Supplementation of Gluten-Free Bread with Some Germinated Legumes Flour

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Abstract: Legume flours, due to their amino acid composition and fiber content are ideal ingredients for improving the nutritional value of gluten-free bread. In this study, the influence of the partial replacement of corn-rice flour by chickpea and sweet lupine flours on the quality characteristics of gluten-free bread was analyzed. The content of nutrients (protein, lipids, ash, dietary fiber and minerals content), amino acid composition, and antinutritional components (tannin, phytic acid and trypsin inhibitor's) were determined in control, germinated and dehulled chickpea and sweet lupine flours. Germination caused increase in crude protein, total dietary fiber, soluble dietary fiber, insoluble dietary fiber and amino acid contents of all the legume samples. Further increase in mentioned parameters was observed after dehulling the germinated legumes. Tannin, phytic acid and trypsin inhibitor's were reduced on germination and more reduction was observed in dehulled over germinated samples. Addition of chickpea or sweet lupine flour to corn-rice flour at 20% level somewhat retarded the increase in the rate of retrogradation (staling) of gluten-free bread. This point was considered very important because of the major economic losses that stale gluten-free bread may entail. The sensory evaluation data demonstrated that, the chickpea or sweet lupine flour can successfully replace corn-rice flour in gluten-free bread up to 20%.
[Yousif, M.R.G. and Safaa, M. Faid. **Supplementation of Gluten-Free Bread with Some Germinated Legumes Flour.** *J Am Sci* 2014;10(3):84-93]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 11

Key words: Coeliac disease - Gluten-free bread - Lupin - Chickpeas - Germination.

1. Introduction

Coeliac disease is a chronic disorder of the small intestine caused by exposure to gluten in the genetically predisposed individuals **(Laurin *et al.,* 2002 and Hamer, 2005)**. It is characterized by a strong immune response to certain amino acid sequences found in the prolamin fractions of wheat, barley, and rye **(Hill *et al.,* 2005)**, resulting in damage to the mucosa of the small intestine and leading to the malabsorption of nutrients, thus adversely affecting all systems of the body **(Feighery, 1999)**. Recently, with the development of sensitive serological tests, it has become possible to evaluate the true prevalence of coeliac disease. It is now regarded as one of the most common genetic diseases, occurring in 1 of 130–300 of the global population **(Fasano and Catassi, 2001 and Fasano *et al.,* 2003)**. The gluten-free diet remains until now the only treatment for coeliac disease **(Sabanis *et al.,* 2009)**. Gluten-free diet includes benefits such as the recovery of the villi of the small intestine and reduced risk of malignant complications **(Seraphin and Mobarhan, 2002)**. However there are growing concerns over the nutritional adequacy of the gluten-free dietary pattern because it is often characterized by an excessive consumption of energy, and a reduced intake of proteins and dietary fiber **(Thompson, 2001 and Thompson *et al.,* 2005)**.

Legumes have been known as "a poor man's meat". They supply protein, complex carbohydrates, fiber and essential vitamins and minerals to the diet, which are low in fat and sodium and contain no cholesterol **(Gomez *et al.,* 2008)**. 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 legumes may have important protective effects on risk for cardiovascular disease **(Anderson and Major, 2002)**. Moreover, legumes 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 fiber 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)**.

The addition of legume to cereal-based products could be a good alternative for increasing the intake of legumes. In addition, 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 **(Minarro *et al.,* 2012)**. Therefore, the combination of

grain with legume proteins would provide better overall essential amino acid balance, helping to combat the world protein calorie malnutrition problem **(Livingstone *et al.,* 1993)**.

Legumes play an important role in the agriculture and diet of many developing countries and are a major source of dietary nutrients for many people. However, their role appears to be limited because of several factors including low protein and starch digestibility **(Negi *et al.,* 2001)**, poor mineral bioavailability **(Kamchan *et al.,* 2004)** and high antinutritional factors **(Ramulu and Udayasekhara, 1997 and Das *et al.,* 1999)**. It has been reported that protein and thiamin **(Sattar *et al.,* 1989)**, mineral bioavailability **(Rao and Prabhavathi, 1982)** and protein and starch digestibility **(Preet and Punia, 2000)** increased, whereas phytic acid **(Sattar *et al.,* 1989 and Ayet *et al.,* 1997)** and tannin **(Savelkoul *et al.,* 1992 and Ayet *et al.,* 1997)** decreased during germination of legumes.

Germination appears to be an inexpensive and effective method of achieving desirable changes in nutritious crops, and germinated seeds have become a widely accepted food item. Germination is known to cause important changes in the biochemical, nutritional and sensory characteristics, and has been claimed to enhance the nutritive value of cereals and legumes. Germination can be considered as a procedure for improving legume digestibility and reducing flatulence properties, which are some of the factors that limit consumption **(Frias *et al.,* 1997)**.

Germinated Legumes are a good source of highly bioavailable proteins, starch, lipids and minerals. Additionally, germinated seeds contain significant amounts of polyphenols with well-documented pro-health properties **(Cevallos-Casals, and Cisneros-Zevallos, 2010)**.

The aim of the present study is to improve the quality and nutritional content of gluten-free bread by using by chickpea and sweet lupine flours into gluten-free formulation and examine the effects of its addition on the quality parameters of the baked end product. The results were compared to a control gluten-free bread formulation without added Legumes flours.

2. Material and Methods

Material:

Chickpea (*Cicer arietinum* L.) and sweet lupine (*Ceratonia siliqua* L.) seeds were obtained from local market. Yellow corn flour was obtained from Egyptian-Italian company for maize products (Maiza), 10th of Ramadan City, Cairo, Egypt. Rice flour was obtained from Sky Live Company for food industry, Giza, Egypt. Xanthan gum was obtained from Sigma chemical Co. (St. Louis, Mo), U.S.A.

Instant active dry yeast (*Saccharomyces cerevisiae*) processed by AKMAYA Co., Turkey, was obtained from the local market. Sugar (sucrose), sunflower oil, and salt were obtained from local market.

Methods:

Germination:

Chickpea (*Cicer arietinum* L.) and sweet lupine (*Ceratonia siliqua* L.) seeds were cleaned from all impurities including broken and diseased seeds, washed and soaked in 4–5 volumes of water (22–25˚C) for 12 h under ambient laboratory conditions. At the end of the period, the water was drained and the seed samples were allowed to germinate under a wet muslin cloth at 22-25˚C, 99% relative humidity and in the dark for 2 and 3 days. The maximum time of germination was fixed in accordance with achieving ~95% sprout seeds. The germination process was evaluated by the percentage of germinated seeds and the sprouted seed were collected and dried in an air dryer oven at 50±5 ˚C for 16–18 h. A portion of germinated samples were dehulled. Ungerminated seeds served as control. All the three samples, (1) control (ungerminated), (2) germinated and (3) dehulled (after germination) were milled to flour in a laboratory mill (3100, Perten Instruments, Sweden). Then, sieved through a 50-mesh screen. The resultant flour was packed in polyethylene bags and stored at (-18˚C) until used according to the method described by **(Fernandez-Orozco *et al.,* 2009)**.

Chemical analysis:

Crude protein, ash, crude fiber and lipid contents were estimated by standard AOAC methods **(AOAC, 2000)**. Total dietary fiber was determined according to **A.O.A.C (2000)**, soluble and insoluble dietary fiber contents were determined by following the enzymatic method **Prosky *et al.* (1988)**.

Mineral contents determined by wet acid-digested, using a nitric acid and perchloric acid mixture (HNO3: HClO4, 5:1 w/v) according to the method described by **Chapman and Pratt (1978)**. Then the total amounts of K, Na, Ca, Mg, Fe, Zn and Mn in the digested samples were determined were determined by atomic absorption spectrophotometer. Whereas phosphorus was determined by spectrophotometer according to the method of **Astm (1975)**.

Tannins was determined according to the method of **Hagerman (1987)**, phytic acid was determined according to the method of **Mohamed *et al.* (1986)**, and trypsin inhibitor's was determined according to the method of **Hamerstrand *et al.* (1981)**.

Amino acid analysis:

Protein hydrolyzate was prepared by treating 300 mg from each treatment with 6N HC1 in an evacuated test tube for 24 hrs at 105˚C. After

evaporation, the dried residue was dissolved in citrate buffer (pH 2.2). Aliquots were analyzed in an LKB Biochrome automatic amino acid analyzer using a buffer system as described by **Zarkdas *et al.* (1993)**. Methionine and cystine + cysteine were analyzed separately after performic acid oxidation and subsequent hydrolysis with HCl **(Khalil and Durani, 1990)**. Tryptophan was determined after alkali (NaOH) hydrolysis by a calorimetric method **(Freidman and Finely, 1971)**.

Rheological properties:

Rheological properties of the various blends were determined by Barbender Visco-Amylograph according to **A.A.C.C (2000)**.

Preparation of free-gluten bread:

Free-gluten bread was prepared according to the method described by **(Sabanis *et al.*, 2009)** with some modification. Preliminary baking was conducted evaluating the control bread formula which consisted of 225 g corn flour, 75 g rice flour, 4.5 g xanthan gum, 264 g water, 6 g dried yeast, 12 g sunflower oil, 12 g sucrose and 6 g of salt. In the trials dehulling germinated legumes flour were added at 10, 20 and 30 g/100 g of (corn, rice flour) weight, for the preparation of different bread samples. Bread doughs were prepared by mixing all ingredients in a 300 g farinograph bowl until they reached maximum development. The yeast was dissolved in warm water (35˚C) and the resulted solution was added to the dry ingredients and finally the oil was added with mixing process for 3 min. The resulted doughs were let to rest for 20 min at 28 – 30˚C then the doughs were sheeted to 2 mm thickness with the help of an aluminium platform. Circles cut of past pieces were done by using of templates with an outer diameter of 20 cm and baked directly at 350 – 400˚C for 40-60 seconds in a pilot plant oven (Food Technology Research Institute, Agricultural Research Center, Giza, Egypt). After baking, loaves were allowed to cool at room temperature before sealed in polyethylene bags to prevent moisture loss.

Evaluation of bread qualities:

Measurement of staling rate:

The staling rates of free-gluten bread were determined by alkaline water retention capacity (AWRC %) as described by **Kitterman and Rubanthaler (1971)**.

Organoleptic evaluation:

Fresh samples of free-gluten bread loaves were organoleptically evaluated according to **Twillman and White (1988)**. The fresh samples were delivered to the panelists within 1 hr after baking. Bread loaves were organoleptically evaluated for rollability, firmness, dryness, teste, odor, color and overall acceptability.

Statistical analysis:

Data were analysis by Analysis of Variance using General Liner Model (GLM) procedure according to the procedure reported by **Sendecor and Cochran (1997)**. Means were separated using Duncan's test at a degree of significance ($P \leq 0.05$). Statistical analyses were made using the producer of the SAS software system program **(SAS, 1997)**.

3. Results and Discussion

Chemical composition:

The proximate composition of different flour samples are presented in Table (1). The results revealed that, the sweet lupine flour was recorded the highest value of crude protein, lipids and crude fiber contents. In compared with, yellow corn, rice and chickpea flours. On the other hand, the highest value of nitrogen free extract (NFE) was recorded for rice flour. While, sweet lupine flour had the lowest NFE value. Such data are in the same line with those obtained by **Yousif (2003)**.

Also, the results in same table showed that crude protein in control legume flour samples ranged from 23.07 to 39.37 g/100 g. There was gradually increased in crude protein after germination and dehulling, respectively. Fat content of control seeds ranged from 5.55 g/100 g in chickpea flour to 8.06 g/100 g in sweet lupine flour. On germination, there was a decrease of fat content, which could be due to total solid loss during soaking prior to germination or use of fat as an energy source in sprouting process. The results are comparable with findings of **Venderstoep (1981) and Ghavidel and Prakash (2007)**. Crude protein and lipids levels were improved after dehulling due to removal of hull portion and concentration of endosperm. The highest nitrogen free extract (NFE) content of legume flour was recorded in chickpea flour (62.12 g/100 g) and the lowest in sweet lupine flour (36.45 g/100 g). These results were in agreement with those reported by **Venderstoep (1981) and Yousif (2003)**. Leaching out of solid matter during pre germination soaking process could be the reason for significant reduction of nitrogen free extract (NFE) content on germination. These results are in agreement with **Ghavidel and Prakash (2007), Kohajdová *et al.* (2011) and Maghaydah *et al.* (2013)**.

Dietary fiber content:

Concerning to the dietary fiber content, results presented in Table (2) showed that, sweet lupine flour contain the highest percentage of total dietary fiber (TDF), soluble dietary fiber (SDF), and insoluble dietary fiber (IDF) which amounted in 37.94, 12.40 and 25.54 g/100g dry basis, respectively. Followed by, 22.37, 2.46 and 19.91 g/100g dry basis for chickpea flour. In contrast, rice flour had the lowest amounts of TDF, SDF and IDF being 1.46, 0.00 and 1.46 g/100g dry basis, respectively. These results are in accordance

with those obtained by **Ghavidel and Prakash (2007)**. Also, there was gradually decreased in TDF, SDF and IDF after germination and dehulling, respectively. The results are comparable with findings of **Ghavidel and Prakash (2007) and Zielinska *et al*. (2008)**.

Table (1): Proximate composition of yellow corn, rice, chickpea and sweet lupine flours (% on dry weight basis).

Samples	*Chemical composition (%)				
	**Crude protein	Lipids	Ash	Crude fiber	Nitrogen free extract (NFE)
Yellow corn flour	8.89 ± 0.02	2.45 ± 0.02	1.07 ± 0.01	1.21 ± 0.01	86.38 ± 0.02
Rice flour	6.96 ± 0.04	0.46 ± 0.03	0.32 ± 0.02	0.22 ± 0.06	92.04 ± 0.04
Chickpea					
Raw	23.07 ± 0.02	5.55 ± 0.02	3.84 ± 0.16	5.42 ± 0.06	62.12 ± 0.14
Germinated	24.81 ± 0.03	5.45 ± 0.02	3.31 ± 0.01	5.04 ± 0.19	61.39 ± 0.32
Dehulled	27.24 ± 0.05	5.87 ± 0.04	2.99 ± 0.02	4.94 ± 0.03	58.96 ± 0.19
Sweet lupine					
Raw	39.37 ± 0.01	8.06 ± 0.10	2.20 ± 0.15	13.92 ± 0.11	36.45 ± 0.45
Germinated	41.60 ± 0.02	7.67 ± 0.07	2.11 ± 0.10	13.35 ± 0.02	35.27 ± 0.02
Dehulled	43.58 ± 0.06	8.72 ± 0.33	1.67 ± 0.14	12.41 ± 0.56	33.62 ± 0.01

*Means of triplicate ± SD.
** Yellow corn and rice flour (N×5.70) while, chickpea and sweet lupine flour (N×6.25).
NFE: Calculated by difference.

Table (2): Total, soluble, insoluble dietary fiber content of yellow corn, rice, chickpea and sweet lupine flours (% on dry weight basis).

Samples	Total dietary fiber (TDF)	Soluble dietary fiber (SDF)	Insoluble dietary fiber (IDF)
Yellow corn flour	10.9 ± 0.38	1.40 ± 0.06	9.50 ± 0.27
Rice flour	1.46 ± 0.14	0.00 ± 0.00	1.46 ± 0.14
Chickpea			
Raw	22.37 ± 0.06	2.46 ± 0.02	19.91 ± 0.04
Germinated	20.81 ± 0.03	1.24 ± 0.08	19.57 ± 0.08
Dehulled	16.23 ± 0.02	0.54 ± 0.01	15.69 ± 0.02
Sweet lupine			
Raw	37.94 ± 0.40	12.40 ± 0.11	25.54 ± 0.24
Germinated	34.16 ± 0.05	11.56 ± 0.02	22.60 ± 0.15
Dehulled	31.05 ± 0.09	9.78 ± 0.01	21.27 ± 0.32

*Means of triplicate ± SD.

Minerals content:

For the minerals content of yellow corn, rice, chickpea and sweet lupine flours, results presented in Table (3), it could be observed that, both of chickpea and sweet lupine flours recorded the highest value for all minerals under investigation than yellow corn and rice flours. These results are in agreement with those reported by **Venderstoep (1981) and Ghavidel and Prakash (2007)**. In addition, the results in same table showed that, there was gradually decreased found in K, Ca, Mg, Na, P, Mn, Fe and Zn contents on germination as present in Table (3). This is easily observable in the lower ash contents obtained in the germinated samples (Table 1). This reduction could be due to leaching of solid matter in soaking water. Further decline in the above mentioned minerals levels after dehulling were observed, which may be contributed to presence of these minerals in hull portion. These results are in agreement with **Giri *et al.* (1981), Das *et al.* (1999), Ghavidel and Prakash (2007) and Zielinska *et al.* (2008)**.

Amino acid composition:

The amino acid requirements are the logical yard-sticks by which protein quality can be measured, and the relative quantities of the various amino acids, in particular the essential amino acids, in the food could be used as reliable estimators of actual protein quality **(Alsmeyer *et al.*, 1974)**. Data presented in Table (4) shows the amino acid composition of raw, germinated and dehulled chickpea and sweet lupine flours. From the obtained data, it could be observed that, both of chickpea and sweet lupine flour protein was rich in essential amino acids such as lysine and isoleucine. Therefore, chickpea and sweet lupine protein could very well complement those protein sources that are low in lysine and tryptophan. The total amino acid in chickpea flour was 86.91 g/100g protein while it was 86.44 g/100g protein in sweet lupine flour.

Germination and dehulling caused a slight increase in total essential amino acids. These results are in good accordance with those reported by **Benchaar *et al.* (1994)**.

Table (3): Minerals content of yellow corn, rice, chickpea and sweet lupine flours (mg/100g on dry weight basis).

Samples	Potassium (K)	Calcium (Ca)	Magnesium (Mg)	Sodium (Na)	Phosphorus (P)	Manganese (Mn)	Iron (Fe)	Zinc (Zn)
Yellow corn flour	325.28	48.83	108.31	54.32	300.42	1.50	4.82	2.65
Rice flour	145.80	146.71	82.75	10.04	182.55	0.80	1.65	0.91
Chickpea								
Raw	3436.29	145.38	136.67	1185.25	491.00	1.64	4.63	3.15
Germinated	2648.81	128.30	125.89	977.44	455.70	1.29	4.06	2.36
Dehulled	2065.54	69.77	105.32	913.63	425.35	1.26	3.48	2.43
Sweet lupine								
Raw	987.39	232.41	172.55	174.35	315.78	1.98	4.10	3.89
Germinated	946.62	219.67	164.71	166.72	307.36	1.90	3.93	3.72
Dehulled	749.22	176.10	128.34	134.09	239.13	1.53	3.12	2.95

Table (4): Amino acid composition of chickpea and sweet lupine flour (g/100g protein)

Amino acids	Chickpea			Sweet lupine		
	Raw	Germinated	Dehulled	Raw	Germinated	Dehulled
Essential Amino Acid						
Lysine	5.04	5.28	5.95	4.57	4.60	5.43
Leucine	7.40	7.44	7.59	7.00	7.21	7.62
Phenyl alanin	4.42	4.61	4.85	2.40	3.57	3.70
Threonine	3.10	3.23	3.78	3.31	3.87	3.98
Isoleucine	4.16	4.18	4.40	3.36	4.00	4.10
Valine	3.55	3.60	3.98	3.34	4.14	4.27
Methionine	1.20	1.54	2.03	1.44	1.53	2.19
Cystine	0.80	0.99	1.42	1.02	1.10	1.40
Tryptophan	0.75	0.88	1.00	0.87	0.89	0.95
Histidine	2.42	2.55	2.61	3.00	3.39	3.45
Tyrosine	1.97	2.65	3.00	3.53	4.43	4.74
Total essential amino acid	34.81	36.95	40.61	33.84	38.73	41.83
Non-Essential Amino Acid						
Glutamic	17.10	17.33	17.51	16.94	17.27	17.50
Aspartic	12.34	13.18	13.86	10.73	10.96	11.35
Proline	4.35	4.75	4.87	4.60	4.78	4.87
Arginine	5.94	6.57	6.81	8.49	8.84	9.14
Glycine	3.70	4.01	4.20	4.16	4.52	4.85
Alanine	4.12	4.59	5.06	3.30	3.91	4.04
Serine	4.55	4.60	5.22	4.38	5.68	6.00
Total non-essential amino acid	52.10	55.03	57.53	52.60	55.96	58.12
Total amino acid	86.91	91.98	98.14	86.44	94.69	99.95

Antinutritional factors:

The antinutritional factors of raw, germinated and dehulled chickpea and sweet lupine flours are shown in Table (5). From the obtained data, it could be observed that, tannin levels in control samples ranged from 201.24 mg/100 g in chickpea flour to 335.73 mg/100 g in sweet lupine flour. Germination reduced the tannin contents of chickpea and sweet lupine flours as previously observed by **Savelkoul *et al.* (1992) and Ghavidel and Prakash (2007)**. Concerning to phytic acid content, it could be observed that, control samples contained considerable amounts of phytic acid being 258.55 and 235.40 mg/100 g for chickpea and sweet lupine flours, respectively. Also, there was gradually decreased in phytic acid content in germinated chickpea and sweet lupine flour samples. The decreased in phytic acid content during germination could be due to increase in phytase activity as reported by **Kyriakidis *et al.* (1998), Egli *et al.* (2002) and Ghavidel and Prakash (2007)**. After dehulling, there

was little phytic acid and tannin detectable in cotyledons, indicating that most of the phytic acid and tannin are present in seed coat. These results are in agreement with **Egli *et al.* (2002) and Ghavidel and Prakash (2007)**. Concerning the trypsin inhibitor activity, results presented in the same table show that, trypsin inhibitor activity was decreased by germination treatment. Similar results were obtained by **Khattak *et al.* (2007)**.

Table (5): Effect of germination and dehulling on antinutritional factors in legume flours (mg/100 g on dry weight basis).

Antinutrional factor	Chickpea			Sweet lupine		
	Raw	Germinated	Dehulled	Raw	Germinated	Dehulled
Tannins	201.24 ± 2.64	113.35 ± 1.58	60.75 ± 1.92	335.73 ± 3.28	181.89 ± 2.33	94.25 ± 0.98
Phytic acid	258.55 ± 1.20	121.43 ± 2.42	75.62 ± 1.74	235.40 ± 1.09	108.22 ± 1.46	73.06 ± 2.12
Trypsin inhibitor's content	21.80 ± 1.83	17.09 ± 2.11	12.41 ± 1.54	98.18 ± 3.59	72.41 ± 1.18	41.69 ± 3.34

*Means of triplicate ± SD.

Bread staling:

Fig. (1) Shows the staling of gluten- free bread samples. It could be observed that, there was a gradual decrease in AWRC% (low freshness) for all different bread samples during storage periods. The lower reduction in staling value (high freshness) was noticed in bread sample which prepared by partial replacement of corn-rice flour with 30% of sweet lupine and chickpea flours, respectively, since the AWRC% reduced from 426 to 242 for bread sample which contained 10% chickpea flour; from 471 to 299 for bread sample contained 20% chickpea flour and from 480 to 326 for bread sample contained 30% chickpea flour after 2 days in comparison to control sample, where its AWRC% reduced from 378 to 215 during the same storage period. These means that replacement of corn-rice flour by 10, 20 or 30% of chickpea or sweet lupine flours caused a considerable improvement in bread freshness in compared to control sample. This may be due to the high water binding capacity of legumes flour. The increased in moisture content of bread samples makes it more tenderness, improved in bread freshness and caused higher AWRC ration. These observations are in agreement with those obtained by **Maleki *et al.* (1980)** reported that bread with high moisture content was initially softer and retained softer up to three days of storage than did bread containing lower moisture. Also, **Rogers *et al.* (1988)** mentioned that, the higher water absorption level results in a softer crumb and a slower rate of bread firming. In the same trend, **Stauffer (2000)** mentioned that, increasing the moisture content of bread increases its shelf life by later the rate of bread firming.

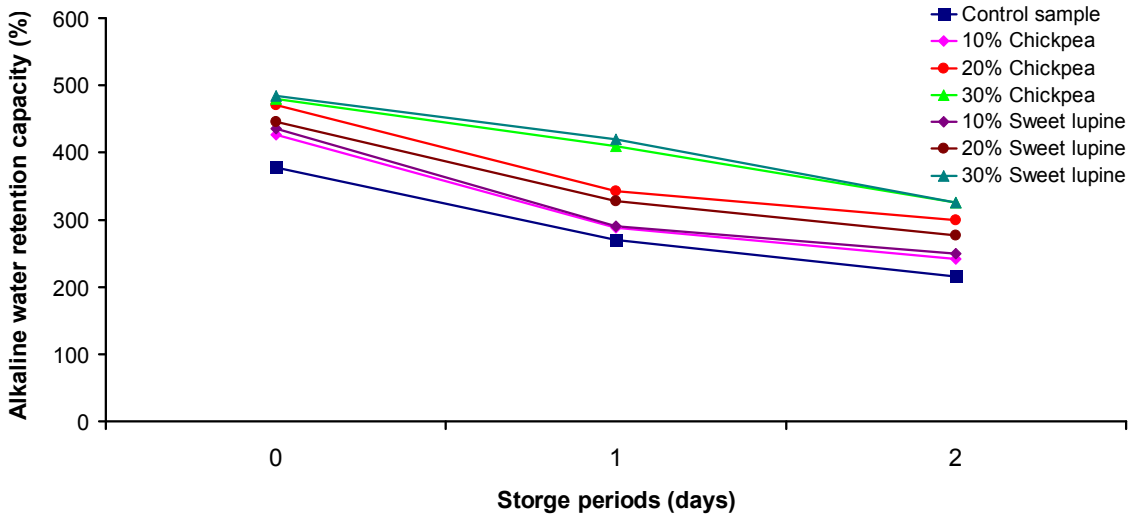

Fig. (1): Alkaline water retention capacity (AWRC %) of gluten-free bread prepared by partial replacement of corn-rice flour by chickpea and sweet lupine flours.

Rheological analysis:

From the results presented in Table (6). It could be observed that, with the increasing the level of replacement of corn-rice flour with chickpea or sweet lupine flour, the transition temperature was slightly increased from 67.5˚C for control sample to 69.0, 72.0 and 75.0˚C for 10, 20 and 30% of chickpea flour, respectively. Also, the transition temperature was recorded 70.5, 74.0 and 76.5˚C, respectively, for 10, 20 and 30% replacement with sweet lupine flour. In addition, the temperature at maximum viscosity was gradually increased with increasing the transition temperature. Concerning to the maximum viscosity, it was recorded 460, 400 and 360 B.U for 10, 20 and 30% of chickpea flour, respectively. In the same time, the maximum viscosity was gradually decreased with increasing the levels of replacement, it was recorded 430, 380 and 300 B.U for 10, 20 and 30% of sweet lupine flour, respectively. In compared with 520 B.U for control sample. Meanwhile, the viscosity at 95˚C was recorded 440, 390 and 350 B.U for 10, 20 and 30% of chickpea flour, respectively. In the same time, the maximum viscosity was gradually decreased with increasing the levels of replacement, it was recorded 420, 360 and 290 B.U for 10, 20 and 30% of sweet lupine flour, respectively. In compared with 500 B.U for control sample.

In addition, the viscosity at 50˚C was recorded 870, 770 and 590 B.U for 10, 20 and 30% of chickpea flour, respectively. In the same time, the maximum viscosity was gradually decreased with increasing the levels of replacement, it was recorded 810, 680 and 460 B.U for 10, 20 and 30% of sweet lupine flour, respectively. In compared with 960 B.U for control sample. Concerning to the set-back viscosity which reflected the degree of retrogradation of amylose. It could be observed that, the control sample had the highest set-back value being 440 B.U followed by 10 and 20% of chickpea and sweet lupine flour, respectively. While, 30% sweet lupine flour had the lower set-back value being 160 B.U.

Table (6):*Visco-Amylogram parameters of corn-rice flour and its blends with chickpea and sweet lupine flours.

Samples	Transition temperature (˚C)	Maximum viscosity (B.U)	Temperature at maximum viscosity (˚C)	Viscosity at 95˚C (B.U)	Viscosity at 50˚C (B.U)	Set-back (B.U)
Control sample	67.5	520	91.5	500	960	440
10% Chickpea	69.0	460	92.0	440	870	410
20% Chickpea	72.0	400	93.0	390	770	370
30% Chickpea	75.0	360	93.5	350	590	230
10% Sweet lupine	70.5	430	93.0	420	810	380
20% Sweet lupine	74.0	380	94.5	360	680	300
30% Sweet lupine	76.5	300	95.0	290	460	160

* on 50 g weight basis.
B.U: Barabender unit.

Sensory evaluation:

The effects of substitution of corn-rice flour with chickpea or sweet lupine flour on gluten-free bread sensory properties and overall acceptability score are presented in Table (7). The results in this Table showed that, there were no significant differences (p >0.05) in rollability and firmness of produced bread between the control sample and 10, 20% level of substitution with chickpea or sweet lupine flour. On the other hand, significant differences (p <0.05) in rollability and firmness between the control sample and 30% substitution level were recorded. Concerning the dryness, no significant difference (p >0.05) was recorded between control sample and bread sample which substituted with 10% chickpea or sweet lupine flour, but there were significant differences (p <0.05) between control sample and bread samples contained 20 and 30% substitution level. For taste, the obtained results indicated that there were significant differences (p <0.05) between control sample and bread sample which substituted with 20 and 30% substitution with chickpea or sweet lupine flour. On the other hand, there were no significant differences (p >0.05) between control sample and bread samples which substituted with 10 and 20% chickpea or sweet lupine flour for bread odor, but there was significant difference (p <0.05) with bread sample which substituted with 30% sweet lupine flour. Also, the obtained results indicated that, there were no significant differences (p >0.05) between control bread sample and all bread samples for bread color. The total scores values were a reflection of all the tested quality attributes and acceptability of the studied bread. These values were calculated from 100 as a sum of received sensory score. The results demonstrated that, the mean total score values of control bread sample which produced by using 100% corn-rice flour was higher than those of other samples and decreased gradually with non significant differences compared with control sample until 20% substitution level with chickpea or sweet lupine flour.

These results are in agreement with those obtained by **Maghaydah *et al.* (2013)**.

Table (7): Sensory evaluation of fresh gluten-free bread prepared by substitution of corn-rice flour with chickpea or sweet lupine flour.

Treatments	Score	Control sample	Chickpea flour			Sweet lupine flour		
			10	20	30	10	20	30
Rollability	**(10)**	9.5^{a}	9.2^{a}	8.8^{a}	7.3^{b}	9.0^{a}	8.7^{a}	7.0^{b}
Firmness	**(10)**	9.0^{a}	9.0^{a}	8.6^{a}	7.5^{b}	8.8^{a}	8.2^{a}	6.8^{b}
Dryness	**(10)**	9.2^{a}	8.8^{a}	7.5^{b}	6.8^{c}	8.6^{a}	7.3^{b}	6.5^{c}
Taste	**(20)**	19.2^{a}	19.0^{a}	18.2^{b}	16.5^{c}	19.0^{a}	17.8^{b}	16.0^{c}
Odor	**(20)**	19.5^{a}	19.2^{a}	19.0^{a}	19.0^{a}	19.3^{a}	19.0^{a}	18.7^{b}
Color	**(20)**	19.5^{a}	19.5^{a}	19.5^{a}	19.2^{a}	19.5^{a}	19.2^{a}	19.0^{a}
Overall acceptability	**(10)**	9.2^{a}	9.0^{a}	8.8^{a}	7.9^{b}	9.0^{a}	8.7^{a}	7.6^{b}
Total Score	**(100)**	95.1^{a}	93.7^{a}	90.4^{a}	84.2^{b}	93.2^{a}	88.9^{a}	81.6^{b}

* Means followed by different letters in the same column are significantly different by Duncan's multiple test ($p<0.05$).

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3/1/2014