



Comparison of the chemical profile of oil extracted from pistacia atlantica subspecies cabulica with pistacia atlantica subspecies mutica

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ABSTRACT

Regarding the increasing amount of edible oil consumption in the world, it is of great significance to take natural oil sources into account. Recently, the consumers' increasing tendency toward changing and improving nutritional diet has led to the development of new and special grain oils, and seeds containing useful fatty acids. Kasor oil (*Pistacia Atlantica* Subsp. *Cabulica*) is one of the vegetable oils enriched with essential fatty acids that can reduce our dependency and provide the healthy oil in our meal. Thus, in this research we intend to examine the estimated amount of oil, fatty acid composition and chemical features of oil extracted from Kasor and compare it with that of Bene (*Pistacia Atlantica* Subsp. *Mutica*). The findings showed that the values under study were different in all features. The following values were obtained from Kasor Oil and Bene Oil, respectively: Iodine value 82.31, 90.16, and Saponification Value 185.72, 194.54, and Peroxide Value 1.53, 2.2, and Anisidine Value 12.45, 15.41, and Totox Value 15.51, 19.81, and Unsaponifiable Matters 0.95, 0.6, and Oxadizibility Measure 2.78, 3.02. Kasor has more oil than Bene. Their fatty acid compositions were different. Kasor oil has higher saturated fatty acid and lower polyunsaturated fatty acid. Both oils are high in minerals and nutritionally very useful. Oleic and Linoleic acids are the dominant fatty acids in both oils and they both can be classified in Oleic-Linoleic acid group.

Keywords: Saponification Value, Anisidine Value, Totox Value, Peroxide Value

INTRODUCTION

Pistacia is the most important genus in the *Anacardiaceae* family which consists of at least eleven species (Whitehouse, 1957) divided into four sections according to leaf characters and nut morphology. Pistachio tree is native to Western Asia and Asia Minor, from Syria to the Caucasus and Afghanistan, forming pure stands at altitudes up to 1000 m (Hadj- Hassan, 2003). Maggs (1973) mentioned that the main Pistachio varieties in the world were taken and spread from Turkey, Iran and Syria. Yaltirik (1967) described *Pistacia* species in Turkey, and found a new species, *P. eurycarpa*, which grows in southeast Turkey, and it may be a hybrid between *P. khinjuk* and *P. atlantica*. On the other hand, Al-Yafi (1978) described a few *P. atlantica* subspecies on the basis of their leaf morphology and retained *P. eurycarpa* as variety of

P. atlantica. *Pistacia* species are very valuable crops for the Mediterranean and North African countries.

Since they are drought resistant plant they can be grown in regions where the annual precipitation is low. Although, *P. atlantica* is a tree with a dense canopy, it grows at altitudes ranging from 100 m to very high elevations (e.g. 3000 m altitude in eastern Algeria) as reported by Khaldi and Khouja. In addition, Muzher (2001) studied *P. atlantica* in the south of Syria and grouped the examined population into 4 genotypes.

Rechinger (1969) identified three pistachio species in Iran including *Pistacia Vera*, *Pistacia Atlantica* and *Pistacia Khinjuk*. These researchers also found three subspecies of *Pistacia Atlantica* that are *Mutica*, *Kurdica* and *Cabulica*. Wild pistachio (species *Pistacia Atlantica*) is a tree, 2-7 meters in height and its jungles are located in the west, east, south and center of Iran and more than 1,200,000 hectares of

Iran Jungles are allocated to it (Soleiman-Beigi and Arzehgar, 2013). Mutica and Kurdica subspecies typically grow from 900 to 2800 meters tall but Cabulica subspecies grows from 50 to 2500 meters tall and is more tolerant of bad weather compared to other two subspecies and their hybrid with Pistacia Vera can help its production conditions (Daneshrad and Aynechi, 1980). The estimated amount of Mutica and Kurdica oils and their fatty acid composition are reported but a comprehensive and complete study of the estimated amount of Cabulica oil and its fatty acid is not done yet, thus, in this research we intend to examine the estimated amount of oil, fatty acid composition and chemical features of oil extracted from Cabulica subspecies and compare it with that of mutica subspecies.

MATERIAL AND METHODS

Procurement of raw material

Kasor (variety of Cabulica) and Bene (variety of Mutica) were both collected from Sirjan heights in Kerman Province. The samples were stored in Polyethylene plasticsin refrigerator to be extracted and examined later. All of the solvents and chemicals with a degree of analytical were bought from Merk Co. Hexane solvent was used to extract oil.

Extraction of oil

After the species were collected, identified and their quality was confirmed, they were dried in shadow by a botanist and then ground in grinder. Oil extraction was done by soxhelt and with a weight/ volume ratio of ¼ with hexane solvent for 6 hours. The solvent was evaporated by a rotary evaporator in about 40°C. The extracted oil was kept in -16 degrees in dark dishes until the intended tests were performed.

Analysis of oil

Fatty Acids composition

Analytical gas chromatography was carried out using a ACME GC-6000 gas chromatography machine, manufactured by Young Lin, Korea, carrier gas Helium, split ratio 1:100, Helium flow rate 0.7 ml min⁻¹ and a flame ionization detector at 280°C. The injector temperature was 250°C and the column temperature was set constant at 198°C for 100 minutes. For analysis, FFAs were first methylated by adding 7 ml of hexane and 2 ml of 2M MeOH/KOH to 15 drops of sample, mixing for 30 seconds and

heating in water bath (50-55°C), while shaking the tube 2-3 times for 15 minutes and then centrifuged at 3000g for 5 minutes. The supernatant was collected and after dehydration by anhydrous Na₂SO₄, 0.6 µL of the sample was injected to the GC apparatus. For the determination of FFAs, the retention time of each sample was compared with standard methyl esters.

Measurement of Oxadizibility

Oxadizibility of oils was measured according to the percentage of C-18 unsaturated fatty acids and based on the following formula:

$$\text{Oxadizibility} = \frac{[(C18:1\%) + 10.3(C18:2\%) + 21.6(C18:3\%)]}{100}$$

in which, C18:1, C18:2 and C18:3 are Oleic, Linoleic and Linolenic acids respectively.

Iodine Value

Iodine value of oils was measured by AOCS method Cd 1-25 (Firestone D, 1993).

Saponification Value

Saponification value of oils was measured by AOCS method Cd 3-25 (Firestone D, 1993).

Anisidine Value

Anisidine value of oils was measured by AOCS method Cd 18-90 (Firestone D, 1993), (White, 1995).

Unsaponifiable Matters

Unsaponifiable matters of oils were measured by AOCS method Cd 6a-40 (Firestone D, 1993).

Peroxide Value

Peroxide value of oils was measured by AOCS method Cd8b-90 (Firestone D, 1993).

Metal Content Measurement

The metal content was measured by CTA 3000 atomic absorption spectrometer, manufactured by CHEM TECH, England.

RESULTS AND DISCUSSION

Fatty Acid profile

Kasor oil extraction results indicated that the amount of oil was 65 % and it had more oil compared to Bene (extracted oil was 50%). Kasor and Bene fatty acid structures are shown in table 1. The oil extracted from

both grains, contained fatty acids common in vegetable oils such as Palmitic, Palmitoleic, Stearic, Oleic, Linoleic, and Linolenic acids. The overall saturated fatty acids of Kasor and Bene (SFA) were 19.47 and 16.94 respectively. The overall unsaturated fatty acids (USFA) for Kasor and Bene were 80.44 and 82.89 respectively. Also, the amount of monounsaturated fatty acids (MUFA) were 60.18 and 59.68, and polyunsaturated fatty acids (PUFA) were 20.26 and 23.21 respectively. USFA to SFA ratios, normally considered as a criterion for oil and fat unsaturation as well as their tendency to lipid oxidizibility (Matthaus B, 2006), for Kasor and Bene were 4.13 and 4.89 respectively. MUFA to SFA ratios were 3.09 and 3.52 and PUFA to SFA ratios were 1.04 and 1.37 respectively. MUFA to PUFA ratios were 2.97 and 2.57 respectively. Higher ratio shows that oil has more oxidation stability and is more suitable for frying process (Table 2). Therefore, Kasor oil has more oxidation stability.

Regarding that Oleic and Linoleic acids comprise more than 70% of fatty acid composition of these oils and the small amount of Linolenic acid (1.1% for Kasor and 0.62 for Bene), these oils can be classified in Oleic-linoleic oil group. Oleic and Linoleic fatty acids are essential for human body (essential fatty acids), therefore, considering their high amount in Kasor and Bene, it can be said that these oils are high in nutritional value and their low amount of Linolenic acid shows that they have more oxidation stability than Oleic-Linolenic oil group.

Saponification Value

Saponification value represents the number of milligrams of Potassium Hydroxide required to saponify 1g of fat under study. This measure can be used to identify oils. In fact, this index shows the chain length of fatty acids of the sample to be tested. In other words, the longer the chain of fatty acids is, the lower the Saponification value of oil is. The Saponification value of Kasor and Bene are 185.72 and 194.54 milligrams of Potassium Hydroxide in 1g of oil, respectively, indicating that their Saponification values were statistically different. The Saponification value of Kasor oil was lower than that of Bene oil. It indicates more molecular weight of Kasor oil fatty acids than that of Bene.

Table 1. Fatty acid composition of kasor and bene oils

Fatty Acids	Kasor Oil	Bene Oil
C14:0	0.067	0.072
C16:0	16.73	13.67
C16:1	3.70	4.12
C17:0	0.07	0.07
C18:0	2.47	2.78
C18:1	56.20	55.25
C18:2	19.11	22.61
C18:3	1.14	0.62
C20:0	-	0.23
C20:1	0.26	0.36
C22:1	0.01	0.01
C24:0	0.04	0.14

Table 2. Fatty acids comparison of Kasor and Bene Oils

Bene Oil	Kasor Oil	Fatty Acids
USFA	80.44	82.89
SFA	19.47	16.94
MUFA	60.18	59.68
PUFA	20.26	23.21
USFA/SFA	4.13	4.89
PUFA/SFA	1.04	1.37
MUFA/SFA	3.09	3.52
MUFA/PUFA	2.97	2.57

Iodine Value

The Iodine value is the mass of Iodine in grams that is consumed by 100 grams of oil or fat. It shows the amount of unsaturation in oils and fats. The iodine value of edible oils has a wide range; in which coconut oil is the most saturated one with Iodine value of 6-11 grams of Iodine molecules per 100

grams of oil and the most unsaturated one is fish oil with Iodine value of 142-176 grams of Iodine molecules per 100 grams of oil [10]. The higher the Iodine value is, the more the oxidative rancidity of oil will be. The Iodine value of Kasor and Bene oils were 82.31 and 90.16 grams of Iodine molecules per 100 grams of oil, indicating that statistically, their Iodine values were different and obviously, the Iodine value of Bene oil is higher than that of Kasor oil due to higher amount of Linoleic fatty acids (22.61 versus 19.11) and lower amount of saturated fatty acids (16.94 versus 19.47) in Bene oil compared to Kasor oil.

Peroxide Value

The primary products of oxidation of lipids are Hydroperoxides which are usually considered as Peroxides. Therefore, it is reasonable to determine the concentration of these peroxides as a measure for oxidation amount. Hydroperoxides do not have any odor and flavor but they are decomposed very fast and produce Aldehydes which have strong bad flavor. Peroxide value is somehow related to the bad flavor imparted by aldehydes and other products of oxidation.

Peroxide value of fat is a measure for its active Oxygen content and it is measured by millimoles of Peroxide or milliequivalent of Oxygen per 1000 grams of fat. The amount of Peroxide in Kasor and Bene oils are 1.53 and 2.2 milliequivalent gram per kilogram, statistically indicating a significant difference between peroxide values of two oils and peroxide value of Kasor oil was lower than that of Bene oil, which shows its suitable Oxidation stability, possibly due to its high unsaponifiable matters that have antioxidant properties and its higher saturated fatty acid.

Anisidine value

Anisidine value measures the amount of Aldehydes (mostly 2-Alkenals and 2, 4-Alkadienals) created by the oxidation of Peroxides. This test was performed based on P-methoxyaniline (Anisidine) color reaction and Aldehydic compounds. This number is normally defined as the optical absorption resulting from 1 gram oil solution reaction with 100 milliliters solution of solvent and reagent which is measured by a wavelength of 350 nm in a 10 mm cell. Anisidine value of Kasor and Bene oils were 12.45 and 15.41 respectively, statistically indicating a difference

between their Anisidine values and Anisidine value of Kasor oil is lower than that of Bene oil. This test is more sensitive to unsaturated aldehydes than saturated ones because the products resulting from unsaturated aldehydes have a lot more absorbance in a wavelength of 350 nm (Shahidi, 2005), thus, as polyunsaturated Aldehydes in Kasor oil are more than polyunsaturated Aldehydes in Bene oil (23.21% versus 20.26%), Anisidine value is higher in Bene oil.

Unsaponifiable Matters

Unsaponifiable matters are the non-glyceride components of oils and fats that are soluble in oil. The amount of these compositions in vegetable oils is more than animal oils. These compositions can't be saponified by alkali. They include Sterols, Tocopherols, Pigments, Polyphenolic compounds etc. Plant Sterols known as Phytosterols, comprise more than 50% of Unsaponifiable matters. Extensive studies have been done on the antioxidant activity of Sterols and they showed that these compounds have a potential antioxidant effect. Tocopherols are the essential components of Unsaponifiable matters of edible oils that have antioxidant activity and are important for human health as vitamin E. Phenolic compounds are a big group of natural plant materials including Flavonoids, Tanins, Antosianins etc., and are found in all parts of a plant. These compounds have a lot of biological features including antioxidant, antimicrobial and anti-inflammatory effects. These compounds play a crucial role in oil stability and preserving human health (Raghavendra and Vijayananda, 2010). As a result, the amount of Unsaponifiable matters is considered as a criterion for oil quality and the more the Unsaponifiable matters of oil are, the better its quality and durability are. The Unsaponifiable values for Kasor and Bene oils are 0.95% and 0.60% respectively that statistically showed a significant difference between unsaponifiable matters of two oils. Unsaponifiable matters of Kasor oil were about 1.6 times more than those of Bene oil that may suggest the suitable stability of this oil and its benefits for human health.

Metal Content

Kasor and Bene oils are high in minerals especially iron. Metal content is shown in table 4. It is noticeable that the amount of iron in Kasor oil is about 5.5 times more than that of Bene oil.

Table 3. Comparison of chemical features of Kasor and Bene Oils

Chemical Test	Kasor Oil	Bene Oil
Saponification Value (mg KOH/mg Oil)	185.72	194.54
Iodine Value (g of I ₂ /100 g)	82.31	90.16
Peroxide Value (meq O ₂ /Kg Oil)	1.53	2.2
Anisidine Value(p-AV)	12.45	15.41
Totox Value	15.51	19.81
Unsaponifiable Matters(%)	0.95	0.6
Oxadizibility Measure	2.78	3.02

Table 4. Mineral profile of Kasor and Bene Oils (mg/Kg oil)

Mineral	Kasor Oil	Bene Oil
Fe	382	69
Pb	26	14
Cu	35	16
As	—	—
Ni	61	—

CONCLUSION

Fruits and grains are essential oil sources for food, industrial and medicinal consumption. Therefore, it is important to research on the new oil sources. As people's knowledge has developed, they have more tendencies towards oils that are healthy in addition to providing energy and flavor. In this research, the chemical structure of Kasor oil was studied according to fatty acid structure, Saponification value, Iodine value, Peroxide value, Anisidine value, Totox value, Unsaponifiable matters, Oxadizibility measure and minerals content. The results showed that Kasor has

more oil than Bene and is more suitable for oil extraction. Their fatty acid compositions were different. Kasor has higher saturated fatty acid and lower polyunsaturated fats. Furthermore, Kasor oil contains more unsaponifiable matters (Sterols, Tocopherols, and Polyphenolic compounds which have significant antioxidant features), therefore, it has more oxidation stability. Both oils have a high amount of minerals particularly iron (Kasor iron is 5 times more than that of Bene) and are nutritionally very useful. Regarding the amount of Oleic and Linoleic fatty acids which comprises more than 70% of fatty acid compositions of these oils and the small amount of Linolenic acid (1.1% for Kasor and 0.62% for Bene), these oils can be classified as Oleic-Linoleic oils. Oleic and Linoleic fatty acids are essential for human body (essential fatty acids), thus, their high amount in Kasor and Bene oils suggests that they have high nutritional value and their small amount of Linolenic acid indicates more oxidation stability compared to Oleic-Linolenic oils.

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Nutritional quality and health benefits of okra (*Abelmoschus esculentus*): A Review

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ABSTRACT

Okra (*Abelmoschus esculentus*) is an economically important vegetable crop grown in tropical and sub-tropical parts of the world. This paper was aimed to review nutritional quality and potential health benefits of edible parts of Okra. Okra is a multipurpose crop due to its various uses of the fresh leaves, buds, flowers, pods, stems and seeds. Okra immature fruits, which are consumed as vegetables, can be used in salads, soups and stews, fresh or dried, fried or boiled. It offers mucilaginous consistency after cooking. Often the extract obtained from the fruit is added to different recipes like stews and sauces to increase the consistency. Okra mucilage has medicinal applications when used as a plasma replacement or blood volume expander. The mucilage of okra binds cholesterol and bile acid carrying toxins dumped into it by the liver. Okra seeds are a potential source of oil, with concentrations varying from 20% to 40%, which consists of linoleic acid up to 47.4%. Okra seed oil is also a rich source of linoleic acid, a polyunsaturated fatty acid essential for human nutrition. Okra has been called “a perfect villager’s vegetable” because of its robust nature, dietary fiber, and distinct seed protein balance of both lysine and tryptophan amino acids. The amino acid composition of okra seed protein is comparable to that of soybean and the protein efficiency ratio is higher than that of soybean and the amino acid pattern of the protein renders it an adequate supplement to legume or cereal based diets. Okra seed is known to be rich in high quality protein especially with regards to its content of essential amino acids relative to other plant protein sources. Okra is a powerhouse of valuable nutrients, nearly half of which is soluble fibre in the form of gums and pectins which help to lower serum cholesterol, reducing the risk of heart diseases. The other fraction of Okra is insoluble fibre, which helps to keep the intestinal tract healthy. Okra is also abundant with several carbohydrates, minerals and vitamins, which plays a vital role in human diet and health. Okra is rich in phenolic compounds with important biological properties like quartering and flavonol derivatives, catechin oligomers and hydroxycinnamic derivatives. Okra is also known for being high in antioxidants activity. Okra has several potential health beneficial effects on some of the important human diseases like cardiovascular disease, type 2 diabetes, digestive diseases and some cancers. Overall, Okra is an important vegetable crop with a diverse array of nutritional quality and potential health benefits.

Key words: Okra, Nutritional, quality, Health, Edible, oil

Introduction

Okra (*Abelmoschus esculentus*) is one of the most widely known and utilized species of the family Malvaceae (Naveed *et al.*, 2009) and an economically important vegetable crop grown in tropical and sub-tropical parts of the world (Oyelade *et al.*, 2003;

Andras *et al.*, 2005; Saifullah and Rabbani, 2009). This crop is one of the most widely known and utilized species of the family Malvaceae (Naveed *et al.*, 2009). Okra plant was previously included in the genus *Hibiscus*. Later, it was designated to *Abelmoschus*,

which is distinguished from the genus *Hibiscus* (Aladele *et al.*, 2008)

Okra originated in Ethiopia (Sathish and Eswar, 2013) and was then propagated in North Africa, in the Mediterranean, in Arabia and India by the 12th century BC (Nzikou *et al.*, 2006). Considering the little contact between Ethiopia and the rest of the world within historic times, it is not surprising that little is known about the early history and distribution of okra. The routes by which okra was taken from Ethiopia to North Africa, the eastern Mediterranean, Arabia, and India, and when, are by no means certain (Tindall, 1983).

Okra is known by many local names in different parts of the world. It is called lady's finger in England, gumbo in the United States of America, guino-gombo in Spanish, guibeiro in Portuguese and bhindi in India (Ndunguru and Rajabu, 2004; Sorapong, 2012). In its origin of Ethiopia it is also called Kenkase (Berta), Andeha (Gumuz), Bamia (Oromica/Amharic). The name Okra probably derives from one of Niger-Congo group of languages (the name for okra in the Twi language is *nkuruma*) (Benjawan *et al.*, 2007). The term okra was in the use of English by the late 18th century (Arapitsas, 2008).

Okra is suitable for cultivation as a garden crop as well as on large commercial farms (Rubatzky and Yamaguchi, 1997). Okra plants are grown commercially in many countries such as India, Japan, Turkey, Iran, western Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Myanmar, Malaysia, Thailand, India, Brazil, Ethiopia & Cyprus and in the Southern United States (Qhureshi, 2007).

Okra is a multipurpose crop due to its various uses of the fresh leaves, buds, flowers, pods, stems and seeds (Mihretu *et al.*, 2014). Okra immature fruits (green seed pods), which are consumed as vegetables, can be used in salads, soups and stews, fresh or dried, fried or boiled (Ndunguru and Rajabu, 2004). It offers mucilaginous consistency after cooking. Often the extract obtained from the fruit is added to different recipes like soups, stews and sauces to increase the consistency. Okra mucilage has medicinal applications when used as a plasma replacement or blood volume expander. The mucilage of okra binds cholesterol and bile acid carrying toxins dumped into it by the liver. The immature pods are also used in making pickle. The entire plant is edible and is used to have several food (Madison, 2008; Maramag, 2013).

Okra seeds are source of oil and protein. Okra seeds have been used on a small scale for oil production. It

can be also used as non-caffeinated substitute for coffee. Okra seeds may be roasted and ground to form a caffeine-free substitute for coffee (Calisir and Yildiz, 2005). Okra also has industrial applications and is used in confectionary (Adetuyi *et al.*, 2011). To promote the use of indigenous vegetables like Okra that have play significant role in mitigate food insecurity and alleviate malnutrition in the country. However, Okra has been considered a minor crop and no attention was paid to its improvement in the international research program in past (Sanjeet *et al.*, 2010).

On the other hand, the demand for vegetable oils is rapidly increasing due to the growing human population and the expanding oil industry with health promoting oil components, the exploration of some underutilized and newer resources of vegetable oils is of much concern (Schalau, 2002). Okra, which is currently grown mainly as a vegetable crop, has potential for cultivation as an essential oilseed crop because okra seeds contain high amount of oil (20-40%) (Sorapong, 2012). However, there is also no comprehensive literature information regarding characteristics of the oils produced from Okra seeds. Therefore, this review was aimed to assess literature regarding the nutritional quality and potential health benefits of edible parts of Okra (*Abelmoschus esculentus*) vegetable. The oil compositions of okra seed was also discussed in order to provide further reliable information about health promoting oil components of Okra seeds.

Nutritional Composition of Okra

Okra is more a diet food than staple and its seeds have been used on a small scale for oil production. Lipid components greatly contribute to the nutritional and sensory value of almost all types of foods. Nature provides a large number of fats that differ in their chemical and functional properties. Four classes of lipids are habitually found in vegetable oils: triacylglycerols, diacylglycerols, polar lipids, and free fatty acids. The fatty acid composition determines the physical properties, stability, and nutritional value of lipids. The most naturally occurring storage lipids are triacylglycerols. Triacylglycerols are natural compounds that consist of saturated and unsaturated fatty acids that differ in the length of their acyl chains and the number and positions of double bonds: saturated, monoenoic, and polyunsaturated fatty acids that differ with respect to detailed fatty acid composition. Monoenoic fatty acids and polyunsaturated fatty acids are structurally distinguished by the presence of repeating methylene units. These units produce an extremely flexible chain that rapidly reorients through conformational states

and constitutes an influential group of molecules that promote health (Vermerris and Nicholson, 2006). Okra seeds from Greece are a potential source of oil, with concentrations varying from 20% to 40% (Sorapong, 2012; Sanjeet *et al.*, 2010), depending on the extraction method. The oil mainly consists of linoleic acid (up to 47.4%) (Andras *et al.*, 2005). Okra seed oil is a rich source of linoleic acid, a polyunsaturated fatty acid essential for human nutrition (Savello *et al.*, 1980).

Proteins play a particularly important role in human nutrition. The amino acid contents, proportions, and their digestibility by humans characterize a protein's biological value (Ewa *et al.*, 2011). Okra has been called "a perfect villager's vegetable" because of its robust nature, dietary fiber, and distinct seed protein balance of both lysine and tryptophan amino acids (unlike the proteins of cereals and pulses) (Sanjeet *et al.*, 2010; Holser and Bost, 2004). The amino acid composition of okra seed protein is comparable to that of soybean and the PER is higher than that of soybean (Adetuyi *et al.*, 2012), and the amino acid pattern of the protein renders it an adequate supplement to legume or cereal based diets (Ndangui *et al.*, 2010). Okra seed is known to be rich in high quality protein especially with regards to its content of essential amino acids relative to other plant protein sources (Oyelade *et al.*, 2003) Hence, it plays a vital role in the human diet (Farinde *et al.*, 2007).

Okra also contains carbohydrates and vitamins (Arapitsas, 2008; Owolarafe and Shotonde, 2004; Gopalan *et al.*, 2007), and plays a vital role in human diet (Saifullah and Rabbani, 2009; Kahlon *et al.*, 2007). Consumption of young immature okra pods is important as fresh fruits, and it can be consumed in different forms (Ndunguru and Rajabu, 2004). Fruits can be boiled, fried or cooked (Akintoye *et al.*, 2011). The composition of okra pods per 100 g edible portion (81% of the product as purchased, ends trimmed) is: water 88.6 g, energy 144.00 kJ (36 kcal), protein 2.10 g, carbohydrate 8.20 g, fat 0.20 g, fibre 1.70 g, Ca 84.00 mg, P 90.00 mg, Fe 1.20 mg, β -carotene 185.00 μ g, riboflavin 0.08 mg, thiamin 0.04 mg, niacin 0.60 mg, ascorbic acid 47.00 mg.

The composition of okra leaves per 100 g edible portion is: water 81.50 g, energy 235.00 kJ (56.00 kcal), protein 4.40 g, fat 0.60 g, carbohydrate 11.30 g, fibre 2.10 g, Ca 532.00 mg, P 70.00 mg, Fe 0.70 mg, ascorbic acid 59.00 mg, β -carotene 385.00 μ g, thiamin 0.25 mg, riboflavin 2.80 mg, niacin 0.20 mg (Gopalan *et al.*, 2007; Varmudy, 2011). Carbohydrates are mainly present in the form of mucilage (Liu *et al.*, 2005; Kumar *et al.*, 2009). That

of young fruits consists of long chain molecules with a molecular weight of about 170,000 made up of sugar units and amino acids. The main components are galactose (25%), rhamnose (22%), galacturonic acid (27%) and amino acids (11%). The mucilage is highly soluble in water. Its solution in water has an intrinsic viscosity value of about 30%.

Potassium, Sodium, Magnesium and Calcium are the principal elements in pods, which contain about 17% seeds. Presence of Iron, Zinc, Manganese and Nickel also has been reported (Moyin-Jesu, 2007). Fresh pods are low in calories (20 per 100 g), practically no fat, high in fiber, and have several valuable nutrients, including about 30% of the recommended levels of vitamin C (16 to 29 mg), 10 to 20% of folate (46 to 88 mg) and about 5% of vitamin A (14 to 20 RAE). Both pod skin (mesocarp) and seeds are excellent source of zinc @80 mg/g (Cook *et al.*, 2000).

Okra seed is mainly composed of oligomeric catechins (2.5 mg/g of seeds) and flavonol derivatives (3.4 mg/g of seeds), while the mesocarp is mainly composed of hydroxycinnamic and quercetin derivatives (0.2 and 0.3 mg/g of skins). Pods and seeds are rich in phenolic compounds with important biological properties like quatering derivatives, catechin oligomers and hydroxycinnamic derivatives (Arapitsas, 2008). These properties, along with the high content of carbohydrates, proteins, glycol-protein, and other dietary elements enhance the importance of this foodstuff in the human diet (Arapitsas, 2008; Manach *et al.*, 2005).

Dried okra sauce (pods mixed with other ingredients and regularly consumed in West Africa) does not provide any beta carotene (vitamin A) or retinol (Avallone *et al.*, 2008). However, fresh okra pods are the most important vegetable source of viscous fiber, an important dietary component to lower cholesterol (Kendall and Jenkins, 2004). Seven-days-old fresh okra pods have the highest concentration of nutrients (Agbo *et al.*, 2008).

Seed as source of edible oil and flour

Okra seeds contain about 20 to 40% oil (Sorapong B. 2012). The bark fibre is easy to extract. It is white to yellow in colour, strong but rather coarse. Tests conducted in China suggest that an alcohol extract of okra leaves can eliminate oxygen free radicals, alleviate renal tubular-interstitial diseases, reduce proteinuria, and improve renal function ((Liu *et al.*, 2005; Kumar *et al.*, 2009). Okra seed can be dried, and the dried seeds are a nutritious material that can be used to prepare vegetable curds, or roasted and ground

to be used as coffee additive or substitute (Agbo *et al.*, 2008).

Okra seed oil yield is comparable to most oil seed crops except oil palm and soybean (Sanjeet *et al.*, 2010). Moreover, okra seed oil has potential hypocholesterolemic effect. The potential for wide cultivation of okra for edible oil as well as for cake is very high (Sanjeet *et al.*, 2010). Okra seed flour could also be used to fortify cereal flour (Adelakun *et al.*, 2008). For example, supplementing maize ogi with okra meal increases protein, ash, oil and fiber content (Akingbala *et al.*, 2003). Okra seed flour has been used to supplement corn flour for a very long time in countries like Egypt to make better quality dough. However, long-term rodent/animal feeding trials would be pertinent before making final recommendations for wider consumption of okra seed flour (Sanjeet *et al.*, 2010).

The enormous nutritional and other biological activities in the pods and seeds were reported by Agbo *et al.*, 2008; Arapitsas, 2008; Kumar *et al.*, 2009). The okra pods were reported to have viscous fiber and lower cholesterol content (Kendall and Jenkins, 2004; Kumar *et al.*, 2010). Okra seeds were determined to have appreciable protein content according to (Akingbala *et al.*, 2003). The variations in polysaccharides found in the mucilage are higher in okra pods according to (Hirose *et al.*, 2004; Sengkhampan *et al.*, 2009).

Green vegetables contain valuable chlorophyll (Ebermann *et al.*, 2006). Chlorophyllin as an important component of chlorophyll was reported for enormous health benefits. The physiological and biochemical activities of phenolic compounds as antioxidant, anti-inflammatory and anti-microbial were also reported by (Ali and Deokule, 2008; Manach *et al.*, 2005; Middleton, 2000; Marinova *et al.*, 2005) proved the higher values of phenolic and flavonoid values, ratios and distributions in some Bulgarian vegetables and fruits. Generally, fruits and vegetables have shown the basic useful properties especially in providing an excellent health and nutritional qualities in the area of prevention and delay in the onset of chronic diseases and the provision of vitamins and enzymes necessary for proper body function (Aman *et al.*, 2005).

Mucilage and its potential

Okra mucilage refers to the thick and slimy substance found in fresh as well as dried pods. Mucilaginous substances are usually concentrated in the pod walls

and are chemically acidic polysaccharides associated with proteins and minerals (Woolfe *et al.*, 1977). Although nature of the polysaccharides varies greatly, neutral sugars rhamnose, galactose and galacturonic acid have been reported often (Hirose *et al.*, 2004; Sengkhampan *et al.*, 2009). The okra mucilage can be extracted as a viscous gum using various procedures. Such diversity in the extraction procedures seems to contribute to the observed variability in the mucilage chemical composition (Ndjouenkeu *et al.*, 1996). Okra mucilage is a renewable and inexpensive source of biodegradable material. Its physical and chemical properties include high water solubility, plasticity, elasticity and viscosity (BeMiller *et al.*, 1993).

Most physical and chemical properties are influenced by factors such as temperature, pH, sugar and salt contents, and storage time (Woolfe *et al.*, 1997; Bhat and Tharanathan, 1987). Okra mucilage has potential for use as food, non-food products, and medicine. Food applications include use as a whipping agent for reconstituted egg whites, as an additive in the formulation of flour-based adhesives, and as an additive in India for clarifying sugarcane juice. Non-food applications include brightening agents in electro deposition of metals, as a deflocculant in paper and fabric production, and as a protectant to reduce friction in pipe-flow (Ndjouenkeu *et al.*, 1996; BeMiller *et al.*, 1993). Polysaccharides can be combined with acrylamide to develop new biodegradable polymeric materials (Mishra *et al.*, 2008). Potential of mucilage for medicinal applications includes uses as an extender of serum albumin (BeMiller *et al.*, 1993), as tablet binder (Ofoefule *et al.*, 2001) and as suspending agent in formulations (Kumar *et al.*, 2009). Okra mucilage is used in Asian medicine as a protective food additive against irritating and inflammatory gastric diseases (Lengsfeld *et al.*, 2004).

Health benefits of okra

In recent years, increasing attention has been paid to the role of diet in human health (Ohr, 2004). The high intake of plant products is associated with a reduced risk of a number of chronic diseases, such as atherosclerosis and cancer (Gosslau and Chen, 2004). These beneficial effects have been partly attributed to the compounds which possess antioxidant activity. The major antioxidants of vegetables are vitamins C and E, carotenoids, and phenolic compounds, especially flavonoids. These antioxidants scavenge radicals and inhibit the chain initiation or break the chain propagation (the second defense line). Vitamin E and carotenoids also contribute to the first defense line against oxidative stress, because they quench

singlet oxygen (Krinsky, 2001). Flavonoids as well as vitamin C showed a protective activity to α -tocopherol in human LDL, and they can also regenerate vitamin E, from the α -chromoxy radical (Davey *et al.*, 200).

Nutrient antioxidants may act together to reduce reactive oxygen species level more effectively than single dietary antioxidants, because they can function as synergists (Rossetto *et al.*, 2002). In addition, a mixture containing both water-soluble and lipid-soluble antioxidants is capable of quenching free radicals in both aqueous and lipid phases (Trombino *et al.*, 2004). For example, with the liposome oxidation method, the activity of combination of quercetin or catechins plus α -tocopherol was significantly higher than the sum of the individual activities. Combinations of α -tocopherol or vitamin C plus phenolic compounds also provided synergistic effects in human erythrocyte membrane ghosts and phosphatidylcholine liposome systems (Liao and Yin, 2000).

Okra seed is rich in protein and unsaturated fatty acids such as linoleic acid (Oyelade *et al.*, 2003). In some countries, okra also is used in folk medicine as antiulcerogenic, gastroprotective, diuretic agents (Gurbuz, 2003). However, little information on antioxidant capabilities of major phenolic compounds from okra seed is available. Okra is also a popular health food due to its high fiber, vitamin C, and folate content. Okra is also a good source of calcium and potassium. Okra pod contains thick slimy polysaccharides, which are used to thicken soups and stews, as an egg white substitute, and as a fat substitute in chocolate bar cookies and in chocolate frozen dairy dessert (Sengkhampan *et al.*, 2009). Okra is also known for being high in antioxidants activity with different parts of the plant (Shui and Peng, 2004). Atawodi *et al.*, (2009) has reported in vitro antioxidant assay of methanol extract of okra fruits. They have done antioxidant/radical scavenging activities by xanthine oxidase and 2-deoxyguanosine methods and reported 50% inhibitory concentration values of 25 and 43 ml. In addition, (Arapitsas, 2008) reported that Okra seed is rich in Phenolic compounds, mainly composed of flavonol derivatives and oligomeric catechins. According to (Khomsug *et al.*, 2010) total phenolic content of pulped and seeds of okra extracts as $10.75 \pm 0.02 \text{ mg GAE/100g extract}$ and $142.48 \pm 0.02 \text{ mg GAE/100g extract}$ which corresponds with scavenging activities. Besides they have also found procyanidin B2 as predominant phenolic compound followed by procyanidin B1 and rutin in seeds. In pulped seed catechin, procyanidin B2, epicatechin and rutin are reported to

be present. It is quite important to see that roasting (1600°C for 10–60 minutes) increased the nutrient composition and antioxidant activity of the seeds whereas pre-treatment (soaking and blanching) increased the nutrient composition, but decreases antioxidant activity (Adelakun *et al.*, 2010), Ansari *et al.* (2005) reported Okra extract as in vitro non-enzymatic inhibitor of lipid peroxidation in liposomes. A. esculentus peel and seed powder contains significant in vivo antioxidant property in streptozotocin-induced diabetic rats.

Administration of different doses of peel and seed powder significantly increased liver, kidney and pancreas superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione levels and decreased thiobarbituric acid reactive substances (TBARS) ($P < 0.001$) levels in diabetic rats compared to diabetic control rats. (Liao *et al.*, 2012) has done a comparative analysis of total phenolics and total flavonoids and antioxidant ability of different organs (flower, fruit, leaf, and seed) and different enrichment fractions of water extracts of the A. esculentus plant. They confirmed fruitful presence of total phenolics and total flavonoids related to antioxidant ability in all the extracts of the plant organs although percentage varied. In flower of okra, highest amount of total phenolic and total flavonoids were found (Liao *et al.*, 2012). This data suggests Okra as a good contributor to the antioxidant status and promising chemopreventive agent as described in several traditional medicines for human race. Okra is abundant with several vitamins, minerals, and nutrients that handles the health advantages the plant provides. Here are a few of okra's health advantages:

Okra contain high fiber, which “helps to stabilize blood sugar by regulating the rate at which sugar is absorbed from the intestinal tract”. Because of fiber along with other nutrition, okra shows useful for minimizing blood sugar levels within the body, assisting along with diabetes. The fiber likewise helps support blood sugar levels level simply by slowing down sugar assimilation through the intestines (Ngoc *et al.*, 2008). The frequent usage of okra might help avoid kidney disease. Within the research, “those who consumed okra every day decreased clinical indications of kidney damage a lot more than the ones that simply consumed a diabetic diet.” This ties along with diabetes, as almost 50% of kidney disease cases are generated by diabetes (Lengsfeld *et al.*, 2004).

Okra is used to treat digestive issues. The polysaccharides present in immature okra pods

possessed considerable antiadhesive properties (i.e. they help remove the adhesive between bacteria and stomach tissue, preventing the cultures from spreading). Okra's polysaccharides were particularly effective at inhibiting the adhesion of *Helicobacter pylori*, a bacterium that dwells in the stomach and can cause gastritis and gastric ulcers if left unchecked. Therefore, eating more okra can keep our stomach clean and create an environment that prevents destructive cultures from flourishing (Messing *et al.*, 2014). Okra is used to support colon health. It smoothly sails down our colon, absorbing all toxins and excess water in its path. Okra is filled with dietary fiber that is required for colon health and digestive health all together. The fiber Okra offers helps to cleanse the intestinal system, letting the colon to operate at higher amounts of effectiveness. In addition, the vitamin A plays a role in wholesome mucous membranes, assisting the digestive system to function adequately (Georgiadisa *et al.*, 2011).

Okra is used to promote healthy skin and blood. One hundred grams of okra also contain approximately 27 percent of our RDI of vitamin C and 50 percent of our RDI of vitamin K. Vitamin C is, of course, an essential antioxidant that aids in the growth and repair of bodily tissues. For this reason, eating more okra can rejuvenate our skin and hair, and also shield us from degenerative diseases associated with long-term free radical damage. Vitamin K, on the other hand, plays an important role in blood clot formation. If you suffer from regular nosebleeds, bleeding gums, heavy menstrual bleeding, or easy bruising, your blood might be too thin. Consider adding more vitamin K-rich foods like okra to your diet to improve your blood's ability to coagulate (Bakre and Jaiyeoba, 2009).

Okra is used to promote a healthy of the pregnancy. An incredibly essential B vitamin for creating and maintaining new cells, foliate is a vital substance for optimum pregnancy. The vitamin aids in preventing birth defects just like spina bifida and enables the baby to develop completely. Vitamin C is additionally required for baby development. Okra is full of both foliate and vitamin C. The high quantity of foliate included in the okra is helpful for the fetus while pregnant. Foliate is a vital nutrient that increases the growth and development of the fetus' brain. The high quantity of folic acid within okra performs a huge role within the neural tube formation of the fetus through the fourth to the 12th week of pregnancy (Zaharuddin *et al.*, 2014).

Okra is used to improve heart health. The soluble fiber within okra helps you to reduce serum cholesterol and therefore decreases the chance of cardiovascular disease. Consuming okra is an efficient method to manage the body's cholesterol level. Okra is additionally loaded with pectin that can help in reducing high blood cholesterol simply by modifying the creation of bile within the intestines ((Ngoc *et al.*, 2008)). Okra is also used to improve good eyesight. The okra pods are fantastic options for Vitamin A and also beta carotene that are both important nourishment for sustaining an excellent eye-sight along with healthy skin (Lengsfeld *et al.*, 2004). Additionally, these types of important nourishment also assist inhibits eye associated illnesses along with problems on the skin. Okra is better ingested when joined along with other healthy veggies. Consuming okra has truly numerous advantages, simply bear in mind to eat natural veggies as opposed to processed veggies (Messing *et al.*, 2014).

Okra is used to control the body's cholesterol level. There are numerous significant illnesses related to high cholesterol level of the entire body. Managing the body's cholesterol level is nearly difficult because it's hard to avoid foods loaded with cholesterol content. One of the better health advantages of consuming okra is definitely the powerful management of the human body's high cholesterol level (Sengkhampan *et al.*, 2009). This healthy vegetable is beneficial in slimming down and also decreasing cholesterol therefore keeps a healthy and also low cholesterol body. Okra have been taken advantage by diet advisors due to these qualities (Zaharuddin *et al.*, 2014).

Generally, okra is used to stabilize blood sugar by regulating the rate at which sugar is absorbed from the intestinal tract. It is a good vegetable for those feeling weak, exhausted, and suffering from depression and it is also used in ulcers, lung inflammation, sore throat as well as irritable bowel. Okra is good for asthma patients and it also normalizes blood sugar and cholesterol levels (Sengkhampan *et al.*, 2009). Previous studies reported that okra polysaccharide possesses anticomplementary and hypoglycemic activity in normal mice (Tomoda *et al.*, 1989). Also, okra polysaccharide lowers cholesterol level in blood and may prevent cancer by its ability to bind bile acids (Lengsfeld *et al.*, 2004; Kahlon *et al.*, 2007). Additionally, Okra seed possess blood glucose normalization and lipid profiles lowering action in diabetic condition (Sabitha *et al.*, 2011).

Conclusion

The information presented here shows the potential nutritional importance of Okra and its role in improved nutrition and health. It is an affordable source of protein, carbohydrates, minerals and vitamins, dietary fibre and health promoting fatty acids. Scientific studies provide some evidence to support the potential beneficial effects of Okra components in lowering the risk for various chronic diseases, although information pertaining to the role of edible plant parts of Okra in disease prevention and the mechanisms of action are limited to date. This is due to the complex nature of disease etiology and various factors impacting their occurrence. It is imperative the scientific community continues to unravel the mechanisms involved in disease prevention and determine how food bio-actives from such foods as Okra can influence human health. Further research, needs to be performed to provide compelling evidence for the direct health benefits of okra consumption. Therefore, promoting the consumption of traditional vegetables such as Okra could provide cheap sources of macro and micronutrients and mineral elements that can improve the nutritional status of resource-poor subsistence farmers in the area in particular and in Ethiopia in general. Furthermore, this vegetable can also be used as an indispensable tool when it comes to reducing the prevalence of malnutrition, especially among resource-constrained urban households in addition to rural household. Consumption of Okra by both low-income and high-income groups can also use as a means of dietary diversification approach.

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Physico-chemical and microbial properties of bread supplemented with sweet potato flour

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ABSTRACT

Sweet potato is a good source of macronutrients like carbohydrates and proteins, has beneficial effects on human health such as improves the immunity and reduces the susceptibility of body to various ailments like cancer and muscular degenerations. Being perishable commodity sweet potato has short shelf life, so it should be converted into suitable product by incorporating into different products suitable for longer period. The basic aim of this research was to prepare sweet potato supplemented breads with best suitable physico-chemical and microbial properties. For this seventeen sweet potato supplemented breads, with and without dough enhancers (DE) were studied after 0, 1, 2, 3 and 4 days interval. The parameters under observation were chemical analysis of wheat and sweet potato flour, proximate composition of prepared breads, physical parameters and microbial assessment. All breads were significantly ($P \leq 0.05$) lighter in color at the last day of storage, while those containing 15% SPF with DE had the highest loaf volumes besides the control. Regarding rheological properties water absorption increased while dough stability and dough development time decreased with the increase of sweet potato flour. Prepared breads decreased in terms of mold with maximum in T0 (2.2733×10^2) & minimum in T7 while total plate count with maximum of T0 (2.443×10^2) and the lowest values T7 (2.1433×10^2) with the increase of sweet potato flour and increase of storage interval. Sweet potato flour supplemented breads have acceptable limits up to 60 % in the presence of dough enhancer and 30 % without dough enhancer.

Key words: Sweet potato, Supplemented Bread, Physico-chemical analysis, Microbial Assessment, Sensory analysis

INTRODUCTION

Sweet potato is a root crop which is grown in all parts of tropical and subtropical regions of the world. It has low product diversity and people are unaware of its potential benefits which accounts for its low utilization (Tewe *et al.*, 2003). It is a good medium for minimizing some health related issues and also play an important role in sustaining food security (Rees *et al.*, 2003). The research on sweet potato processing has reason to say that there is a lot more in sweet potatoes than its starch. It is a good source of natural colorants and antioxidants. The shelf life of sweet potato can be extended by processing it in different value added products and ultimately it could be used in different products which will help in increasing the intake of many nutrients (Katan and De Roos, 2004). Aside from being rich source of carbohydrates and other nutrients, it is thought that wheat is nutritionally poor as it is deficient in essential amino acids such as threonine and lysine. Hence, nutritional value of wheat product should be increased by supplementation with

staples such as pulses, cereals and sweet potato Flours (Ranhotra, 2001). Sweet potato flour in different concentrations has been substituted by various researchers in bread. Bread is high nutritional product which has worldwide consumption. Different recipes, formulations and specific storage conditions are used to improve the nutritional quality and to extend the shelf life of bread. Many additives are used to enhance the acceptability and shelf life of bread (WHO 2005).

Van Hal *et al.* (2000) have supplemented various levels of sweet potato flour (SPF) in specialty bread recipe but due to least amount of gluten causes either small percentages of SPF to be added. It resulted a loss in textural properties and low loaf volume and decreased consumer choice. This lack of gluten the addition of SPF in bread at different levels is suppressed. So, we need additional ingredients which aid to improve the quality of sweet potato supplemented bread (Bovell-Benjamin, 2007). Dough Improvers or dough conditioners are usually added in bread in very minute quantity to get ideal physical

characteristics of bread such as loaf volume, texture, flavor, shelf-life and overall acceptability. There is no knowledge regarding the use of dough improver in sweet potato bread. It was reported that a dough improver could be a health loving alternative having potential to improve the total quality characteristics of sweet potato breads (Hathorn, 2008). Dough enhancer influenced the end characteristics of the sweet potato supplemented breads, which are used up to various levels (Mandala, 2004). The current study is designed to evaluate the physico-chemical, textural and microbial properties of sweet potato supplemented wheat bread and to enhance nutritional value of bread.

MATERIAL AND METHOD

Procurement of raw material

Wheat (Lasani 2008) and red skin variety sweet potato were purchased from local market of Faisalabad Pakistan.

Flour processing

Wheat flour

Wheat flour was prepared in Brabender Senior Quadrumate Mill (Germany) at National Institute of Food Science and Technology, Faisalabad. The grains of wheat were subjected to tempering at a 14.5 % moisture level. The tempering of wheat was carried out in plastic containers at room temperature for 24 hours for equilibrate the moisture content within the grains. The amount of water required for tempering was calculated by following the expression given below as per procedure given in AACC (2000) method No. 26-95.

Sweet potato flour

The sweet potato were dehydrated and processed into flour. The flour was packed in polyethylene bags and was stored in airtight containers. Sweet potatoes, purchased from a local grocery store were washed, dried, and weighed, hand-peeled, shredded, and dehydrated at 70 °C for 12 h. The dehydrated sweet potatoes were processed into flour following the procedures described by (Dansby and Bovell-Benjamin 2003). The flours were put into polyethylene bags, which were placed into paper bags, and stored in airtight containers at 22±2°C until it was used in product.

Composite Flour

Different levels of sweet potato flour were added in wheat flour according to treatment plan and composite flour was analyzed for rheological properties by using

farinograph (method No. 54-21) following the procedures given in AACC (2000). Farinographic characteristics includes water absorption, dough development time, dough stability and mixing tolerance index.

Bread preparation

The breads were prepared according to the AACC (2000) straight dough method No 10-10B. The following ingredients (g/100 g flour basis) were used: water (calculated to obtain a dough Lab absorption value of 500 FU), instant dry yeast (1g/100 g), ascorbic acid (0.01 g/100 g) and salt (1.8 g/ 100 g) sweet potato flour according to treatments plan given in Table 1. Control bread with no sweet potato flour was also prepared. All samples were prepared in triplicate and were analyzed for given below tests after 0, 1, 2, 3 and 4 days interval.

Chemical Analysis of bread

The prepared bread samples were analyzed for moisture, ash, crude fat, crude fiber, and crude protein using procedure as outlined by AACC (2000).

Physical analysis

The color of bread was determined with the help of Color meter II (Neuhauser colour test II, Neotec). The sample reading was compared with standards described by Rocha and Morais, (2003).

Loaf volume

Loaf volume was measured by rapeseed displacement method (Greene and Bovell-Benjamin, 2004).

Texture

Texture is an important parameter to check the quality of products. The textural study was analyzed by using Texture analyzer TA-XT plus as described by Piga *et al.* (2005).

Water activity

The ratio between the vapor pressure of the food itself, when in a completely undisturbed balance with the surrounding air media, and the vapor pressure of distilled water under identical conditions is called the water activity of a food. The water activity was determined by using Hygropalm water activity meter as described by Piga *et al.* (2005).

Microbial examination

Counting of molds in bread after 0, 1, 2, 3 and 4 days of storage was performed by the agar plate method on agar medium, whereas counting of bacteria were performed on nutrient agar medium using the plate count method followed by Tarar *et al.* (2010).

Sensory evaluation of bread

The prepared samples were assessed for quality and acceptability of color, texture, flavor, taste, chewing ability and overall acceptability by the method as described by Lawless and Heymann (1998).

Statistical Analysis

The data obtained for each parameter were subjected to statistical analysis to determine the level of significance using analysis of variance in ANOVA as described by Steel *et al.* (1997) and means were further compared through turkey range test to determine best treatment using IBM SPSS v21.

RESULT AND DISCUSSION

Rheological Studies

The results of various parameters of rheological studies have been shown in table 2. The parameters are discussed as follow:

Water Absorption

The highest water absorption is in the T8 and the lowest is in the T0. Here T1, T3, T5 and T7 containing dough improver have somewhat higher water absorption than the T2, T4, T6 and T8 containing no dough improver. As the level of SPF increased the water absorption increased T1<, T2<, T3<, T4<, T5<, T6< and T7 respectively which is in accordance with Hamed *et al.* (1973).

Dough Development Time

The mean values of dough development time varied from 4.50min to 6.20min among different levels of SPF addition. The highest dough development time was recorded in the T0 wheat flour (4.50min.). The lowest dough development time was found in the T8 (1.90 min.). The dough development time decreased with decrease in the level of gluten quality T1>, T2>, T3>, T4>, T5>, T6> and T7 respectively. Here T1, T3, T5 and T7 containing dough improver have somewhat higher dough development time than the T2, T4, T6 and T8 containing no dough improver. The results of present study are in line with the findings of Hamed *et al.* (1973) who found the same results.

Dough Stability

The mean values of dough stability varied from 3.38 to 10.54 among varying levels of SPF added. The highest mean value of dough stability is for T0 10.54 and the lowest is for the T8 which is 3.38. Here T1, T3, T5 and T7 containing dough improver have somewhat higher dough stability than the T2, T4, T6 and T8 containing no dough improver. An overall decreasing trend of dough stability was observed with

decrease of protein contents T1>, T2>, T3>, T4>, T5>, T6> and T7 respectively which is in accordance with Hamed *et al.* (1973).

Mixing Tolerance Index

The mean values regarding the mixing tolerance index of different wheat varieties is given in Table 4.2.1.4b. The highest mean value of mixing tolerance index is for T7 168.67 and the lowest is for the T0 which is 35.33. Here T1, T3, T5 and T7 containing dough improver have somewhat higher mixing tolerance index than the T2, T4, T6 and T8 containing no dough improver. The present study is in accordance with the study of Hamed *et al.* (1973).

Chemical analysis of bread

Mean values regarding the moisture, ash and protein are shown in Table 3. The mean values of moisture were decreasing in the treatments with the highest value of T7 (33.29) and the lowest value is T3 (29.19). During storage moisture is also decreasing from 32.69 at 0 day to 29.63 at the 4th day which is in accordance with (Sidhu *et al.*, 1997). Among the interaction of days into treatments the highest mean value is of T7 35.22 at 0 day and the lowest was 30.07 in T3 on the 4th Day of storage. The moisture contents consistently decreasing during storage but the moisture loss was very low. Dough improver helps to retain moisture and resulted in less moisture loss. Present study of moisture is in accordance with (Patelet *et al.*, 2005).

The mean values of ash were increasing in the treatments with the highest value of T7 (2.357) and the lowest value is T3 (2.32) while the ash values at 1, 2 and 3 days were 2.32, 2.32 and 2.32 respectively which is similar to (Dhingra and Jood, 2001). The interaction of days into treatments is non-significant and the ash level remains nearly constant which is in accordance with Srivastava, Meyer, Rao and Seibel (2002). The mean values of protein were decreasing in the treatments with the highest value of T0 (9.35) which is wheat bread and T1 (9.33) have the highest protein in the composite flour bread and the lowest value is T7 (7.09). During storage, it also ranged from 8.59 to 8.360 at the end of 4th day. While the protein values at 1, 2 and 3 days 8.60 which is similar to (Georgopoulos *et al.*, 2004). The interaction of days into treatments is non-significant and the protein level remains nearly constant.

During storage and interaction of days into treatments, no effect on fat was observed and fat remained non-significant during storage. As we increase sweet potato, fat decreased but in present study fat level increased which is explained by comment that due to the presence dough improver the fat retaining capacity

is increased. These results are in accordance with Green (2003).

The mean values of fiber were increasing in the treatments with the highest value of T7 (2.37) and the lowest value is T0 (2.03) but T1 next higher to T0 having fiber content T2 (2.28). During storage and interaction of both days and treatments, fiber remained constant having range of 2.29-2.3 while the fiber values at 1, 2 and 3 days were 2.29, 2.29 and 2.3 respectively which is similar to Dhingra and Jood (2001).

Physical properties

Color

The means regarding L* values of bread are presented in Table 4. The L* values for bread T₀ (control), L* values means range from 63.08 to 47.51 for T1 and T8 respectively and 78.19 (T₀) for wheat bread. The treatments containing dough improver are T1, T3, T5, and T7, have higher L* while T2, T4, T6 and T8 do not contain dough improver and have lower L* values. During storage is also Changes from 57.17 (0) day to 54.0 at the end of 3th day, it means that changes constant during storage. While the L* values at 1, 2 and 3 days are decreasing which is similar to the findings. Interaction of days and treatments is non-significant a little variation occurred in every treatment which is in accordance with Farvili *et al.* (1997). The interaction of days into treatments is non-significant and the fiber level remains nearly constant.

Loaf volume

Mean values regarding the treatment are shown in Table 5. The mean values of loaf volume were decreasing in the treatments with the highest value of T0 (2.37) and the lowest value is T8 (2.03) but T1 next higher to T0 having loaf volume T2 (2.28). The treatments containing dough improver are T1, T3, T5, and T7, have higher loaf volume while T2, T4, T6 and T8 do not contain dough improver that is why little lower loaf volume have comparatively. During storage loaf volume was highly significantly affected and having range of 649- 620 at 0 and 4 day respectively. While the loaf volume values at 1, 2 and 3 days were 644, 633.81 and 620.78 respectively which is similar to (Green and Bovell Benjamin, 2004). The interaction of days into treatments is highly significant and volume decreases constantly. The mean value of loaf volume at 0 day for T0 is 9.56 while T8 has 7.09 fiber which in accordance with Srivastava *et al.*, (2002).

Texture

The mean values (Table 5) showed that bread hardness ranged from 3628.9 to 6045.8g among bread of

various wheat varieties. The highest bread hardness i.e. 6045.8g. The lowest bread hardness 3628.9g was recorded in wheat variety Iqbal-91. The bread hardness of all the varieties showed significant results among each other. These results are in agreement with Mohamed *et al.*, (2008) who found the same results. Similar textural and crumb grain profiles have been stated previously by means of sensorial and instrumental studies of breads (Basman *et al.*, 2002).

The water activity (aw)

The maximum water activity was found in 15 % SPF/DE bread (0.90) while the minimum water activity was found in 30%SPF/NDE (0.88). The increase in protein (gluten) affects the water activity. The water activity was decreased with decrease of protein But due to high water entrapped the water activity of T8 (60%SPF/NDE) was significantly different. During the storage, water activity changes were minor (0) day has water activity mean of 0.9 while at the end of 4th day water activity was .88 with minor changes which are explained in another study by (DeMan *et al.*, 1999), who observed that white bread after one day of storage had water activity 0.942 – 0.95. Overall, the aw for the breads between the beginning and the ending of this study was negligible.

Microbial properties

Total Plate and mold Count

The studies on the bread on the nutrient agar were analyzed. The data revealed that the results were highly significant among the treatments the maximum no. of microbial count was observed by the T0 (2.443×10^2) and the lowest values are shown by T7 (2.1433×10^2) which contain 60% SPF/DE. The microbial count the bread after 0, 1, 2, 3, 4 interval were also performed. On the first day 5×10^1 CFU/g bacterial count was observed which increased to 2.5×10^2 CFU/g on the subsequent study which is in accordance with Lazaridou *et al.* (2009). The study on the mold count on the potato dextrose medium were analyzed. The maximum no of mold were observed in T0 (2.2733×10^2) and the lowest was observed in the T7 which contain 60% SPF.DE, The analysis of variance showed highly significant results the results were supported by karaoglu *et al.* (2005).

Sensory evaluation

Mean values regarding the treatment are shown in table 7. The mean values of volume were decreasing in the treatments with the highest value of T0 (8.1) and the lowest value is T8 (3.3) but T1 next higher to T0 having volume (7.1). The treatments containing dough improver are T1, T3, T5, and T7, have higher volume while T2, T4, T6 and T8 do not contain dough

Table 1. Treatment plan for composite flour of sweet potato and wheat flour

Treatment	Wheat flour	Sweet potato flour	Dough enhancer
T ₀	100	0	-
T ₁	85	15	Present
T ₂	85	15	Absent
T ₃	70	30	Present
T ₄	70	30	Absent
T ₅	55	45	Present
T ₆	55	45	Absent
T ₇	40	60	Present
T ₈	40	60	Absent

Table 2. Mean values of rheological parameters of composite flour

Treatment	Water absorption	Dough stability	Dough Development	Mixing Tolerance Index
T0	57.3g	10.54a	4.50a	35i
T1	59.7f	9.00b	3.89b	62g
T2	61.1e	8.45c	3.62c	58h
T3	62.1d	7.45d	3.15d	93e
T4	63.0c	6.23e	3.03e	89f
T5	63.9b	4.70f	2.46f	153.6c
T6	64.0b	4.02h	2.22g	145d
T7	64.9a	4.21g	2.01h	168.6a
T8	65.2a	3.3i	1.90i	160b

Table 3. Mean values of moisture, ash, crude protein, fat content and crude fiber of sweet potato supplemented bread during storage

Treatment	Moisture content	Ash Content	Protein Content	Fat Content	Crude Fiber
T0	31.65c	0.7095f	9.65a	1.76a	2.03f
T1	31.63c	2.32de	9.36b	1.04f	2.29d
T2	29.19f	2.29de	9.33b	1.03f	2.26e
T3	31.88c	2.34b	8.98c	1.23c	2.33c
T4	30.34e	2.31e	8.98c	1.10e	2.30d
T5	32.78b	2.34bc	8.42d	1.21c	2.36d
T6	30.95b	2.31e	8.39d	1.17d	2.33c
T7	33.29a	2.36a	7.18e	1.31b	2.39 a
T8	31.00a	2.33cd	7.09e	1.22c	2.37ab

Table 4. Mean values of L*, a* & b* values of sweet potato supplemented bread during storage

Treatment	L* Value	a* value	b* value
T0	78.19a	-0.06f	15.75c
T1	63.08b	3.2e	14.07h
T2	55.36c	3.2e	16.46a
T3	54.9c	4.9e	15.00f
T4	52.7c	4.9e	15.44d
T5	52.6cd	5.7c	15.40e
T6	50.9cd	5.7c	14.99f
T7	50.5cd	6.1b	14.34g
T8	47.51d	6.7a	14.88b

Table 5. Mean values of Loaf volume, texture and water activity of sweet potato supplemented bread during storage

Treatments	Loaf Volume	Texture	Water Activity
T0	817.87a	3998.9i	0.91a
T1	769.67b	4174.7h	0.90ab
T2	723.13c	4361.3 g	0.90abc
T3	696.00d	4508.0 f	0.89bc
T4	646.13e	4660.3 e	0.88c
T5	625.60f	4721.0 d	0.89bc
T6	568.80g	4887.7c	0.89bc
T7	465.73h	5002.3b	0.89bc
T8	391.13i	5299.5a	0.89bc

Table 6. Mean values of Total Plate Count and Fungal Growth of sweet potato supplemented bread during storage

Treatments	Total Plate Count	Fungal Growth
T0	1.70f	2.03f
T1	2.32de	2.29d
T2	2.28de	2.26e
T3	2.30b	2.33c
T4	2.31e	2.30d
T5	2.34bc	2.36d
T6	2.31e	2.33c
T7	2.35a	2.39 a
T8	2.33cd	2.37ab

Table 7. Mean values of Volume, Color and Symmetry of sweet potato supplemented bread during storage

Treatments	Volume	Color	Symmetry
T0	8.1a	6.53a	3.78a
T1	7.1b	6.53a	3.46ab
T2	6.1c	5.86b	3.20b
T3	7.1b	5.60b	2.66c
T4	5.8cd	5.13c	2.80 c
T5	5.45d	5.06c	2.66c
T6	4.8 d	4.86 c	2.46cd
T7	3.7f	4.46d	2.26d
T8	3.3f	4.06d	2.13d

improver that is why comparatively less volume has. During storage loaf volume was decreasing from 6.18 (T0) to 5.29 (T8). While the loaf volumes values at 1, 2 and 3 days were 5.88, 5.6 and 5.4 respectively which is similar to (Grosh and Wieser, 1999). Mean values regarding the treatment are shown in table 7. The mean values of crust color were increasing in the treatments with the highest value of T0 (6.5) and the lowest value is T8 (4.06) but T1 is similar to T0 having crust color (6.5). The treatments containing dough improver are T1, T3, T5, and T7, have higher value of crust color while T2, T4, T6 and T8 do not contain dough improver that is why little lower value of crust color have comparatively. During storage value of crust color was highly significantly affected by SPF level having range of 6.07 at 1 day while 4.59 at the 4 day of storage .while the value of crust color values at 1, 2 and 3 days were 5.44, 5.42 and 5.20 respectively which is similar to (Dhingra and Jood, 2001). Mean regarding symmetry are shown in table 7 that were decreased with increasing treatments with the highest value of T0 (3.78) and the lowest value is T8 (2.13) but T1 next higher to T0 having value T1 (3.46). The treatments containing dough improver are T1, T3, T5, and T7, have higher scores while T2, T4, T6 and T8 do not contain dough improver that is why little lower score have comparatively. During storage symmetry of form was highly significantly affected by various levels of SPF. While the scores at 1, 2 and 3 days were 3, 2.75 and 2.61 respectively.

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Comparative studies on the shelf stability of different types of apple jams

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ABSTRACT

The aim of the study was to evaluate a suitable combination of apple pulp and sucrose solution for the preparation of apple jam, stored at ambient temperature. The treatments were T₀, T₁ and T₂. The samples were filled in the bottles and evaluation was carried out for total period of 60 days. Physicochemical analysis, pH, brix,⁰ acidity and sensory characteristics of; color, taste, texture and overall acceptability (using Larmond scale) were evaluated at 15 days interval. The TSS of apple pulp was 14 brix⁰ when sucrose solution was added in different ratio; The pH of apple jam was increased from 3.84 to 4.98 for the period of storage. Maximum reduced was perceived in T₂ (3.91%), in compare minimum fall was observed in T₀ (3.87%). Then TSS of apple jam was increased from 64 to 67.67 during storage. Maximum increased was observed T₀ (6.45%), while lowest value was observed in followed by T₁ (4.5%). The Titratable acidity of apple jam was increased from 0.2 to 0.4 for the period of storage. Supreme increased was perceived in T₀ (53.33%), while lowest raise was observed in T₂ (34.042%). The storage intervals had effect on the mean scores for organolyptic assessment. Mean scores of juries for the color of apple jam was reduced from 6.90 to 6.30 for the period of storage. Supreme decreased was perceived in T₂ (22.08%), while lowest fall was observed in T₀ (18.75%). Mean totals of juries for the taste of apple jam was reduced from 7.50 to 6.17 for the period of storage. Maximum reduced was detected in T₂ (20%), while lowest fall was observed in T₀ (14.29%). Mean scores of judges for the texture of apple jam was reduced from 8 to 6.21 for the period of storage. Maximum decreased was perceived in T₀ (24.05%), while lowest fall was observed in T₀ (18.52%). Mean scores of juries for the overall acceptability of apple jam was reduced from 8.23 to 8.40 throughout the storage. Maximum decreased was perceived in T₅ (50%) and T₄ (50%) followed by T₂ (25%), while lowest fall was observed in T₂ (20.24%). Statistically result was showed that the treatment T₀ (Tarnab jam) was found most acceptable in terms of physicochemical and organoleptically.

Keywords: Apple Jam, storage, Physico-chemical analysis, organolyptic analysis

INTRODUCTION

Apples are one of the most consumed fruits worldwide and are consumed fresh or in processed forms such as jam, juice or dried. Apples contain over 84% water, a variety of vitamins (except vitamin B complex), minerals (K, Mg, Ca, Na), trace elements (Zn, Mn, Cu, Fe, B, F, Se, Mo) and have a high fiber content. Due to the varied and well balanced composition of apples, they have the potential to prevent digestive cancers, colon and liver cancers, coronary heart disease, lung function disorder and asthma (Feliciano *et al.*, 2010).

Apple can be made into fruit leather by using apple juice concentrate (AJC) instead of glucose and sucrose. In this way, the AJC could be used to give a natural sweet taste to the fruit leather. Addition of blackcurrant concentrate (BCC) to the apple fruit leather would enhance the nutritional quality of the product. Moreover, incorporation of pectin would

improve the physicochemical and sensory properties of the product (Vijayanand *et al.*, 2000).

Jam is defined as a semi-solid mixture, obtained upon cooking the fruits puree with sugar. Jam is an intermediate moisture food prepared by boiling fruit pulp with sugar (sucrose), pectin, acid, and other ingredients (preservative, coloring, and flavoring materials) to a reasonably thick consistency, firm enough to hold the fruits tissues in position. Jam should contain more than 68.5% total soluble solid (TSS) and at least 45% fruit whereas, the codex Alimentarius Commission specify that the finished jam should contain more than 65% TSS (Baker *et al.* 2005) Jam, jellies and preserves are manufactured as one of the important fruit products in industries and based on the high solids-high acid principal. Jam are of the two kinds one prepared from a single fruit and another prepared from the combination of two or more fruits (Manay *et al.*, 2005) Citric acid is considered

necessary to correct the balance which is needed in jam and jelly production. Lime and lemon juice are high in citric acid therefore they can be used as a replacement of citric acid in jam manufacture (Desrosier *et al.*, 1978).

Perishable fruits are available as seasonal surpluses during certain parts of the year in different regions and are wasted in large quantities due to absence of facilities and know – how for proper handling , distribution , marketing and storage. Massive amount perishable fruits produced during a particular season results in abundance in the market and become scarce during other season (Agarwal *et al.*, 2005).

Sugar constitutes more than 40% of total weight, an effect that is essential for the physical, chemical and microbiological stability; provides body; improves appearance and makes gelation of pectin possible. The added sugar acts as a dehydrating agent for the pectin molecules, permitting closer contact between the chain molecules reduces water activity to below 0.8, thus the spoilage organisms in jam do not survive (Hyvonen and Torma, 1983). Guava is normally consumed fresh as dessert fruit that is pleasantly sweet and refreshing in flavour. The whole fruit is edible along with skin. It is considered as one of the most delicious and luscious fruit. Excellent salad, pudding, jam, jelly, cheese, canned fruit, RTS, nectar, squash, ice cream and toffees can be made from guava fruit (Jain and Asati, 2004).

MATERIALS AND METHODS

The research was conducted in the laboratory of the section of Food Science and Technology, Agriculture research institute (ARI) Tarnab, Peshawar.

Selection of apple jam

The Tarnab Apple jam compared to the multinational and local company apple jams. The products were purchased from the confined market of Peshawar and were brought to the laboratory of the Food Science and Technology section of ARI (Agriculture research institute). Tarnab, Peshawar.

Preparation of the samples

These Samples were analyzed for physiochemical and organoleptically. The analysis were carried out at the laboratory of the Food Science and Technology section of ARI (Agriculture research institute). Tarnab, Peshawar at 15 days interval for a total period of 60 days.

Proposed plan of study

Comparative study of ARI (Agriculture, Institute, Peshawar, Tarnab, Peshawar), local and multinational company jam

T₀ (ARI, Tarnab, Peshawar jam)

T₁ (multinational company jam)

T₂ (local company company jam)

Physicochemical Analysis

pH

pH was determined by digital pH meter according to standard method of AOAC (2010). The pH meter was standardized by using pH four and seven buffer solutions. Distilled water was used to wash the electrodes and were dried with tissue paper before measuring pH of sample.

Total Soluble Solids

The total soluble solids (TSS) were determined at room temperature by standard method of AOAC (2010), using hand refractometer. Before operating, temperature of the equipment was adjusted to room temperature. The samples were placed between the two lower prisms and then the connecting arm was rotated until the critical way centered in the eyepiece, reading was taken directly in^obrix.

Titrate acidity

Titrate acidity was determined by standard method of AOAC (2010).The percent acidity was calculated by dissolving 10 gram of ber jam with distilled water making the volume up to 100 ml. 10 ml of sample was taken for titration against 0.1 N NaOH using phenolphthalein as an indicator.

Organolyptic evaluation

Selected samples of apple jam were evaluated organoleptically for color, flavor, texture and Overall acceptability by the method as described by Larmond (1977). Samples were presented to trained judges to compare them and assign them score between 1-9, where 1 represent extremely disliked and 9 represent extremely liked.

Statistical analysis

All the data concerning treatments and storage interval were statistically analyzed by means of 2 factorial CRD (Completely randomize Design) and the means were separated by applying LSD Test at 5% probability level as described by Steel and Torrie (1996).

RESULTS and DISSCUSTION

pH of jams

The statistical analysis showed significantly ($P < 0.05$) results which may be due to the effect of treatment and storage intervals on the pH value of apple jam during storage. The mean were separated by applying LSD test at 5% probability level (Table 1). The initial pH value of Tarnab jam, Multinational jam & Local jam was T_0 (3.81), T_1 (3.82), and T_2 (3.83) which was gradually increased to 3.94, 3.95 and 3.99 respectively during storage (Table 1). The mean values for intervals were increased from 3.84e to 3.98a during storage. The maximum mean value T_2 (3.92a) was noted in local jam, while the minimum value T_0 (3.87c) was recorded in local jam. During storage highest fall in pH was recorded Tarnab jam T_0 (3.41%), in compare minimum fall was observed in Tarnab jam T_2 (3.18%).

Total solouble solids (TSS)

The statistical analysis showed significantly ($P < 0.05$) results which may be due to the effect of treatment and storage intervals on the TSS value of apple jam during storage. The mean were separated by applying LSD test at 5% probability level (Table 2). The initial TSS value of Tarnab jam, Multinational jam & Local jam was T_0 (62), T_1 (66), and T_2 (64) which was gradually decreased to 66, 69 and 68 respectively during storage (Table 2). The mean values for intervals were increased from 64d to 67.67a during storage. The maximum mean value T_1 (67b) was noted in multinational jam, while the minimum value T_0 (64c) was recorded in Tarnab jam. During storage highest fall in TSS was recorded Tarnab jam T_0 (6.45%), in compare minimum fall was observed in Tarnab jam T_1 (4.55%).

Acidity (%)

The statistical analysis showed significantly ($P < 0.05$) results which may be due to the effect of treatment and storage intervals on the acidity value of apple jam during storage. The mean were separated by applying LSD test at 5% probability level (Table 3). The initial acidity value of Tarnab jam, Multinational jam & Local jam were T_0 (0.21) and T_1 (0.22) and T_2 (0.31) which was gradually increased to 0.45, 0.041 and 0.47 respectively during storage (Table 3). The mean values for intervals were increased from 0.25c to 0.44a during storage. The maximum mean value T_0 (0.4b) was noted in Tarnab jam, while the minimum value T_1 (0.4a) was recorded in multinational jam. During storage highest fall in acidity was recorded local jam T_0 (53.33%), in compare minimum fall was observed in Tarnab jam T_2 (34.042%).

Color

The statistical analysis showed significantly ($P < 0.05$) results which may be due to the effect of treatment and storage intervals on the color value of apple jam during storage. The mean were separated by applying LSD test at 5% probability level (Table 4). The initial color value of Tarnab jam, Multinational jam & Local jam was T_0 (8) and T_1 (8) and T_2 (7.7) which was gradually decreased to 6.5, 6.4 and 6 respectively during storage (Table 4). The mean values for intervals were decreased from 7.90d to 6.30a during storage. The maximum mean value T_0 (7.02b) was noted in Tarnab jam, while the minimum value T_2 (6.72a) was recorded in local jam. During storage highest fall in color was recorded local jam T_2 (22.08%), in compare minimum fall was observed in Tarnab jam T_0 (18.75%).

Taste

The statistical analysis showed significantly ($P < 0.05$) results which may be due to the effect of treatment and storage intervals on the taste value of apple jam during storage. The mean were separated by applying LSD test at 5% probability level (Table 5) The initial taste value of Tarnab jam, Multinational jam & Local jam was T_0 (7), T_1 (8), and T_2 (7.5) which was gradually decreased to 6, 6.5 and 6 respectively during storage (Table 5). The mean values for intervals were decreased from 7.50c to 6.17a during storage. The maximum mean value T_1 (6.70a) was noted in multinational jam, while the minimum value T_2 (6.50a) was recorded in local jam. During storage highest fall in taste was recorded local jam T_2 (20%), in compare minimum fall was observed in Tarnab jam T_0 (14.29%).

Texture

The initial texture value of Tarnab jam, Multinational jam & Local jam was T_0 (8.1), T_1 (8), and T_2 (7.9) which was gradually decreased to 6.6, 6.2 and 6 respectively during storage (Table 6). The mean values for intervals were decreased from 8c to 6.27a during storage. The maximum mean value T_0 (7.14) was noted in Tarnab jam, while the minimum value T_2 (6.76b) was recorded in local jam. During storage highest fall in texture was recorded local jam T_2 (24.08%), in compare minimum fall was observed in Tarnab jam T_0 (18.52%) (Table5). The statistical analysis showed significantly ($P < 0.05$) results which may be due to the effect of treatment and storage intervals on texture of apple jam during storage. The mean were separated by applying LSD test at 5% probability level (Table 6).

Table I. Effect of the pH value of apple jam during storage

Treatment	storage intervals					% decrease	Means
	0	15	30	45	60		
T ₀	3.81	3.82	3.85	3.92	3.94	3.41	3.87c
T ₁	3.82	3.85	3.87	3.93	3.95	3.40	3.88b
T ₂	3.83	3.87	3.91	3.95	3.99	4.18	3.91a
Means	3.84e	3.85d	3.93c	3.95b	3.98a		

Values having different alphabetical letters are significantly (P<0.05)

Table 2. Effect of the TSS value of apple jam during storage

Treatment	storage intervals					% Dec	Mean
	0	15	30	45	60		
T ₀	62	63	64	65	66	6.45	64.00c
T ₁	66	67	67	68	69	4.55	67.40b
T ₂	64	64	66	67	68	6.25	65.80a
Mean	64.00d	64.67d	65.67c	66.67b	67.67a		

Values having different alphabetical letters are significantly (P<0.05)

Table 3. Effect of the acidity value of apple jam during storage

Treatment	storage intervals					% increase	Means
	0	15	30	45	60		
T ₀	0.21	0.31	0.42	0.43	0.45	53.33	0.36b
T ₁	0.22	0.23	0.33	0.34	0.41	46.34	0.31a
T ₂	0.31	0.32	0.37	0.42	0.47	34.04	0.38a
Means	0.25c	0.29c	0.37b	0.40ab	0.44a		

Values having different alphabetical letters are significantly (P<0.05)

Table 4. Effect of the color value of jam during storage

Treatment	storage intervals					% decrease	Mean
	0	15	30	45	60		
T ₀	8	7	7	6.6	6.5	18.75	7.02b
T ₁	8	7	6.7	6.5	6.4	20.00	6.92a
T ₂	7.7	7	6.6	6.3	6	22.08	6.72a
Means	7.90d	7.00d	6.77c	6.47b	6.30a		

Values having different alphabetical letters are significantly (P<0.05)

Table 5. Effect of the taste value of jam during storage

Treatments	storage intervals					% decrease	Mean
	0	15	30	45	60		
T ₀	7	7	7	6	6	14.29	6.60a
T ₁	8	7	6	6	6.5	18.75	6.70a
T ₂	7.5	7	6	6	6	20.00	6.50a
Means	7.50c	7.00c	6.33bc	6.00ab	6.17a		

Values having different alphabetical letters are significantly (P<0.05)

Table 6. Effect of the taste value of jam during storage

Treatment	storage intervals					% decrease	Means
	0	15	30	45	60		
T ₀	8.1	7.1	7.2	6.7	6.6	18.52	7.14b
T ₁	8	7	6.5	6.4	6.2	22.50	6.82b
T ₂	7.9	7	6.6	6.3	6	24.05	6.76a
Means	8.00c	7.03c	6.77b	6.47b	6.27a		

Values having different alphabetical letters are significantly (P<0.05)

Table 7. Effect of the overall acceptability of jam during storage

Treatments	storage intervals					% decrease	Means
	0	15	30	45	60		
T ₀	8.4	7	7	6.8	6.7	20.24	7.18b
T ₁	8.3	7	6	6	6.5	21.69	6.76b
T ₂	8	7	6	6	6	25.00	6.60a
Mean	8.23c	7.00c	6.33c	6.27b	6.40a		

Values having different alphabetical letters are significantly (P<0.05)

Overall acceptability

The statistical analysis showed significantly (P < 0.05) results which may be due to the effect of treatment and storage intervals on the overall acceptability of apple jam during storage. The mean were separated by applying LSD test at 5% probability level (Table 7). The initial overall acceptability value of Tarnab jam, Multinational jam & Local jam was T₀ (8.4), T₁ (8.3), and T₂ (8) which was gradually decreased to 6.7, 6.5 and 6 respectively during storage (Table 7). The mean values for intervals were decreased from 8.23c to 6.40a during storage. The maximum mean value T₀ (7.18b) was noted in Tarnab jam, while the minimum value T₂ (6.60a) was recorded in local jam. During storage highest fall in overall acceptability was recorded in local jam T₂ (50%), in compare minimum fall was observed in Tarnab jam (T₀).

CONCLUSION

On the basis of different parameters and analysis, it was observed that the statistically result was showed that the T₀ (Tarnab jam) was found most acceptable both Physiochemically and organoleptically

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Physico-chemical and organolaptic evaluation of gluten free chapatti made from mung bean and rice composite flour

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ABSTRACT

Gluten free chapatti developed from rice and mung bean composite flour was subjected to Physico-chemical and organolaptic evaluation. The chapatti was developed from the mung bean and rice flour at different ratios (20:80, 40:60, 60:40, 80:20, 100:00, and 00:100). The chapatti made from whole rice/mung bean flour was treated as control. Sensorial quality was measured by 9-point hedonic scale. The chapatti developed from mung bean: rice flour ratio of 80:20%, contained 19.07% moisture, 2.82% ash, 7.57% fat, 1.37% fiber and 16.76% protein. The chapatti prepared from the whole mung bean flour contained highest moisture, ash, crude fiber and crude protein; while fat content was highest in chapatti prepared from mung bean: rice flour ratio of 20:80%. The chapatti prepared from mung bean: rice flour ratio of 60:40% achieved highest score for color (8.37) and taste (7.97); while chapatti of mung bean: rice flour ratio of 80:20% was superior in flavor (8.10), texture (8.23) and overall acceptability (8.50). Chapatti prepared from whole mung bean flour contained highest moisture, ash, crude fiber and crude protein; while fat was highest in mung bean: rice ratio of 80:20%. For chapatti with higher physico-chemical properties and superior flavor, texture and overall acceptability, 80:20% mung bean: rice flour ratio is suggested.

Key words: Gluten free chapatti, Rice, Mung bean, Physico-chemical properties, Organolaptic evaluation.

INTRODUCTION

In recent years there has been growing demand for gluten free products driven in part by the increasing prevalence of allergic reactions and intolerance, most notable celiac disease. Although there is no evidence to suggest a single definitive threshold, a daily gluten intake of less than 10 mg is unlikely to cause significant histological abnormalities (Palma *et al.* 2009; Akobeng and Thomas, 2008; Anderson, 2011). Wheat intolerance is perhaps the most common and a diet free of wheat gluten and incorporating other cereals such as barley, oats and legumes can be used to manage the condition. In contrast some extreme forms of celiac disease requires adherence to a strict gluten free diet regime (excluding any wheat like components) as part of a care and restoration program (Holmes *et al.*, 2001). The celiac disease prevalence remains to be significantly under estimated (Fasano and Catassi, 2001) because of diagnostic problems. The factors responsible for that primary life-long intolerance in genetically predisposed individuals are wheat gliadins and prolamins of rye, legumes, rice, barley and possibly oats (Murray *et al.* 1999). The reaction to gluten ingestion by patients suffering from that chronic disease is inflammation of the small intestine leading to the malabsorption of several important nutrients

including minerals, folic acid and fat-soluble vitamins (Kelly *et al.*, 1990; Holmes *et al.*, 2001), likely due to the development of new serological tests (Hischenhuber *et al.*, 2006; de la Barca *et al.*, 2010). Availability of manufactured gluten-free foods in Pakistan is limited. Gluten-free foods imported from foreign countries are very expensive and will never be affordable for masses. Therefore, efforts must be made in getting the food producers and suppliers to create gluten-free foods from locally available ingredients (Rashid and Khan, 2009). The chapatti from gluten free flour has proved to be highly beneficial from human health view point. Legumes mung bean and cowpea supply protein, complex carbohydrates, fiber and essential vitamins and minerals to the diet, which are low in fat and sodium and contain no cholesterol. Mung bean flour contains high graded vegetable proteins and satisfactory level of minerals and vitamins. Mung bean may be the first choice of farmers due to its good taste, easy digestibility, better palatability and acceptable market price (Kabir, 2001). The products from the legumes are considered as low glycaemic index food and low glycaemic index foods is very important in the dietary treatment of diabetes mellitus, increases satiety, facilitate the control of food intake and has other health benefits for healthy subject in

terms of post-prandial glucose and lipid metabolism (Lin, 1999). Regular consumption of pulses may have important protective effects on risk for cardiovascular disease. Due to social reasons, the people in the sub-continent heavily depend on pulses a source of proteins, vitamins and minerals in the daily diet (Tateishi, 1996). Similarly, Rice (*Oriza sativa* L.) is also a major source of food for a significant Pakistani population; and its consumption has increased with increasing human population (Waheed *et al.* 2012). The rice produces gluten free flour and a detailed analysis of nutrient content of rice suggests that the nutrition value of rice varies based on a number of factors (Welch *et al.* 2010). Unless great care is taken, a gluten-free diet can lack the vitamins, minerals, and fiber which are found in wheat and barley. However, this can be mitigated through the consumption of rice and legume grains. Many gluten-free products are not fortified or enriched by such nutrients. Therefore, the present study was carried out on the development and evaluation of gluten free chapatti from rice and mung bean flour. Rice flour is naturally gluten-free, rich in carbohydrates and low in fat. Rice flour and mung bean flour was used as gluten free flour; and hence examined the effects of substitution of rice flour with mung bean flour at various ratios on the physical, textural, and sensory characteristics of gluten free chapatti. Chapatti from whole rice flour and whole mung bean flour were used as control flour composition. The aim of the study was to develop gluten free chapatti using flour mixes of rice and mung bean and analyze for chemical and organoleptic quality of the final product (chapatti).

MATERIALS and METHODS

The studies were carried out at FSPDI (Food Science & Product Development Institute), NARC, Islamabad to evaluate gluten free chapatti made from rice and mung bean flour at different ratios for physico-chemical properties and organoleptic quality. The chapatti was developed from the rice and mung bean flour at different ratios (20:80, 40:60, 60:40, 80:20, 100:00, and 00:100). Rice was obtained with the courtesy of FSPDI Islamabad and the mung bean was purchased from the local market of Islamabad. The samples were packed in plastic bags, labeled and brought to the FSPDI laboratory (NARC), Islamabad. The dough was developed from the flour mixed as per the treatments; while the organoleptic analysis of the final product (chapatti) was done by constituting a panel of judges. The experimental procedures are briefly described as under:

Milling of raw materials

Before milling, the rice and mung bean seed samples were cleaned and washed. Rice and mung bean samples were milled separately in China Mill to prepare rice and mung bean flour and sieved separately.

Development of dough

The dough was made by mixing individual samples with predetermined amount of water for three minutes in mixer and then was allowed to rest for 20 minutes before making dough balls. Dough pieces were rounded and rolled to attain a uniform thickness. Sheet was made by this dough and time to complete it was 2 minutes. The chapatti was cooked on hot plate and after baking from one side it was turned over and baked from the other side. Chapatti was puffed on open flame for 2 to 3 seconds.

Chapatti formulation

Recipe was finalized or optimized after a lot of trials. Now this is optimized recipe.

S. No	Ingredients	Amount in gm/ml
1.	Flour	50g
2.	Salt	0.5g
3.	Baking Powder	0.5g
4.	Water	As per water absorption
5.	Oil	2g
6.	Xanthan gum	1.5g
7.	Zera	0.3g

Chemical analysis

The chapatti samples were analyzed for different chemical parameters as per the procedures and methods given below.

Moisture content (%)

The moisture content of chapatti in different treatment groups was determined by using the procedure described in AACC (2000), method No.44-15A.

Ash content (%): The ash content of chapatti in different treatment groups was determined by the procedure as described by AACC (2000).

Crude fat (%)

The crude fat of chapatti in different treatment groups was determined by using n-hexane as solvent in butchi extraction system according to method as described by AACC (2000), method No.30-20.

Crude protein (%)

Crude protein of chapatti in different treatment groups was determined by auto Kjeldhal analyzer according to the method as described by AACC (2000), method No.46.10. Butchi Auto Kjeldahl Analyzer was used, the instrument automatically performed distillation and titration. Nitrogen % and crude protein % were displayed on the instrument.

Crude fiber

For crude fiber analysis in different treatment groups, 2g sample were digested with 1.25% H₂SO₄ followed by 1.25% NaOH solution (AACC, 2000) Method No.32-10, using the following formula was used.

$$\text{Crude fiber\%} = \frac{\text{Weight loss on ignition}}{\text{Weight of sample}} \times 100$$

Organoleptic evaluation of chapatti

The sensory evaluation was carried out by the panel of 10 judges for various attributes i.e. color, taste, flavor, texture and overall acceptability by a 9-point hedonic scale (Amerine *et al.*, (1965).

Statistical analysis

The data obtained was subjected to Analysis of variance (ANOVA) according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Moisture content (%)

The highest moisture content (21.07%) in chapatti was determined when it was formatted from 100% mung bean flour; followed by 20.76% moisture content in chapatti prepared by 100% rice flour. The moisture in chapatti simultaneously decreased to 19.36, 19.07 and 18.06 % when it was formatted from mung bean: rice flour at the ratio of 20:80, 80:20 and 40:60 %, respectively. However, the minimum moisture content in chapatti (17.80%) was determined when it was prepared from mung bean: rice flour ratio of 60: 40 %. The effect of mung bean: rice flour ratio on chapatti moisture content was inconsistent as it shows moisture similarity under mung bean: rice flour ratios of 80:20 and 20:80 %, and lowest in 60:40 % ratio. However, it was obvious that the moisture content reduced in chapatti when it was prepared from mung bean: rice mixed flour and increased when the chapatti was prepared from whole mung bean or rice flours. Anjum *et al.* (2007) who determined moisture content in the similar range and concluded that the moisture content of chapatti was mainly associated with the ratio of flour from different grain species. Kadam *et al.* (2012) reported 15-18 percent moisture in chapatti from cereal: legume mix flours. Hence, it is quite

clear that moisture content of chapatti is mainly influenced by the ratio of flour mixes.

Ash content (%)

Significant (P<0.05) effect of mung bean: rice flour ratios on ash content of chapatti were observed. The highest ash content (3.28%) in chapatti was obtained when it was prepared from 100% mung bean flour; while ash content in chapatti simultaneously decreased to 2.82, 2.24 and 1.78 % when it was prepared from mung bean: rice flour at the ratio of 80:20, 60:40 and 40:60 %, respectively. The ash content in chapatti further decreased to 1.48% under mung bean: rice flour ratio of 20:80 %; while the minimum ash content in chapatti was determined when it was prepared from whole (100%) rice flour. There was a linear effect of mung bean: rice flour ratios on the chapatti ash content; and with increasing the mung bean flour ratio, the ash content was also increased. It was observed that the ash content was higher in mung bean flour as compared to rice flour and chapatti from whole mung bean flour contained 3.28% ash against 1.26% ash in chapatti prepared from whole rice flour. The ash content of chapatti determined by Kadam *et al.* (2012) was 2.08 to 2.70 percent against 0.80 to 3.28 percent in the present study. In the present study the increase in ash content was mainly associated with the increase in mung bean flour ratio and decreased simultaneously with increase in the rice flour ratio. Yadaz *et al.* (2012) also found similar results from their experiment and reported that legumes contained higher ash contents as compared to cereals and mixing legume grain flour with cereals for chapatti development resulted in higher ash contents. Yazynina *et al.* (2008) and Mariotti *et al.* (2011) have also partially supported the findings of the present research and indicated that ash content in the chapatti is associated with the grain species used for the chapatti flour.

Fat content (%)

The fat content of chapatti developed from different ratios of mung bean: rice flour was significant (P<0.05) and highest fat content (8.66%) in chapatti was determined when it was prepared from mung bean: rice flour ratio of 40:60 %; followed by mung bean: rice flour ratio of 80:20, 20:80 and 60:40 % resulting average fat content of 7.57, 7.22 and 7.08 %, respectively. The fat content in chapatti decreased considerably to 5.69 and 5.14 % when chapatti was made from whole rice and whole mung bean flour, respectively. The results clearly indicated some interactive effect of mung bean and rice flour combination and regardless the ratio of flours, mixing of mung bean and rice flours resulted in increased fat content in chapatti. The fat content in chapatti showed similarity when it was prepared from whole mung bean (5.14%) and whole rice flour (5.69%). Moreover, similarity in fat content in chapatti (P>0.05) was also observed amongst mung bean: rice ratios of 20:80, 60:40 and 80:20 %. The results of Gujral *et al.* (2012) and Qayyum *et al.* (2012) also

showed similarity regarding fat content of chapatti they developed from flour mixes of cereals and legume grains. However, Kadam *et al.* (2012) reported fat content of chapatti in the range of 1.53 to 3.45 percent. The higher fat content in chapatti in the present study was mainly associated with the use of cooking oil in dough as well as at the time of baking on chapatti.

Crude fiber content (%)

The highest crude fiber content (1.60%) was found in chapatti when it was made from whole mung bean flour (100:00%); followed by crude fiber content of 1.37, 0.95 and 0.62 % determined in chapatti prepared from mung bean: rice flour ratio of 80:20, 60:40 and 40:60 %, respectively. The crude fiber content in chapatti decreased to 0.29 % when chapatti was made from mung bean: rice flour ratio of 20:80 %; while the lowest crude fiber content of 0.11 % was determined in chapatti made from whole rice flour. The crude fiber content in chapatti prepared from whole mung bean was higher (1.60%) than the whole rice chapatti (0.11%). Generally, it is believed that crude fiber in human diet is beneficial for improving digestibility; hence, mung bean flour contained higher fiber contents than rice flour based chapatti. The crude fiber content of chapatti is markedly influenced by the ratio of flour mixes (Anjum *et al.* 2007; Yadav *et al.* 2012); while Kadam *et al.* (2012) have reported 1.24 to 2.05 percent which was close to the fiber content determined in the present study. However, it is believed that variation in the fiber content is associated with the ratio of flour mixes.

Crude protein content (%)

The maximum crude protein content (18.43%) was determined in whole mung bean flour chapatti (100:00%); followed by crude protein contents of 16.76, 14.29 and 12.24 % determined in chapatti prepared from mung bean: rice flour ratio of 80:20, 60:40 and 40:60 %, respectively. The crude protein content in chapatti declined to 11.08 % when chapatti was made from mung bean: rice flour ratio of 20:80 %; while the lowest crude protein content of 6.56 % was determined in chapatti made from whole rice flour (00:100%). The crude protein content in chapatti prepared from whole mung bean was higher (18.43%) than the whole rice chapatti (6.56%). The results clearly suggested that mung bean flour contains high protein contents and its inclusion in the rice or wheat based chapatti will be highly beneficial for the human health. The protein content in chapatti is generally associated with the grain species (Cappa *et al.* 2013; Anjum *et al.* 2007; Curiel *et al.* 2014) and Kadam *et al.* (2012) reported 11.8 to 15.37 percent crude protein in chapatti produced from cereals and legume flour mixes. The variation in the crude protein of chapatti determined in the present experiment as compared to past workers was mainly linked with the ratio of the flour mixes or use of different grains.

Color of gluten free chapatti

The chapatti color ranked 1st with maximum score of 8.37 when it was developed from mung bean: rice flour ratio of 60:40 %, followed by chapatti made from mung bean: rice flour ratios of 100:00, 80:20 and 00:100 % with average scores of 7.53, 7.37 and 7.03, respectively. Lower scores were awarded by the judges to the product (chapatti) prepared from mung bean: rice flour ratios of 40:60 (6.93) and 20:80 % (6.42), lowest being under 20:80 mung bean flour ratio. Statistically, the similarity ($P>0.05$) in color (as perceived by the panel of judges) was observed in chapatti made from mung bean: rice flour ratios of 100:00 and 80:20 40 %; while chapatti made from mung bean: rice ratios of 40:60 and 00:100 % also showed similarity in color quality. The study suggested that color of chapatti made from mung bean: rice flour ratio of 60:40 was extremely liked by the panel of judges. Gujral *et al.* (2012) reported that the chapatti prepared from various flour mixes contained varied color and other sensorial qualities. The score on color of gluten free chapatti was from the judges was associated with the grain species used for achieving flour and recipes finalized for the chapatti.

Taste of gluten free chapatti

Chapatti in taste ranked 1st with maximum score of 7.97 when it was prepared from mung bean: rice flour ratio of 60:40 %, followed by chapatti made from mung bean: rice flour ratios of 80:20, 40:60 and 20:80 % with average scores of 7.23, 7.10 and 6.80, respectively. Lower scores were awarded by the judges to the taste of chapatti prepared from whole mung bean flour (6.53) and lowest score on chapatti taste was awarded when it was prepared from whole rice flour. It was observed that mung bean: rice flour ratios of 60:40 produced extremely tasty chapatti, followed by mung bean: rice flour ratio of 80:20 %. The chapatti prepared from whole mung bean flour or whole rice flour could not highly satisfy to the judges. Hence, the mixing of mung bean and rice flour at the ratio of 60:40 was an appreciable combination for producing chapatti of highest taste quality. The differences in taste of chapatti were statistically non-significant ($P>0.05$) when prepared from mung bean: rice flour ratios of 40:60, 20:80, 100:00 and 00:100 or between 80:20 and 60:40%. The score on the taste of chapatti in this study was further supported by Shahzadi *et al.* (2005) who reported that chapatti taste was influenced by the storage period and blending of various legumes.

Flavor of gluten free chapatti

The chapatti made from mung bean: rice flour ratio of 80:20 % achieved 8.10 score out of 9, followed by chapatti made from

mung bean: rice flour ratios of 60:40, 40:60 and 20:80 % achieving 7.00, 6.83 and 6.83, respectively. Lower scores were awarded by the judges to chapatti on its flavor when it was prepared from whole mung bean flour (6.33) and minimum score on chapatti flavor was awarded when it was prepared from whole rice flour. This indicated that chapatti flavor improved to a maximum extent when it was developed from mung bean: rice flour ratios of 80:20. Relatively, low scores for flavor were obtained by chapatti prepared from whole mung bean flour or whole rice flour. Thus, the maximum chapatti quality in regards to its flavor can be achieved by mixing mung bean and rice flour at the ratio of 80:20 %. Statistically, the differences in flavor of chapatti were non-significant ($P>0.05$) when prepared from mung bean: rice flour ratios of 60:40, 40:60 and 20:80 %. The protein content in milled rice ranges from 4 to 14% and mean protein 6.3 to 9.2%; while cowpea and mung bean protein. Sensoric attributes of chapatti such flavor decreased during the storage period and blending of various legumes (Shahzadi *et al.* 2005). Gujral *et al.* (2012) also partially agreed the findings of the present research and stated that flavor of the chapatti is mainly depended upon grain species and quantities of mixes.

Texture of gluten free chapatti

As perceived by the judges, the texture quality of chapatti made from mung bean: rice flour ratio of 80:20 % was highest scoring 8.23 points out of 9, followed by chapatti made from mung bean: rice flour ratios of 60:40, 40:60 and 20:80 % achieving scores of 6.93, 6.63 and 6.63 points, respectively. Relatively, lower scores were awarded by the judges to chapatti on its texture when it was prepared from whole mung bean flour (6.43) and minimum score on chapatti texture was awarded when it was prepared from whole rice flour (5.27). According to the sensory analysis, the chapatti quality in terms of its texture was highest when made from mung bean: rice flour ratios of 80:20. There were marked differences in scores for texture when chapatti was prepared from whole mung bean flour (6.43) and whole rice flour (5.27). This indicates that in

texture, mung bean flour played more effective role to achieve improved texture of chapatti. The differences in texture of chapatti were statistically non-significant ($P>0.05$) when prepared from mung bean: rice flour ratios of 60:40, 40:60 and 20:80 %. Gujral *et al.* (2012) also reported similar trend of findings to that of the present research regarding the texture of chapatti they prepared from flour mixes. Shahzadi *et al.* (2005) determined sensoric attributes of chapatti and found that overall acceptability texture of chapatti was affected by the storage period and blending of chapatti.

Overall acceptability of gluten free chapatti

The overall acceptability of chapatti made from mung bean: rice flour ratio of 80:20 % was highest achieving 8.50 hedonic points out of 9, followed by chapatti made from mung bean: rice flour ratios of 60:40, 40:60 and 20:80 % achieving scores of 7.27, 7.27 and 6.90 points, respectively. Relatively, lower scores were awarded by the judges to chapatti on its overall acceptability when it was prepared from whole mung bean flour (6.47) and minimum score on chapatti overall acceptability was awarded when it was prepared from whole rice flour (5.97). According to the sensory analysis, the chapatti quality in terms of its overall acceptability was highest when made from mung bean: rice flour ratios of 80:20. There were marked differences in scores for overall acceptability when chapatti was made from whole mung bean flour (6.47) and whole rice flour (5.97). This indicates that in overall acceptability was more in case of mung bean flour inclusion than the rice flour. The differences in overall acceptability of chapatti were statistically non-significant ($P>0.05$) when prepared from mung bean: rice flour ratios of 60:40, 40:60 and 20:80 %. The overall acceptability of the chapatti was decreased with the decrease in mung bean flour ratio and such observation is further supported by Yadav *et al.* (2012) who reported that overall acceptability of chapatti on the basis of 9 point hedonic scale was in the range of 6.8 ± 0.2 to 8.4 ± 0.2 and the trend of overall acceptability followed the findings of the present research for this parameter.

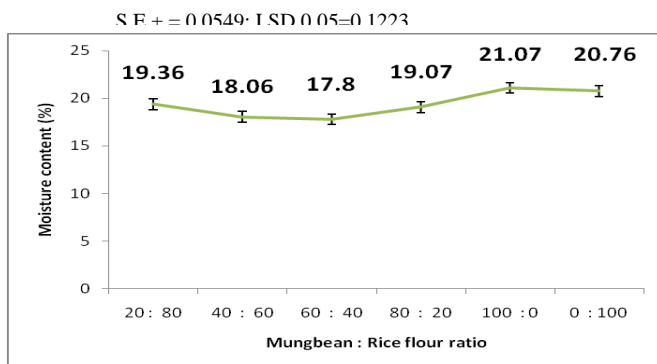


Figure1. Mean moisture content (%) of mung bean and rice

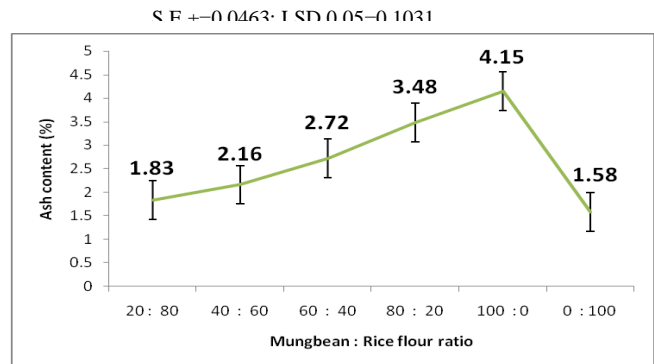


Figure2. Mean ash content (%) of mung bean and rice

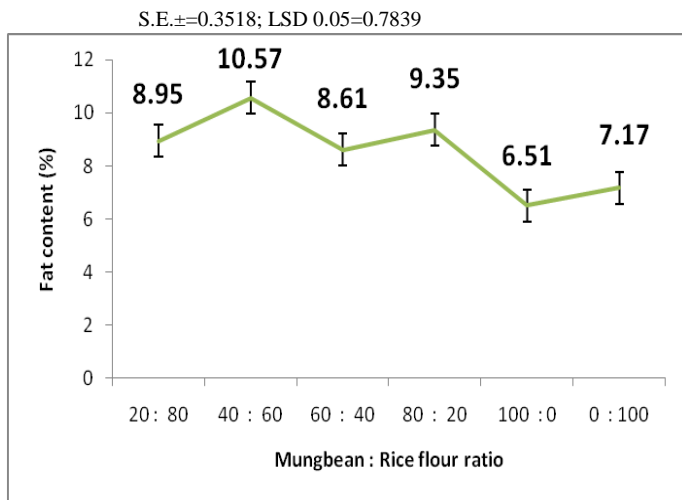


Figure 3. Mean fat content (%) of mung bean and rice chanatti at various ratios

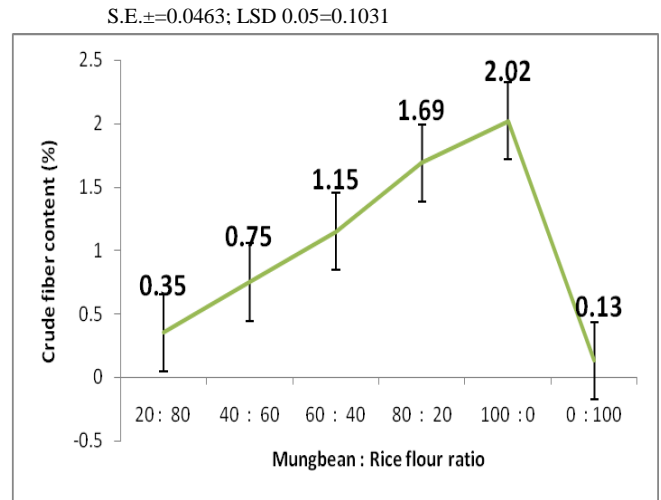


Figure 4. Mean fibre content (%) of mung bean and rice chanatti at various ratios

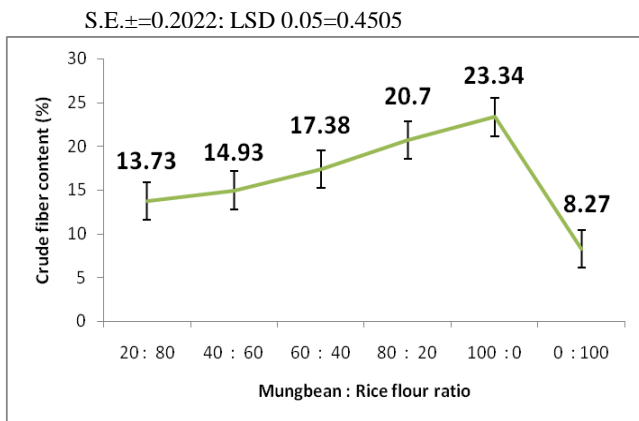


Figure 5. Mean crude protein content (%) of mung bean and rice chanatti at various ratios

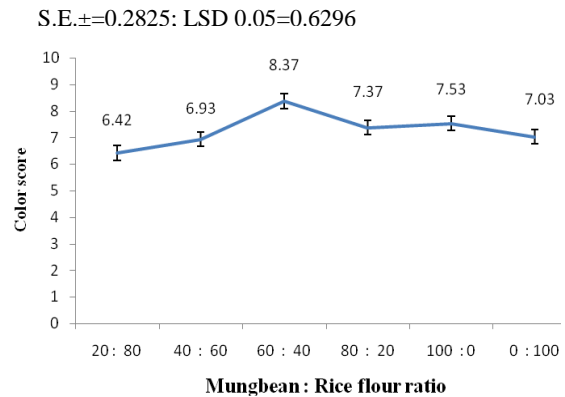


Figure 6. Score on color of mung bean and rice flour chanatti at various ratios

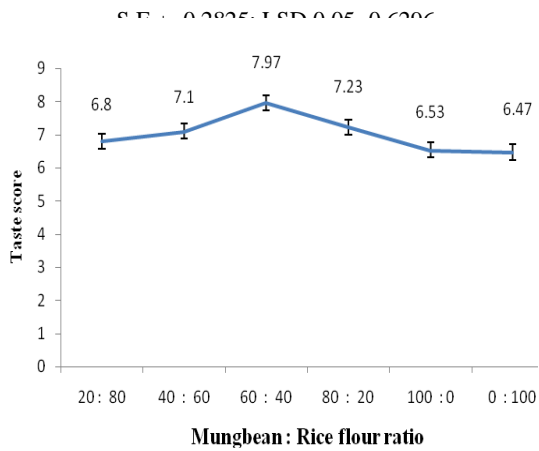


Figure 7. Score on taste of mung bean and rice flour chapatti at various ratios

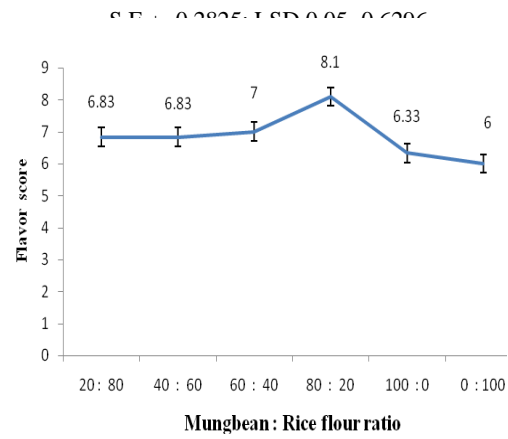


Figure 8. Score on flavor of mung bean and rice flour chapatti at various ratios

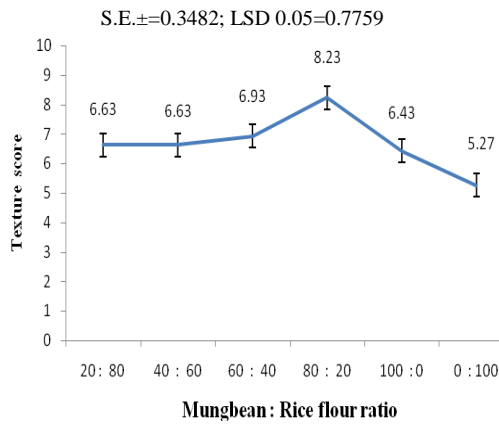


Figure 8. Score on texture of mung bean and rice flour chapatti at various ratios

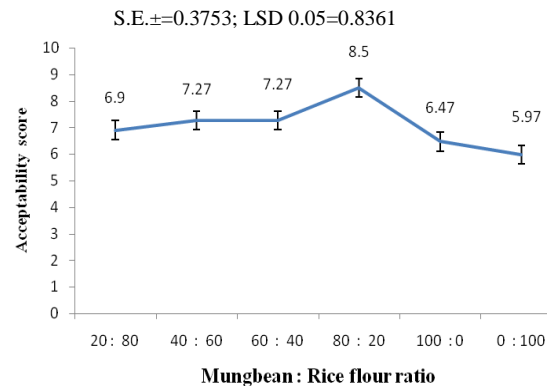


Figure 9. Score on acceptability of mung bean and rice flour chapatti at various ratios

CONCLUSION

The chapatti prepared from the whole mung bean flour contained highest moisture, ash, crude fiber and crude protein; while fat content was highest in chapatti prepared from mung bean: rice flour ratio of 20:80 %. The chapatti made from the mung bean: rice flour ratio of 60:40 % achieved highest score for color and taste; while chapatti prepared from mung bean: rice flour ratio of 80:20 %

obtained highest score on flavor, texture and overall acceptability. For preparing chapatti with higher physico-chemical properties, 80:20% mung bean: rice flour ratio may be maintained. For preparing chapatti with superior flavor, texture and overall acceptability, mung bean: rice flour ratio should be maintained as 80:20 %. Xanthan gum may be used to achieve dough for extended keeping quality of chapatti prepared from mung bean: rice composite flour.

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Comparative study of bread prepared with and without germinated soyabean (*Glycine max*) flour

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ABSTRACT

The supplementation of wheat flour with high lysine legume flours has positive effects on the nutritional value of bread. In present study, germinated and ungerminated soya flour blends were prepared and supplemented in bread in variable proportions (10% and 20% of each) to check its impact on quality and sensory attributes of bread. The results showed that there was a significant increase in protein, ash and crude fat contents due to increase in the level of germinated and ungerminated soya flour. However, the moisture and crude fibre contents of composite flours containing germinated and ungerminated soya flour decreased with increased level of supplementation. Mean values for physical analysis (loaf volume, specific volume, weight loss and force for texture) were significantly higher in breads prepared with germinated soya bean flour. The scores assigned to sensory parameters of breads like volume, color of crust, symmetry, color of crumb, texture, taste and aroma decreased significantly by increasing the level of germinated and ungerminated soya flour in wheat flour while color of crust and taste slightly improved. The scores given to overall acceptability of bread prepared from composite flour supplemented with 10% germinated soya flour was highest (8 out of 9).

Key words: Composite bread, protein energy malnutrition, supplementation, amino acid profile, grain legumes

INTRODUCTION

Bread is an important cereal product which is consumed globally in relatively large amounts (Bakke and Vickers, 2007). Type of bread and its production techniques vary greatly from region to region (Martin, 2004). It is the basic source of macro-nutrients for the people. However, white bread is considered poor in nutrition owing to its deficiency of some essential amino acids such as lysine, threonine and tryptophan as well as dietary fiber (Aghamirzaei *et al.*, 2013).

A lot of research work have been carried out and still continue to increase the use of composite flour in which flour made from crops locally grown, having high protein contents replace a part of wheat flour for bread production (Giami *et al.*, 2004). Other ingredients which may be suitable for addition include flours of other cereals, malt flour, legume flour, soya flour, emulsifiers, fat, yeast foods, milk and milk products, gluten and fruits (Dhingra and Jood, 2001). Soya bean (*Glycine max*), a famous legume, belongs to family Leguminosae and sub family Papilionideae. It is an important source of protein for both human and animal consumption and is also an important source of edible oils and fats

(Alabi *et al.*, 2001). The protein content of soybean is about 2 times of most other legumes, 4 times of wheat grain, 6 times of rice grain, 12 times of milk and 4 times of egg (Alam *et al.*, 2013). Since soybeans contain reasonable amounts of these anti-nutritional factors, an appropriate and convenient method of processing is required in order to render them safe, palatable, digestible and nutritious (Lasekan *et al.*, 2004). Germination is a method of soaking and leaving the legume seeds until they start to germinate. This process is associated with increase in the nutritional attributes of soya bean (Kumar *et al.*, 2010). Germinated soya flour supplemented to wheat flour increases the aesthetic acceptability and also improves the mouth feel of the product (Malomo *et al.*, 2012). Our objective is to produce wheat sprouted soya flour bread, evaluate the effects of germinated and ungerminated soya flour on the loaf qualities, establish sensory qualities of the loaf and determine the optimum level of germinated and ungerminated soya flour for acceptable loaf with consumers' point of view.

MATERIALS AND METHODS

Procurement of raw materials

The present study was conducted in the Post Graduate Research Lab of National Institute of Food Science and Technology, University of Agriculture, Faisalabad. Soya bean was purchased from local market and then they were subjected to sample preparation. Chemicals and standards used for present study were purchased from Merck (Merck, KGaA, Darmstadt, Germany) and Sigma-aldrich (Sigma-aldrich, Tokyo, Japan).

Germination

Soya bean seeds were washed and cleaned with tap water before soaking for 6 h in room temperature (28°C). After 6 hr, samples were placed under wet muslin cloth and left soaked for 48 h in room temperature (28°C) without direct contact with sun light (Yasmin *et al.*, 2008).

Milling of grains

The procured wheat, ungerminated and germinated soya bean grains were subjected to cleaning and tempering before milling. The flour for the production of bread was obtained by milling the wheat grains in C.W. Brabender Quadrumat Junior Lab Mills made in Duisburg, Germany.

Chemical analyses of wheat flour and legumes

Wheat, ungerminated and germinated soya bean flour were analyzed for ash, moisture, crude protein, crude fiber, crude fat and nitrogen free extract following the prescribed methods of AACC (2000).

Formulating the bread recipe

Different bread formulations were made with different concentrations of germinated and ungerminated soya bean flour as described in Table No. 1. The soy/wheat blends were called T₀, T₁, T₂, T₃ and T₄ as follows:

Bread preparation

Bread was prepared using the above mentioned formulation for all the treatments using standard procedure (AACC, 2000) method No. 10-10B.

Analysis of bread

Chemical analysis

Wheat, ungerminated and germinated soya bean flour were analyzed for ash, moisture, crude protein, crude

fiber, crude fat and nitrogen free extract following the prescribed methods of AACC (2000).

Physicochemical analysis

Color

The color of bread was determined with the help of color meter color test II, (serial no. 95808, made in Germany) according to method of Lara *et al.*, (2010).

Loaf volume

Loaf volume was measured by rapeseed displacement method (Greene and Bovell-Benjamin, 2004).

Specific volume

The specific volume of bread was determined by following the protocol of Peressini and Sensidoni (2009).

Weight loss

Dough and baked loaf was weighed and percent weight loss was calculated by following the guidelines of Majzoobi *et al.* (2011).

Texture analysis

Textural analysis of bread was carried out by using Texture Analyzer (TA-XT plus Texture Analyzer by texture technologies corp and by stable micro systems made in Hamilton, Canada) according to method of Piga *et al.*, (2005).

Sensory Analysis

Sensory analysis of product was performed following the method described by Lawless and Heymann, (1998).

Statistical analysis

The obtained data was subjected to statistical analysis to determine the level of significance and to draw valid conclusions (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

Proximate analysis of bread

Proximate analysis of each treatment was done and mean values are shown in Table No. 2 Analysis of variance for the moisture presented significant variation in various treatments. The lowest value of moisture content was for T₀ (31.2%) and highest value of moisture content was in T₄ (34.83%). This increase in moisture content can partially be attributed to the fact that soya flour has high water binding capacity. Increase in moisture content is associated with increase in dietary fibre contents

(Maneju *et al.*, 2011). There was a gradual increase in protein content of bread with increase in concentration of germinated and ungerminated soya flour. The highest protein content was in T₄ (12.65%) and the lowest in T₀ (8.95%) followed by T₁, T₂ and T₃ having 9.36%, 10.22% and 10.23% protein content respectively. The content of crude fiber in T₀, T₁, T₂, T₃ and T₄ was 1.8%, 2.77% and 3.67%, 1.89% and 2.04% respectively. The crude fibre most likely from the hull of soy beans (Islam *et al.*, 2007). According to Gomez *et al.* (2002), the main problem of dietary fibre addition in baking process is the reduction of loaf volume and the different texture of the breads obtained. These deleterious effects of fiber on dough structure and loaf volume are due to the dilution of gluten network which impairs gas retention rather than gas production (Dewettinck *et al.*, 2008).

The lowest value of crude fat was showed by T₀ (3.35%) and highest value was showed by T₄ (4.98%). While the values of crude fat of T₁, T₂, T₃ were 4.36%, 3.80% and 3.25% respectively. Mean values for crude fat demonstrated that by increasing level of soybean in bread, fat contents going to increase. Soya bean, from which the soya-flour was produced is an oil seed, must have contributed most of the oil content to the product. Higher oil contents ill affect the shelf life stability of bread (Weiss, 2000). The results showed that T₀ has 1.87% ash contents followed by T₁ 1.91%, T₂ 1.94%, T₃ 1.94% and T₄ has 1.99%. It reveals that the ash content of bread increases by increase in soya flour level while ash contents are lower in germinated soy bread than ungerminated soy bread. The mean value of NFE content of different formulated bread was 52.83%, 48.54%, 44.31%, 48.69% and 45.98% of T₀, T₁, T₂, T₃ and T₄ respectively. The results showed that NFE contents decreased with the increased level of soya flour in bread. The same trend for proximate composition of soy-wheat composite bread was reported in the findings of Ndife *et al.* (2011) and Jideani and Onwubali (2009).

Physical analysis of soy composite bread

Different physical analyses were carried out for composite bread. Mean values regarding physical parameters are shown in Table No.3. The highest mean for L* value (lightness property) was observed in T₀ (68.29) and lowest in T₄ (61.55). Mean L* values reported that lightness of bread increased by the addition of germinated and ungerminated soya flour. The analysis of variance regarding a* value (redness property) of bread color at different levels of soya flour was also significant. Reported mean values showed that with the addition of soya flour, b* value (yellowness property) of bread color was greatly affected. The volume of bread was in negative correlation with the level of soya flour in composite bread. The highest value (963 cm³) for volume of bread was for the T₀ (treatment containing 100% wheat flour) and the lowest (780 cm³) in T₄ (20% ungerminated soya flour). Our mean values are similar to the findings of Malomo *et al.*, (2012), who found that the volume of bread decreased with addition of soya flour. It defined that the specific volume has decreasing trend with the addition of soya flour. Bread with 100% wheat flour had 4.70 cm³/g while T₁, T₂, T₃ and T₄ bread with had 4.55 cm³/g, 4.23 cm³/g, 4.20 cm³/g and 3.98 cm³/g respectively. Weight loss of bread after baking was highest (16%) for bread which had 20% addition of ungerminated soya flour. With the addition of 10% germinated soya flour, the weight loss was 3%. Bread hardness ranged from 1.90 kg to 2.40 kg among bread of various levels of germinated and ungerminated soya flour. The highest hardness showed by the bread prepared from 20% germinated soya flour and lowest for T₀. As gluten contents are responsible for hardness of bread, germinated and ungerminated soya flour have greatly influence on gluten properties of bread. Soya flour has different water binding capacity from wheat, which affects the moisture level of bread and gluten development. Due to improper development of gluten, the texture of bread became soft by every addition of soya flour specially ungerminated soya flour. This water absorption might be because of more water retention by these blends as a result of higher protein content

Table 1. Treatment plan

Treatments	Wheat Flour %	Germinated Soya flour %	Ungerminated Soya flour %
T ₀	100	0	0
T ₁	90	10	0
T ₂	80	20	0
T ₃	90	0	10
T ₄	80	0	20

Table 2. Proximate composition of soy composite bread

Proximate composition	T ₀	T ₁	T ₂	T ₃	T ₄
Moisture	31.2c	32.17bc	32.58b	33.9a	34.83a
Ash	1.87b	1.94ab	1.99ab	1.91ab	1.94a
Crude protein	8.95d	9.36c	10.23b	10.22b	12.65a
Crude fiber	1.8e	2.77b	3.67a	1.89d	2.04c
Crude fat	3.35d	4.36d	4.80b	4.25c	4.98a
NFE	52.83a	48.54b	44.31c	48.69b	45.98c

Means carrying same letters are not significantly different

Table 3. Physicochemical analysis of soy composite bread

Treatment	L*	a*	b*	Volume(cm ³)	Specific volume	Wt. loss (%)	Texture
T ₀	68.29a	1.13e	13.61d	963a	4.70a	15.96a	1.98d
T ₁	62.53b	2.69b	15.78c	945a	4.55a	15.06b	2.11c
T ₂	63.88b	2.21c	15.41c	908b	4.23b	14.77b	2.34a
T ₃	62.12b	1.75d	16.71b	895b	4.20b	14.98b	2.25b
T ₄	61.55b	3.22a	18.20a	780c	3.98c	14.12c	2.30ab

Means carrying same letters are not significantly different

Table 4. Sensory evaluation of soy composite bread

Treatments	Volume	Symmetry	Crust color	Crumb color	Taste	Texture	Aroma	Overall acceptability
T ₀	8.0a	8.0a	7.60c	8.10a	7.60a	7.80a	7.80a	7.80a
T ₁	7.80ab	7.90a	8.40ab	7.40b	7.80a	7.40b	7.70a	8.00a
T ₂	7.30bc	7.80ab	8.60a	6.90cd	7.60a	7.40b	7.0c	7.70a
T ₃	7.40c	7.60bc	8.30b	7.10c	7.20b	7.30b	7.40b	7.30b
T ₄	6.80d	7.50c	8.40ab	6.70d	6.90c	6.90c	6.70d	7.00b

Means carrying same letters are not significantly different

as compared with wheat flour. Selvaraj & Shurpalekar (1982) also reported that water absorption capacity increases by almost 1% when every 2% of soy flour in wheat flour was increased.

Sensory Evaluation

Mean values showing score for sensory attributes are presented in Table No. 4. The volume of bread was in negative correlation with the level of supplementation of germinated and ungerminated soya flour in wheat flour. The highest score for volume of bread was for the T₀. For color of crust, T₂ showed highest mean score. The mean score for color of crust of bread increased as we increased the level of soya flour into wheat flour. The crust color becomes darker as we increased the level of soya flour into wheat flour for bread making. Darker brown bread appearance could be due to the increase in fiber contents (Hu et al., 2007). Browning of the breads may also be due to caramelization and millard reactions because the protein contributed by soya bean flour must have reacted with sugars during baking (Dhingra and Jood, 2001).

T₀ showed highest mean score for bread symmetry (8.0) followed by T₁ (7.90), T₂ (7.80), T₃ (7.60) and T₄ (7.50). Our results reported that the addition of soya flour into bread provides lower symmetry than 100% wheat flour bread. Reduction in the bread symmetry is due to the lack of gluten proteins in the soya flour which is necessary for better texture of bread. Supplementation of germinated and ungerminated soya flour in bread and crumb color had negative correlation with each other. The addition of soya flour slightly affected the taste of

bread. panelist complained of beany taste and aroma from the soya bean flour in the composite breads. Serrem *et al.* (2011) resulted that substitutions of soya bean flour into wheat bread were associated with beany taste, aroma and after taste. T₀ showed mean score 7.80 followed by 7.40, 7.40, 7.30 and 6.90 for T₁, T₂, T₃ and T₄ for the texture of bread, respectively. Hard texture due to increased fiber from wheat bran substitution was resulted by Eiman *et al.* (2008). The baking conditions, state of the flour components, such as starch, fibres, protein (gluten) and the amount of water absorbed during dough mixing, all are responsible for the final texture of the breads (Gomez *et al.*, 2003). The highest score obtained by the treatments for aroma was T₀ (7.80) followed by 7.70, 7.0, 7.40 and 6.70 for T₁, T₂, T₃ and T₄ respectively.

Over all acceptability

For over all acceptability, all the treatments were assigned above average scores so all the treatments were acceptable. However, 10% germinated soy-wheat bread was assigned highest scores (8) and 20% ungerminated soy- wheat bread got lowest marks (7). T₀, T₁ and T₂ were non-significant to each other but significantly different from T₃ and T₄. Our results are in close conformity with the findings of Malomo *et al.*, (2012), Conforti & Davis (2006) and Sabanis & Tzia (2009). Baking properties of composite flour are effected and the organoleptic characteristics of the bread due to the dilution of the gluten (Jideani and Onwubali, 2009).

CONCLUSION

Bread was supplemented with soya bean due to its unique amino acid profile. Germination was done to remove anti nutritional substances. Bread with germinated soya bean gave better nutritional and rheological properties than non-germinated soya bean (especially at 10% substitution level of germinated soybean flour) and bread produced from non-germinated flour showed better textural properties and pleasant taste so it can be inferred that germinated soybean flour is more desirable by bakers. Addition of soya flour beyond 10% is responsible for loss of rheological structure of bread. Rheological characters of bread which were lost from germinated soybean flour can be improved by the adding the appropriate additives. These potentials can be helpful in improving the nutritional quality of bread, lowering the protein energy malnutrition, decreasing the cost of production and consequently contributing to ensure food security.

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