113.2 \pm 2.3	174.7 \pm 6.1	472.5 \pm 2.7	61.50 \pm 3.6	13.50 \pm 3.2	13.1 \pm 1.2
Group 3	119.9 \pm 2.9	171.3 \pm 4.9	434.0 \pm 3.1	51.40 \pm 1.9	12.40 \pm 2.1	11.8 \pm 1.3
Group 4	117.3 \pm 1.34	169.0 \pm 4.5	430.5 \pm 5.3	49.70 \pm 3.9	12.30 \pm 1.5	11.5 \pm 1.7
Group 5	116.3 \pm 1.54	155.8 \pm 6.1	402.5 \pm 2.7	37.50 \pm 3.6	11.50 \pm 3.2	9.3 \pm 1.2
Group 6	112.9 \pm 2.28	146.3 \pm 4.9	364.0 \pm 3.1	31.40 \pm 1.9	10.40 \pm 2.1	8.6 \pm 1.3

Control A*: Normal rats fed on a basal diet

Group 3: Diabetic rats fed on basal diet + 15 % lupine flour

Control B*: Diabetic rats fed on basal diet

Group 4: Diabetic rats fed on basal diet + 5 % lupine fiber

Group 1: Diabetic rats fed on basal diet + 5 % lupine flour

Group 5: Diabetic rats fed on basal diet + 10 % lupine fiber

Group 2: Diabetic rats fed on basal diet + 10 % lupine flour

Group 6: Diabetic rats fed on basal diet + 15 % lupine fiber

8.2. Biochemical analysis

8.2.1. Glucose level

For serum glucose, the present study in Table (28) showed that the levels of serum glucose of alloxan diabetic group was increased approximately 2 fold (225.20 ± 4.1) compared with normal control (99.96 ± 0.9). Feeding of alloxan diabetic groups showed significantly reduction in glucose levels by: 25.59 %, 32.37 %, and 31.90 % for lupine flour at 5, 10 and 15 % respectively, 28.36 %, 38.11 % and 47.75 % for lupine fiber at 5, 10 and 15 % respectively as compared to alloxan diabetic control.

The results were in agreement with finding of Mansour et al., (2002), who found that treated diabetic rats with 75mg\day\100g body wt. of lupine for 4 weeks reduced the glucose levels by 59 % as compared to diabetic alloxan rats. Abdel-Salam and Abdel-Megeid, (1998), found that raw and blanched lupine at 5 and 10 % have hypoglycemic effect and blanched lupine have more effect than raw as compared to diabetic control. The hypoglycaemic effect of lupine flour and lupine fiber may be due to the active constituents such as alkaloids, flavonoids, tannins, quinovic acid and its glycocidic derivatives, saponins and triterpenoid saponins (Pollmann et al., 1997). Other phenomenon due to saponins effect have hypoglycaemic activity, which may be due to the inhibition of hepatic gluconogenesis (Kubo et al., 2000). The effect of lupine may be due to the increase levels of serum insulin (Eskander and Won Jun, 1995), and also may be due to the enhancement of peripheral metabolism of glucose (Skim et al., 1999). The effects of lupine fiber on the diabetic symptoms in streptozotocin induced diabetic rats showed a decreased glucose levels in urine and lowering plasma glucose (Yamamoto et al., 2000).

8.2.2. Serum cholesterol and total lipids

In diabetes mellitus, hypercholesterolemia is a common complication, which is thought to be secondary to accumulation of triacylglycerol rich lipoproteins due to impaired activity of lipoprotein lipase (Kingman, 1991).

Table (28) showed that serum total cholesterol and total lipids were significantly increased in alloxan diabetic group by 113.97 % and 78.4 % respectively. Hypocholesterolemic and hypolipidemic effect were found in groups fed on lupine flour and lupine fiber. These findings are in accordance with those obtained by some investigators as Newairy et al., (2002) who reported that diabetic rats treated with terms reduced the level of cholesterol and total lipids as compared to diabetic rats. There is strong evidence in rats to suggest that the soluble polysaccharides present in the hypoglycemic plants were fermented in the colon producing short

chain fatty acids, notably, propionic acid, it has an inhibitory effect and reducing cholesterol synthesis (Chen and Anderson, 1986).

Table 28: Glucose, cholesterol and total lipid contents (mg/100 ml) of healthy and diabetic rats fed on basal diet supplemented with different levels of lupine flour (LF) and lupine fiber (L-fiber).

Diets	Glucose	Cholesterol	Total lipids
Control A*	99.96 ± 0.9	89.93 ± 6.3	950.40 ± 24.4
Control B*	225.20 ± 4.1	192.43 ± 8.8	1695.83 ± 17.6
Group 1	167.57 ± 1.7	169.93 ± 14.0	1478.53 ± 13.97
Group 2	152.30 ± 1.8	134.00 ± 15.4	1267.33 ± 35.6
Group 3	153.35 ± 2.3	119.01 ± 10.5	1062.34 ± 40.0
Group 4	161.32 ± 1.3	159.09 ± 4.5	1330.5 ± 25.32
Group 5	139.37 ± 1.5	125.85 ± 6.1	1102.5 ± 32.71
Group 6	119.91 ± 2.2	106.36 ± 4.9	964.0 ± 63.15

Control A*: Normal rats fed on a basal diet

Group 3: Diabetic rats fed on basal diet + 15 % lupine flour

Control B*: Diabetic rats fed on basal diet

Group 4: Diabetic rats fed on basal diet + 5 % lupine fiber

Group 1: Diabetic rats fed on basal diet + 5 % lupine flour

Group 5: Diabetic rats fed on basal diet + 10 % lupine fiber

Group 2: Diabetic rats fed on basal diet + 10 % lupine flour

Group 6: Diabetic rats fed on basal diet + 15 % lupine fiber

Also, it has been shown that dietary propionate reduce total plasma cholesterol (Bush and Milligan, 1971). Other observation stated that some saponins lowered both total and LDL-cholesterol levels in the plasma hypercholesterolemic animals (Sidhu et al., 1987), also increased the excretion of cholesterol to 65 % (Sim et al., 1984). This phenomenon regard to saponins stimulated lipoprotein lipase activity and it indicated that rats receiving germinated lupine might stimulate enzymes relating to the metabolism of lipids including cholesterol (Sim et al., 1984). Koo, (1983) reported that saponins had been shown to prevent atherosclerosis in experimental

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animals. El Shewey, (2000) indicated that rats receiving germinated lupine had significantly lowering levels of total cholesterol.

Dike et al., (2001), reported that fermented carob present a significant lowering levels of cholesterol. Haber, (2002) reported that carob pod fiber can significantly reduce cholesterol levels especially low density lipoprotein \ cholesterol in hypercholesterolemia people. Zunft et al., (2001), reported that patients with hypercholesterolemia (total cholesterol (232-302 mg\dl) consuming 15 g of fiber\day as a supplement to their regular diet, after 4 weeks reduction of 7.1 % and 10 % in mean total cholesterol and LDL cholesterol and after 6 weeks showed 7.8 % and 12.2 % in both respectively.

9. Conclusion

The objectives of this study were to investigate technological and rheological properties, chemical composition (proteins, lipids, starch, sugars, fiber, ash, amino acids, total phenolic, flavonoids, flavonols contents) and antioxidant capacity (DPPH and ABTS). The potential use of different sweet lupine seed derivatives (flour and fiber) at different concentration (5, 10 and 15 %) for baking applications (bread and cake) and the influence of lupine addition on the rheological properties (empirical rheology and fundamental) of dough and quality of final products were also described. Effects of different sweet lupine seed derivatives (flour and fiber) at different concentration (5, 10 and 15 %) on diabetic rats were also studied.

The lupine flour showed higher levels of moisture, crude protein, ash, crude fat and dietary fiber than the wheat flour. Conversely, wheat flour showed higher levels of starch. These results confirmed by statistical analysis, which highly significant differences ($P < 0.05$) were observed between the two type of flours. Mean protein and dietary fiber increased with increasing amount of lupine flour added to be 13.73 ± 0.24 , 14.75 ± 0.27 , 16.28 ± 0.31 and 4.66 ± 0.27 , 6.61 ± 0.43 8.57 ± 0.60 g/100 g for substituting wheat flour with lupine flour at 5, 10 and 15 %, respectively on dry weight basis. There was no significant difference between wheat flour and supplemented flour with different concentration of lupine for moisture, ash and fat content.

The lupine fiber showed higher levels ash, crude fat and dietary fiber than the wheat flour. Conversely, wheat flour showed higher levels of moisture, crude protein and starch. These results confirmed by statistical analysis, which highly significant differences ($P < 0.05$) were observed between wheat flour and lupine fiber. Mean dietary fiber increased with increasing amount of lupine fiber added to be 6.82 ± 0.32 , 10.95 ± 0.53 and 15.07 ± 0.75 for substituting wheat flour with lupine flour at 5, 10 and 15 %, respectively on dry weight basis. There was no significant difference between wheat flour and supplemented flour with different concentration of lupine fiber for moisture, ash and fat content.

The results for the amino acid content of wheat flour (WF) and lupine flour (LF) showed that the essential amino acids (lysine, threonine, isoleucine, phenylalanine and tryptophane) in lupine flour were higher than those in wheat flour except methionine content which was higher in wheat flour (1.7 g/kg). From the results we can noticed that lupine flour is rich with arginine and histidine (36.13 and 5.89 g/kg respectively).

Conclusion

The lupine flour showed higher levels of total phenolic and total flavonoids than the wheat flour. Conversely, wheat flour showed higher levels of total flavonols. These results confirmed by statistical analysis, which highly significant differences ($P < 0.05$) were observed between the two types of flours. Total phenolic and total flavonoids increased with increasing amount of lupine flour added to be 132.17 ± 0.58 , 142.5 ± 7.10 , 156.53 ± 3.88 ($\mu\text{g GAE/g DW}$) and 7.67 ± 1.27 , 7.93 ± 0.06 , 8.4 ± 0.52 ($\mu\text{g QE/g DW}$) for substituting wheat flour with lupine flour at 5, 10 and 15 %, respectively on dry weight basis. Results clearly indicate that lupine flour exhibited higher antioxidant activity with DPPH and ABTS than the wheat flour. The antioxidant activity increased with increasing amount of lupine flour added to be 5.1 ± 0.10 , 6.04 ± 0.77 , 7.16 ± 0.26 in DPPH and 29.41 ± 0.37 , 31.09 ± 0.00 , 32.35 ± 0.37 in ABTS respectively, for substituting wheat flour with lupine flour at 5, 10 and 15 %, respectively on dry weight basis. The same trend was observed with the antioxidant activity for lupine fiber in DPPH and ABTS tests. After that, physico-chemical and material science examination were done.

Supplementation of wheat flour with lupine flour or lupine fiber increased the water required for optimum bread making absorption ($p < 0.05$) (from 56.1 % for wheat flour to 57.2 % and 68.5 % for the 30 % lupine flour or lupine fiber respectively). The time required for the control dough to reach 500 FU consistency was also modified by lupine flour addition. During this phase of mixing, the water hydrates the flour components and the dough is developed. Dough development time (DDT) was significantly higher ($p < 0.05$) for all wheat-lupine flour or fiber blends than control (2.5 min), also between lupine samples significant difference was observed at different concentration ($p > 0.05$). Regarding dough stability, it appears that the dough sample containing 5 % lupine exhibited higher stability and resistance to mechanical mixing values than the control, while it decreased as the substitute level increases from 10 % to 15 %.

A rheometer Paar Physica UDS 200 (Physica®, Anton Paar GmbH, Austria Europe) using a plate-plate system (measure system MP 31) was needed for measuring the rheological properties of dough samples. In the double logarithmic representation of the deformation-dependent behavior of the studied dough is pronounced with a dominant LVR solid behavior ($G' > G''$ or detect loss factor < 1), followed by a decrease of both moduli and an increase in the loss factor with structural break ($\tan \delta = 1$). The overall structure and macro-structure is experiencing a "break down", will be completely destroyed. A sub-structure is not available. The destruction of deformation γ_z with $G' = G''$ is located at wheat flour-dough at 0.6, with 0.5 lupine

Conclusion

flour dough. The shape of the curves shows a linear viscoelastic behavior in the area of $10^{-4} \leq \gamma \leq 10^{-3}$. The results of oscillation tests showed that the moduli (G' , G'' and G^*) with increasing lupine flour content in the mixtures in comparison to the wheat flour with a deformation of $\gamma = 10^{-3}$ decreased slightly. A stable dough structure in hibernation is determined based on the dominance of the wheat flour in the blends. The addition of lupine fiber caused a shift of curves G' and G'' towards higher values, while curve $\tan \delta$ moved towards lower values. The data indicate that the additions applied caused an increase in wheat flour dough elasticity (G') and viscosity (G'').

In the double logarithmic representation of the deformation-dependent behavior of the studied batter is pronounced with a dominant LVR behavior ($G' > G''$ or detect loss factor < 1), followed by a decrease of both module and an increase in the loss factor with structural break ($\tan \delta = 1$). The shape of the curves shows a linear viscoelastic behavior in the area of $10^{-4} \leq \gamma \leq 10^{-3}$. Made for the implementation of the frequency sweep defining the boundary of the LVR with $\gamma = 10^{-3}$. Compared to the wheat flour batter should be noted that despite the higher protein content, the higher level structure and the relatively larger particulation at lupine-batter quickly leads to a structural instability of the batter, recognizable by the curve of $\tan \delta$. By all doughs, the curves of G' and G'' run nearly parallel in frequency sweep and G' was greater than G'' . This indicates the distinctive solid state characteristics of all changes. The dough promoted dispersion and net-gel-like structure.

Dough handling was not affected at low levels up to 10 % supplementation, but beyond 10 % level of lupine flour supplementation, the dough became sticky and was difficult to process. The dough surface of the wheat dough and the blend with 5 % and 10 % were classified as "normal" and "still normal" respectively. The blend with 15 % was described as "sticky". While, the dough surface of the wheat dough and the blend with 5, 10 and 15 % lupine fiber were classified as "normal". Particle system, no gel-like system special relations, depended on concentration and temperature.

The volume of the control bread sample was significantly higher than that of samples incorporating lupine flour or fiber ($p < 0.05$). This effect is probably related to the decreased elasticity of dough resulting from lupine addition.

Conclusion

The crust color of samples was affected by the replacement of wheat flour with lupine flour or fiber. In general, as lupine flour or fiber level increased, the crust color became darker as measured by the colorimeter.

Substitution of lupine flour or fiber at 5, 10 or 15 % leads to reduced bread making potential degree of reduction depends on the substituent level. However, substitution at 5 and 10 % lupine flour or fiber gives parameter values at least as good as the control sample and produces acceptable bread in terms of weight, volume, crumb structure and color. Also, it was found that cake which was fortified with different levels of lupine flour had non significant difference in taste, odour and overall acceptability as compared to unfortified cake (control).

At last, the feeding of alloxan diabetic rats groups showed significantly reduction in glucose levels by: 25.59 %, 32.37 %, and 31.90 % for lupine flour at 5, 10 and 15 % respectively, 28.36 %, 38.11 % and 47.75 % for lupine fiber at 5, 10 and 15 % respectively as compared to alloxan diabetic control. Hypocholesterolemic and hypolipidemic effect were found in groups fed on lupine flour and lupine fiber.

Finally, it can be conclude that lupine flour or fiber can be used successfully as hypoglycemic agents in bakery products. This could be utilized for the development of composite blends from locally produced lupine at small scale industry level as value-add products.

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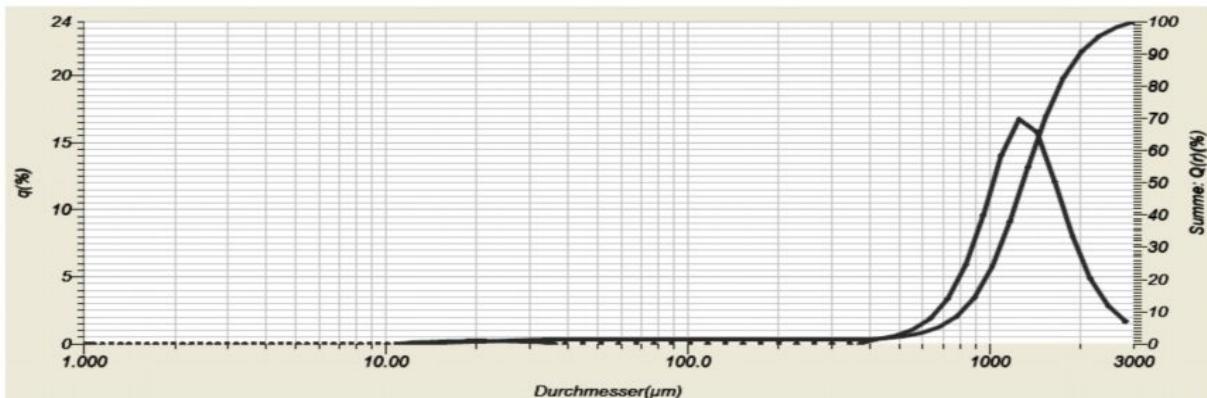
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11. Appendix

HORIBA Laser Scattering Particle Size Distribution Analyzer LA-950 gemessen an der TU Berlin, FG Lebensmittelrheologie

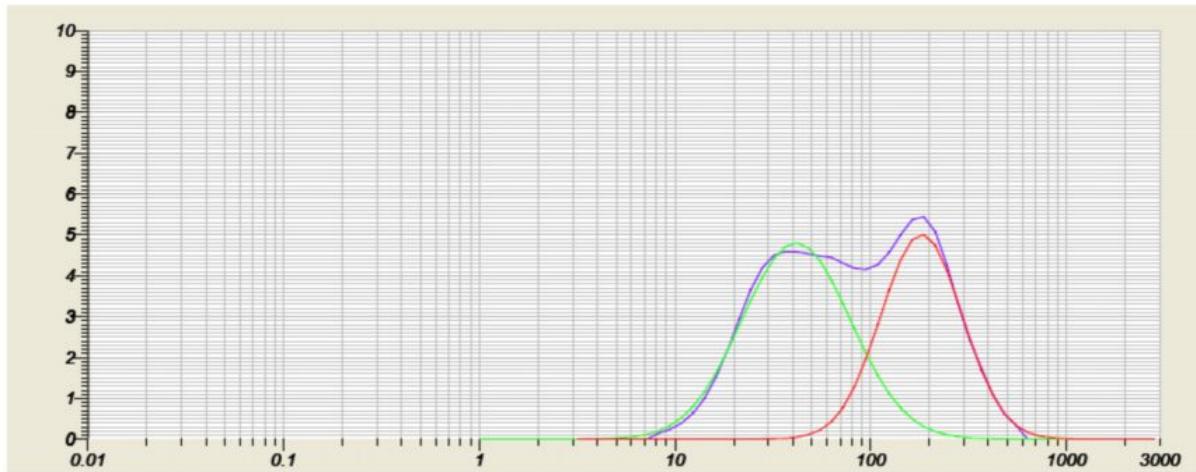
Probenname	Mehl	Median	1286.66248(µm)
ID#	201202141327373	Durchschnittswert	1344.10144(µm)
Datenbezeichnung	LF1000	Standardabweichung	487.5477(µm)
Transmission (R)	97.7(%)	Geometr. Mittelwert	1214.7972(µm)
Druckluft	0.1 MPa	Geometr. Standardabweichung	1.8099(µm)
Rinne	Auto	Modalwert	1258.7028(µm)
Auflösung	Manuell	Spanne	AUS
Anzahl Iterationen	15	x(Q)-Wert	(2)10.00 (%) - 803.9055(µm)
Art der Verteilung	Volumen		(9)90.00 (%) - 1986.7583(µm)
Brechungsindex (R)	Fraunhofer Kernel[Fraunhofer Diffraction	Spez. Oberfläche	91.619(cm²/cm³)
Material	WF		



Nr.	Durchmesser(µm)	q(%)	Summe: Q(r)(%)	Nr.	Durchmesser(µm)	q(%)	Summe: Q(r)(%)	Nr.	Durchmesser(µm)	q(%)	Summe: Q(r)(%)
1	1.005	0.000	0.000	26	29.907	0.165	1.245	51	890.116	5.862	14.400
2	1.151	0.000	0.000	27	34.255	0.129	1.373	52	1019.515	9.622	24.022
3	1.318	0.000	0.000	28	39.234	0.000	1.373	53	1167.725	14.019	38.040
4	1.610	0.000	0.000	29	44.938	0.000	1.373	54	1337.481	16.736	54.776
5	1.729	0.000	0.000	30	51.471	0.000	1.373	55	1531.914	15.784	70.560
6	1.981	0.000	0.000	31	58.953	0.000	1.373	56	1754.613	12.099	82.659
7	2.269	0.000	0.000	32	67.523	0.000	1.373	57	2009.687	8.019	90.678
8	2.609	0.000	0.000	33	77.339	0.000	1.373	58	2301.841	4.831	95.509
9	2.976	0.000	0.000	34	88.583	0.000	1.373	59	2636.487	2.836	98.345
10	3.409	0.000	0.000	35	101.460	0.000	1.373	60	3000.000	1.055	100.000
11	3.905	0.000	0.000	36	116.210	0.000	1.373				
12	4.472	0.000	0.000	37	133.103	0.000	1.373				
13	5.122	0.000	0.000	38	152.453	0.000	1.373				
14	5.867	0.000	0.000	39	174.616	0.000	1.373				
15	6.720	0.000	0.000	40	200.000	0.000	1.373				
16	7.697	0.000	0.000	41	229.075	0.000	1.373				
17	8.816	0.000	0.000	42	262.376	0.000	1.373				
18	10.097	0.000	0.000	43	300.518	0.000	1.373				
19	11.565	0.000	0.000	44	344.206	0.000	1.373				
20	13.246	0.117	0.117	45	394.244	0.000	1.373				
21	15.172	0.154	0.271	46	451.556	0.335	1.708				
22	17.377	0.188	0.459	47	517.200	0.579	2.287				
23	19.904	0.211	0.669	48	592.387	1.029	3.317				
24	22.797	0.214	0.884	49	678.504	1.868	5.184				
25	26.111	0.197	1.080	50	777.141	3.353	8.538				

HORIBA

Laser Scattering Particle Size Distribution Analyzer LA-950



	Statistics	Sample	Dist1	Dist2	Dist3	Residual
1	D50 (μm)	81.775	41.6057	182.089
2	D10 (μm)	23.6288	18.2715	98.4951
3	D90 (μm)	257.708	94.7588	336.292
4	Average (μm)	116.891	51.0685	204.044
5	Mode (μm)	186.178	41.9724	186.498
6	StdDev (μm)	100.563	36.3186	103.304
7	Span (μm)
8	AreaRatio(Sample)	...	0.56704	0.441894	...	0.0432612
9	AreaRatio(:Dist1)	1.78354	...	0.779299	...	0.078293
10	AreaRatio(:Dist2)	2.28299	1.28321	0.0978996
11	AreaRatio(:Dist3)
12	AreaRatio(Residual)	23.1154	13.1074	10.2145
13	CumOnPer_0.10 μm (%)	0	0	0
14	CumOnPer_0.50 μm (%)	0	0	0
15	CumOnPer_1.00 μm (%)	0	0	0
16	CumOnPer_5.00 μm (%)	0	0.0469989	0
17	CumOnPer_10.00 μm (%)	0.361791	1.29034	0
18	CumOnPer_50.00 μm (%)	34.0949	61.2892	0.348628
19	CumOnPer_100.00 μm (%)	56.1628	91.4868	10.4983
20	CumOnPer_500.00 μm (%)	99.5179	100	98.2358
21	CumOnPer_1000.00 μm (%)	100	100	99.9812
22	CumOnPer_0.00 μm (%)	0	0	0
23	PerOnCum_10.00 % (μm)	23.6288	18.2715	98.4951
24	PerOnCum_20.00 % (μm)	32.8667	24.2318	121.511
25	PerOnCum_30.00 % (μm)	44.2357	29.7544	141.471
26	PerOnCum_40.00 % (μm)	59.7824	35.3886	161.178
27	PerOnCum_60.00 % (μm)	113.032	48.9504	205.574
28	PerOnCum_70.00 % (μm)	150.63	58.191	234.007
29	PerOnCum_80.00 % (μm)	193.886	71.4286	272.872
30	PerOnCum_90.00 % (μm)	257.708	94.7588	336.292
31	PerOnCum_0.00 % (μm)	0.01	0.01	0.01
32	PerOnCum_0.00 % (μm)	0.01	0.01	0.01

ID# : 201202141326369
Name Ergebnisdatei : LF<C>
Probenname : Mehl
Transmission (R) : 96.4(%)
Rinne : Auto
Druckluft : 0.3 MPa
Art der Verteilung : Volumen
Brechungsindex (R) : Fraunhofer Kernel[Fraunhofer Diffraction Sample(0.000 - 0.000i)]
Anzahl Iterationen : 15
Datenaufnahme Probe (LD) : 23000
Algorithmus : Standard

Median : 81.77499(μm) D(v,0.1) : 23.62878(μm)
Durchschnittswert : 116.89052(μm) D(v,0.5) : 81.77499(μm)
Modalwert : 186.1763(μm) D(v,0.9) : 257.70752(μm)
Spanne : AUS

HORIBA

Laser Scattering Particle Size Distribution Analyzer LA-950

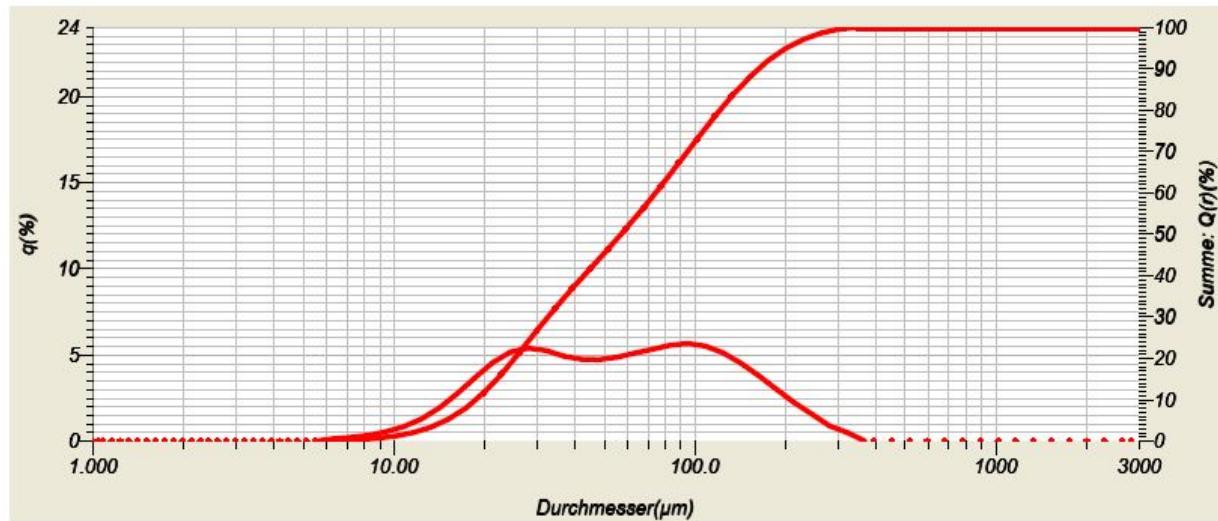
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 ID# : 201202141327367
 Datenbezeichnung : WF
 Transmission (R) : 96.3(%)
 Auflösung : Manuell
 Anzahl Iterationen : 15
 Art der Verteilung : Volumen
 Brechungsindex (R) : Fraunhofer Kernel[Fraunhofer Diffraction Sample(0.000 - 0.000i)]
 Material : WF

Median : 56.87588(µm)
 Durchschnittswert : 76.11115(µm)
 Standardabweichung : 60.9822(µm)
 Geometr. Mittelwert : 55.4021(µm)
 Geometr. Standardabweichung : 2.2764(µm)
 Modalwert : 94.7588(µm)
 Spanne : AUS
 x(Q)-Wert : (2)10.00 (%) - 18.7035(µm)
 : (9)90.00 (%) - 163.3784(µm)

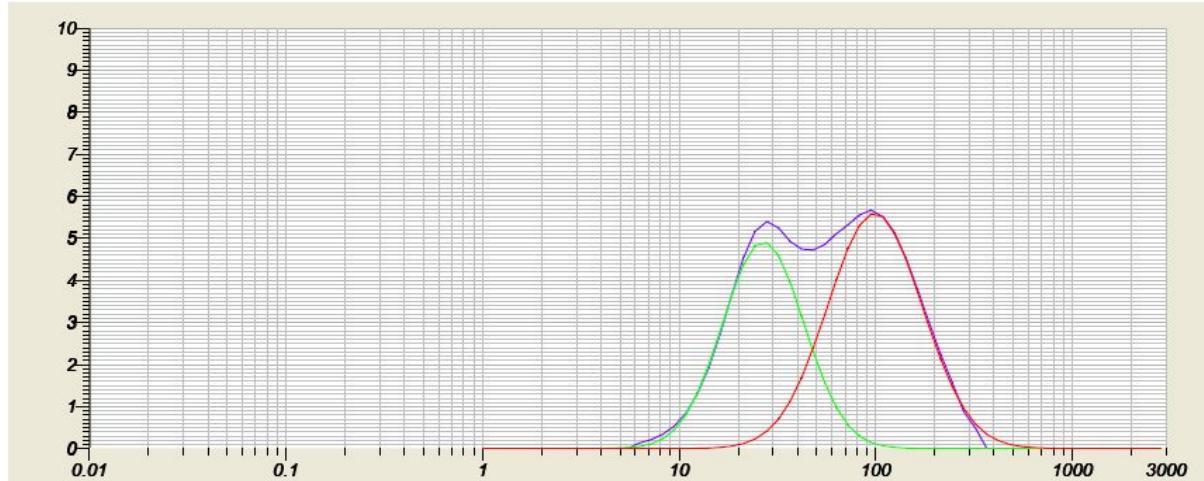
Peak # 1 (1.0000 - 51.4707 µm)
 Median : 27.33236(µm)
 Durchschnittswert : 28.51263(µm)
 x(Q)-Wert : (2)10.00 (%) - 14.5289(µm)
 : (9)90.00 (%) - 45.0374(µm)
 Modalwert : 27.9728(µm)
 Std. Abw. : 11.0291(µm)

Peak # 2 (51.4707 - 3000.0000 µm)
 Median : 102.20184(µm)
 Durchschnittswert : 117.36233(µm)
 x(Q)-Wert : (2)10.00 (%) - 59.7453(µm)
 : (9)90.00 (%) - 198.0715(µm)
 Modalwert : 94.7588(µm)
 Std. Abw. : 56.3132(µm)

Breiten/Längenverhältnis
 Peak 1:2 : 3.739



HORIBA Laser Scattering Particle Size Distribution Analyzer LA-950



	Statistics	Sample	Dist1	Dist2	Dist3	Residual
1	D50 (μm)	56.8769	26.8098	99.0222	---	---
2	D10 (μm)	18.7035	14.5281	48.5528	---	---
3	D90 (μm)	163.378	49.4603	201.119	---	---
4	Average (μm)	76.1112	30.0102	115.432	---	---
5	Mode (μm)	94.7588	27.8636	95.0448	---	---
6	StdDev (μm)	60.9822	15.1114	69.1186	---	---
7	Span (μm)	---	---	---	---	---
8	AreaRatio(Sample)	---	0.431802	0.571772	---	0.0295526
9	AreaRatio(:Dist1)	2.31695	---	1.32477	---	0.0884719
10	AreaRatio(:Dist2)	1.74895	0.754851	---	---	0.061698
11	AreaRatio(:Dist3)	---	---	---	---	---
12	AreaRatio(Residual)	33.838	14.6045	19.3476	---	---
13	CumOnPer 0.10 μm (%)	0	0	0	---	---
14	CumOnPer 0.50 μm (%)	0	0	0	---	---
15	CumOnPer 1.00 μm (%)	0	0	0	---	---
16	CumOnPer 5.00 μm (%)	0	0.0210068	0	---	---
17	CumOnPer 10.00 μm (%)	1.16556	1.89377	0.0016782	---	---
18	CumOnPer 50.00 μm (%)	45.42	90.4355	10.9035	---	---
19	CumOnPer 100.00 μm (%)	72.3133	99.7184	50.7059	---	---
20	CumOnPer 500.00 μm (%)	100	100	99.8217	---	---
21	CumOnPer 1000.00 μm (%)	100	100	100	---	---
22	CumOnPer 0.00 μm (%)	0	0	0	---	---
23	PerOnCum 10.00 % (μm)	18.7035	14.5281	48.5528	---	---
24	PerOnCum 20.00 % (μm)	25.1699	17.9384	62.0227	---	---
25	PerOnCum 30.00 % (μm)	32.4983	20.8611	73.9895	---	---
26	PerOnCum 40.00 % (μm)	42.77942	23.751	86.0348	---	---
27	PerOnCum 60.00 % (μm)	74.0471	30.2381	113.951	---	---
28	PerOnCum 70.00 % (μm)	94.6072	34.3727	132.335	---	---
29	PerOnCum 80.00 % (μm)	121.156	40.0185	158.056	---	---
30	PerOnCum 90.00 % (μm)	163.378	49.4603	201.119	---	---
31	PerOnCum 0.00 % (μm)	0.01	0.01	0.01	---	---
32	PerOnCum 0.00 % (μm)	0.01	0.01	0.01	---	---

ID# : 201202141327367
Name Ergebnisdatei : WF<C>
Probenname : Mehl
Transmission (R) : 96.3(%)
Rinne : Auto
Druckluft : 0.3 MPa
Art der Verteilung : Volumen
Brechungsindex (R) : Fraunhofer Kernel[Fraunhofer Diffraction Sample(0.000 - 0.000i)]
Anzahl Iterationen : 15
Datenaufnahme Probe (LD) : 25000
Algorithmus : Standard

Median : 56.87588(µm) D(v,0.1) : 18.70349(µm)
Durchschnittswert : 76.11115(µm) D(v,0.5) : 56.87588(µm)
Modalwert : 94.7588(µm) D(v,0.9) : 163.37837(µm)
Spanne : AUS