Density-dependent Accumulation of Heavy Metals in Spring Wheat (*Triticum Aestivum*) and the Risk Assessment from Weak Alkaline Soils, Northwest of China

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Abstract

In order to investigate the effects of planting density on accumulation of heavy metals in spring wheat, a weak alkaline agricultural district mainly contaminated by heavy metals Cu, Zn, Pb and Cd, was chosen in 2014. Five planting densities (114×10^4, 174×10^4, 249×10^4, 369×10^4 and 519×10^4 plants hectare^{-1}, respectively) were designed and the level of heavy metals in soil and in various parts (root, stem, leaf, husk and grain) of spring wheat were detected, and then the health risk to human health was assessed via hazard quotient. The results indicate that concentrations of Pb and Cd in each part of spring wheat plant are negatively correlated with planting density, similar but weaker trends are observed for those of Cu and Zn. The hazard quotient of heavy metals through food chain is markedly reduced with increasing planting density, especially for Pb and Cd at higher planting densities (369×10^4 and 519×10^4 plants hectare^{-1}). Conclusively, the present study shows that suitable planting density could be a feasible cultivation method for ensuring the safety of wheat products and human health on slightly polluted alkaline soils.

Keywords

Planting Density; Heavy Metals; Spring Wheat; Health Risk; Weak Alkaline Soil

Introduction

Heavy metals pollution in soil has been a worldwide environmental problem because of human activities [17]. Owing to their non-biodegradable, non-thermal degradable and persistant toxic characters, heavy metals are easy to stay in soil and may enter into ground water [6, 12], be taken up by plants and accumulate in human body through food chain, then posing threats to human health [2, 11]. As the second largest crop in the world, wheat as the main food resource for locals is widely grown in northern China [1]. However, wheat grain is no longer safe for consumption because of high soil heavy metals [5]. Although measures have been taken by governments at all levels for protecting the grain crop security, the responses from local farmers are not positive enough for the consideration of yield and economic benefits. So it is urgent to take action to explore a relatively safer planting pattern on spring wheat, especially in slightly polluted and low yield farmland regions.

Moreover, the characteristics of the plant itself to enrich heavy metals have been extensively studied, confirming that the ability essentially depends on plant species. About more than 450 plant species are found to accumulate extraordinarily high amounts of heavy metals, which are termed hyperaccumulators and have the potential to be used for phytoremediation [14]. Wheat belongs to non-hyperaccumulators, but still accumulates high amount of heavy metals, especially in grains [10]. Besides, plant genotypes differ in their uptake, translocation, accumulation of trace metals [4], and similar phenomenon was observed in wheat plants and asparagus bean cultivars [9, 22]. Recently, Hansi et al. found, in lab, that the concentration of Cu in barley shoot would reduce with increasing planting density [8]. All these researches provide important information for breeding crop cultivar that is of low ability to accumulate heavy metals under certain conditions. Nevertheless, it can be different even for the same species to accumulate heavy metals in various types of soil.

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Therefore, the present study aims to (1) make clear about the accumulation ability of spring wheat on heavy metals under different planting densities; (2) clarify whether changing planting densities can be a useful cultivation method for safe production on slightly polluted alkaline soil.

Materials and Methods

Study Area and Planting Density Design

The present study was carried out in Baiyin (103°54′-104°24′E, 36°14′-36°47′N), China. The district of Baiyin is a non-ferrous metal mining and smelting base where the agricultural population is about 1.3 million. Due to the great population and low annual rainfall (about 180 - 450 mm), sewage water was once used for field irrigation, which was the main reason leading to accumulation of heavy metals Cu, Zn, Pb and Cd in local agricultural soil.

Spring wheat (*Triticum aestivum*) is the main crop for local inhabitants, and the variety of Longchun 26 was chosen for sowing. Planting density of local fields was 300 - 400×10^4 plants hectare^{-1}. So, the ideal densities of 150×10^4 (D1), 200×10^4 (D2), 300×10^4 (D3), 400×10^4 (D4) and 550×10^4 (D5) plants hectare^{-1} were designed with each designing plot as 3 m^2 (1.5 m × 2 m) and three replicates. However, the practical planting densities were investigated to be 114×10^4 (D1), 174×10^4 (D2), 249×10^4 (D3), 369×10^4 (D4) and 519×10^4 (D5) plants hectare^{-1}. The field was homogenized before seeding. In order to be realistic, the designed plots were handled in the same way as local people did.

Sample Preparation and Chemical Analysis

Spring wheat was sowed on March 22, 2014 and harvested on June 30, 2014. The whole plant was collected and taken to lab with polyethylene bags. After that, samples were rinsed with tab water and washed three times with deionized water. Then wheat plants were divided into root, stem, leaf, husk and grain. Finally, various parts were dried at 70°C in a constant tempreture oven for 48 h and dry matters were determined. Meanwhile, corresponding topsoils (0~20 cm) were sampled when sampling the plant, dried at room temperature and ground to pass through a 0.15 mm nylon sieve. At least 6 plants were collected from each plot and mixed into a composite sample.

Soil pH was measured in H_2O (1:2.5, soil:water ratio, w/v). Plant sample about 0.5 g was added to 50 ml teflon crucible, digested with HNO_3-$HClO_4$ till the solution became transparent. The solution obtained was cooled and filtered through Whatman neutral filter paper. The filtered solution was then diluted to 25 ml and kept at ambient temperature for further analysis. For soil sample, 0.5 g dry matter was used for digestion with 10 ml HCl, 10 ml HNO_3, 5 ml $HClO_4$ and 3 ml HF. When the mixture became transparent, it was cooled, filtered with Whatman neutral paper and diluted to 25 ml with deionized water.

Determination of the heavy metals in solution was performed on atomic absorption spectrophotometer (AAS, ZEEnit 700P, Analytik Jena, Germany). To ensure the reliability of the detecting results, reagent blanks were used to correct the instrument readings. Quality controls similar to the digestion procedure of plant sample and soil were carried out in digestion of Certified Reference Materials for the Chemical Composition of Biological Sample (GBW 10052, GSB - 30) and Soil Sample (GBW 07402, GSS - 2). The relatively standard deviation (% RSD) was < 5% and the recovery rate was > 95%.

Health Risk Assessment

The noncarcinogenic health risk from consumption of wheat grain is expressed in hazard quotient (HQ) by Eq.(1) [5]. If HQ < 1, the population unlikely to suffer adverse effects. The higher the HQ, the more attention should be paid to.

$$HQ = \frac{DIM}{RfD} \tag{1}$$

Where, RfD is the reference dose of individual metal and is defined as the maximum permissible level of metal that is unlikely to pose deleterious effects on human health. RfD are based on 0.04, 0.3, 0.004 and 0.001 mg/kg/day for Cu, Zn, Pb and Cd, respectively [23]; DIM is the daily intake of individual metal and is expressed in Eq.(2).

$$DIM = \frac{C_{metal} \times D_{intake}}{BW} \tag{2}$$

Where, C_{metal} represents the concentration of individual metal in wheat grain, D_{intake} represents the daily intake rate of grain (0.47 kg /person/ day) and BW represents the average body weight of 65 kg for adults.

Bioconcentration factor (BCF) is an index of accumulation degree of metal in plants, and is calculated by Eq.(3).

$$BCF = \frac{C_{mt}}{C_{ms}} \tag{3}$$

Where, C_{mt} represents the concentration of individual metal in whole aerial part (shoot) or any organ or tissue; C_{ms} is the concentration of individual metal in soil.

Statistical Analysis

All data were computed with statistical package SPSS 19.0 (IBM, Amonk, NY, USA). One-way ANOVA test (LSD) was performed to assess the influence of different planting densities on metal concentrations in wheat plant and soil ($p<0.05$). The histograms were generated by OriginLab pro 9.0 (OriginLab. Inc., USA).

Results and Discussion

Heavy Metal Concentration in Soil

From Table 1, it can be seen that the soil is slightly alkaline, and the total concentrations of the four selected elements are lower than the environmental quality standard for soils of China (GB15618-1995), except for Cd, and the concentrations of metals are also lower than that from the previous work done in this region (60.05 mg/kg, 230.66 mg/kg, 100.7 mg/kg, 5.7 mg/kg for Cu, Zn, Pb and Cd, respectively) [5]. However, concentrations of all the four elements exceeded their background values, indicating that over the past few decades the environment-unfriendly development of industry and agriculture might cause the local soil polluted. The reduced content of heavy metals in this region respects to that of the last ten years may be due to annually crop plant uptake, runoff wash out and leaching [18].

TABLE 1. SOIL pH, SOIL HEAVY METAL CONCENTRATIONS (mg/kg, DW; n=5).

	pH	Cu	Zn	Pb	Cd
Data from the present study	7.75	43.86±3.64	147.68±27.24	54.99±4.71	1.23±0.35
Permissible limits of Chinese standards[a]	>7.5	100	300	350	1
Soil element background values[b]	-	24.1	68.5	18.8	0.116

a: Set by Environment Protection Administration of China(GB15618-1995)
b: From Chinese Soil Element Background Values (1990)

Heavy Metal Concentration in Spring Wheat

It can be seen that the concentrations of four heavy metals in any part of spring wheat from five planting densities are all below the thresholds of plant toxicity [3]. Zn has the highest concentration followed by Cu, and they are two or more times higher than Pb and Cd, which may be due to the essentiality of Cu and Zn for plant physiology, while Pb and Cd are the stress factors to photosynthesis, chlorophyll synthesis and antioxidant enzymes metabolism [15, 16]. Grain always has the lowest concentrations of Cu, Pb and Cd in all plant parts from D1 to D5, except for Zn, which is in consistent with Nan et al.'s report about heavy metal accumulation in wheat grain planted on alkaline soil [13].

The heavy metal concentrations varied with the various parts of wheat (Figure 1), for Cu: root > husk > leaf > stem > grain; for Zn: root > grain > leaf > husk > stem; for Cd: root > leaf > stem > husk > grain and for Pb: root > leaf > husk > grain > stem. The present results are partly different with the former reports about Cu [19], Pb and Cd [20]. Reasons for these differences may be the soil properties, plant species or cultivars and ratio of the bioavailable heavy metals in soil [21].

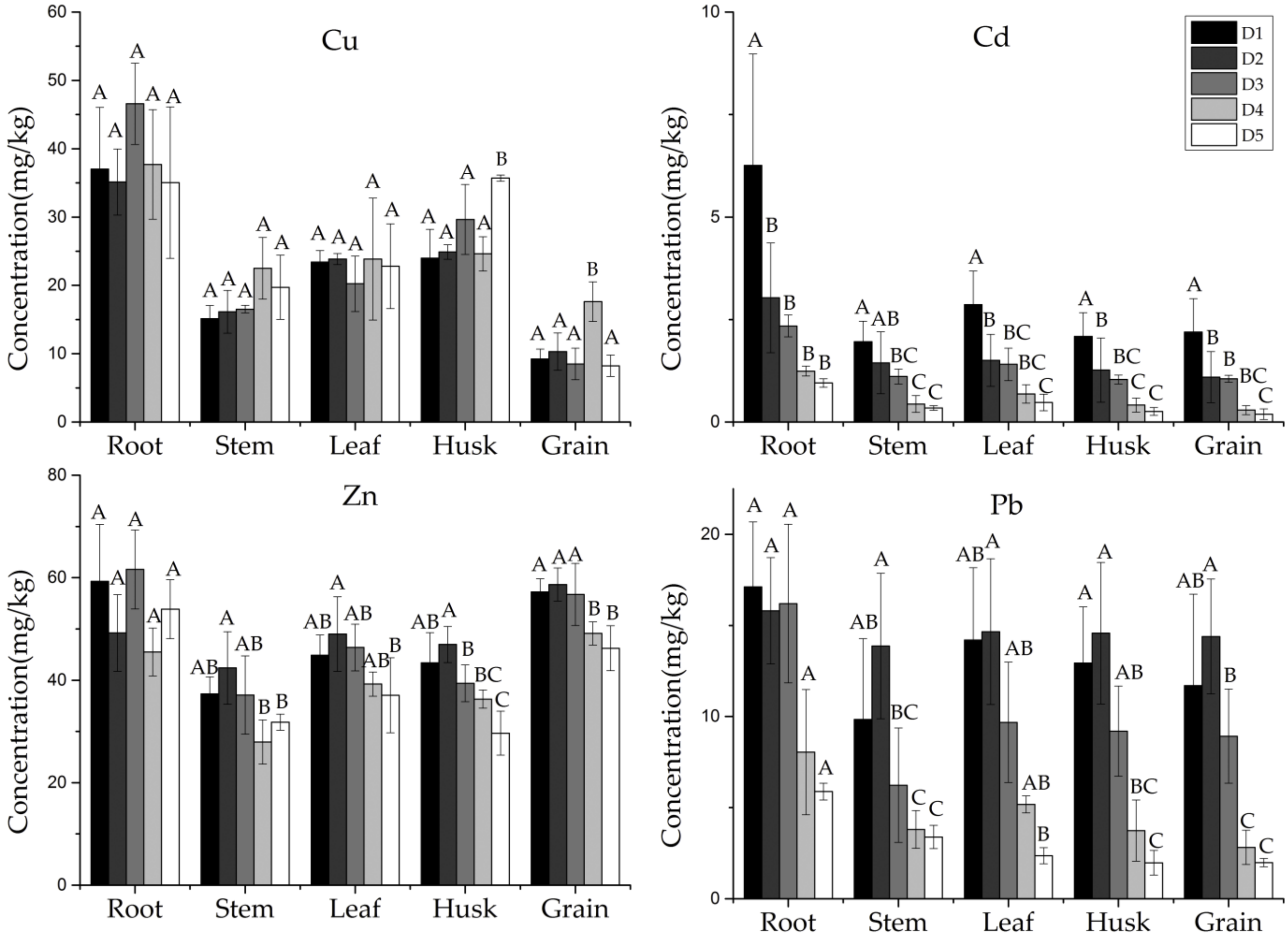

FIGURE 1. HEAVY METAL CONCENTRATION IN EACH PART OF SPRING WHEAT FROM DIFFERENT PLANTING DENSITIES IN BAIYIN DISTRICT. VALUES WITH DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT AT P<0.05(A, B AND C INDICATE DIFFERENCES AMONG DIFFERENT PLANTING DENSITIES).

TABLE 2. PEARSON'S CORRELATION BETWEEN PLANTING DENSITY AND TISSUES OF SPRING WHEAT (n=5).

		Root	Stem	Leaf	Husk	Grain
Cu	Planting density	-0.650	0.303	-0.300	0.681**	0.090
Zn		-0.131	-0.554*	-0.614*	-0.834**	-0.787**
Pb		-0.616*	-0.708*	-0.650**	-0.803**	-0.823**
Cd		-0.731**	-0.824**	-0.790**	-0.808**	-0.784**

* and ** mean difference or significant difference at p<0.05 or p<0.01(2 tailed).

Among the experimental densities of D1-D5, it is showed that the concentrations of heavy metals Pb, Cd and Zn in wheat are negatively related with the wheat planting density (Table 2), which suggests that in the selected five densities the higher the density, the lower the concentration of Pb, Cd and Zn in wheat tissues (except for Zn in root), while there is no distinct regularity of Cu content in wheat.

Feasibility of Reducing Heavy Metal Content in Wheat Grain

Heavy metals in grain of different plant densities are shown in Table 3. In all planting densities, Cu concentration, except for D4, is under the limit of China Standards. Only a significant decrease is observed at D5, though it is slightly lowered compared to that at D1. Meanwhile, Zn concentration at D1, D2 and D3 is beyond the limit (50 mg/kg), then it goes to the safe range at D4 and D5. Similar but much obvious tendency is found in Pb and Cd of grain (Table 3). Although they are still above the limits at the highest density (D5), the concentrations have already decreased about 78% and 75% for Pb and Cd , respectively, compared to previous work done in the same region [5]. Above all, the higher the planting density is, the lower the Pb and Cd concentrations in wheat grain are, which means elevating planting density has a significant effect on reduction of Pb and Cd and a weaker effect on Cu and Zn in wheat grain.

The safety of grain is directly related to the consumers' health. Therefore, the assessment of health risks to inhabitants posed by heavy metals via intake of grain is done (Table 4). HQs of Cu, Zn Pb and Cd are above 1 at all five planting densities, indicating there are potential noncarcinogenic effects to the consumers. Pb has the highest HQ followed by Cd in all densities, while a significant decrease of HQ_{Pb} is found with the increasing of planting density, and the integrative risk also significantly decreased from D3 to D5 respect to the previous work (21.98 of Pb and 6.72 of Cd in local planting density [5]. So planting density could be considered as an alternative cultivation way for safer production on slightly polluted soils.

However, yield is under consideration as well as grain safety. As is known, production would increase with increasing planting density in certain ranges [7]. In present study, the grain production has elevated with planting density, getting to the maximum at D4 and going down at D5 (Table 4), but no significance appears among the five selected densities ($p<0.05$). Meanwhile, the HQs of Pb and Cd at D4 and D5 significantly declined compared to that in D1, D2 and D3, thus, planting density is again confirmed to be feasible for safer production on slightly contaminated soils. In addition, further study is required for the most appropriate density, which will keep health risk and yield in balance.

TABLE 3. CONCENTRATIONS OF HEAVY METALS IN WHEAT GRAIN. (mg/kg, DW; n=3)

Densities	Cu	Zn	Pb	Cd
	Mean±StDev	Mean±StDev	Mean±StDev	Mean±StDev
D1	9.24±1.43^{a}	57.25±2.55^{a}	11.70±0.81^{ab}	2.19±0.81^{a}
D2	10.31±2.71^{a}	58.66±3.25^{a}	14.39±3.15^{a}	1.09±0.62^{b}
D3	8.50±2.31^{a}	56.75±6.06^{a}	8.92±2.58^{b}	1.06±0.08^{b}
D4	17.62±8.89^{b}	49.12±2.26^{b}	2.82±0.94^{c}	0.29±0.11^{bc}
D5	8.26±1.56^{b}	46.23±4.37^{b}	1.99±1.22^{c}	0.19±0.13^{c}
Limits	10^{m}	50^{n}	0.2^{x}	0.1^{x}

$^{a,\ b,\ c}$ mean significant differences among different densities ($p<0.05$).
$^{m,\ n,\ x}$ represent the permissible limits of China Standards GB15199-94, 13106-91 and 2762-2012, respectively.

TABLE 4. HQ OF HEAVY METALS IN GRAIN TO INHABITANTS AND YIELDS(KG/HA) OF WHEAT GRAIN IN DIFFERENT DENSITIES

Densities	Yields(n=3)	HQ			
		Cu	Zn	Pb	Cd
D1	4153.8±745.8^{a}	1.67^{a}	1.37^{a}	21.2^{ab}	15.8^{a}
D2	2918.6±571.9^{a}	1.86^{a}	1.41^{a}	26.0^{a}	7.90^{b}
D3	5477.2±1082.2^{a}	1.54^{a}	1.36^{a}	16.13^{b}	7.64^{b}
D4	5541.2±802.6^{a}	3.18^{a}	1.18^{b}	5.10^{c}	2.09^{bc}
D5	5330.1±2325.6^{a}	1.48^{a}	1.11^{b}	3.59^{c}	1.37^{c}

$^{a,\ b,\ c}$ mean significant differences among different densities ($p<0.05$).

Effect of Planting Density on BCFs

BCFs of the four metals in grain followed the sequence of Cd > Zn > Cu > Pb (Table 5), which is different from Dai et al's result of Zn > Cd > Cu > Pb [5]. The difference of BCFs may be due to cultivars, which could cause variety of ability to accumulate heavy metals. The BCFs vary in different parts of spring wheat, but only the BCFs of Cd are relatively higher, especially in root at all densities (except Cu in root at D3) are > 1 (values in bold, Table 5), suggesting that Longchun 26 could be an ideal excluder for Cd. Thus, planting density negatively affect the bioaccumulating ability of wheat for heavy metals, meanwhile, can ensure the safety of the edible part.

TABLE 5. THE BCFS OF WHEAT TISSUES

Elements	Densities	BCFs				
		Root	Stem	Leaf	Husk	Grain
Cu	D1	0.93	0.38	0.59	0.6	0.23
	D2	0.76	0.35	0.52	0.54	0.22
	D3	**1.02**	0.36	0.45	0.66	0.19
	D4	0.9	0.54	0.57	0.59	0.42
	D5	0.95	0.54	0.62	0.97	0.22
Zn	D1	0.35	0.22	0.27	0.26	0.34
	D2	0.31	0.27	0.31	0.29	0.37
	D3	0.41	0.25	0.31	0.26	0.38
	D4	0.34	0.21	0.29	0.27	0.37
	D5	0.33	0.21	0.23	0.19	0.3
Pb	D1	0.25	0.14	0.20	0.19	0.17
	D2	0.25	0.21	0.23	0.23	0.22
	D3	0.29	0.11	0.17	0.16	0.16
	D4	0.15	0.07	0.1	0.07	0.05
	D5	0.12	0.07	0.05	0.04	0.04
Cd	D1	**3.3**	0.98	**1.44**	**1.05**	**1.09**
	D2	**1.9**	0.91	0.95	0.8	0.69
	D3	**1.8**	0.83	**1.04**	0.78	0.81
	D4	**1.3**	0.47	0.70	0.42	0.30
	D5	**1.2**	0.43	0.62	0.32	0.25

Conclusions

The present study aims to make sure the effect of different planting densities on the accumulation of heavy metals in spring wheat. The results have shown that with the increasing of planting density, the concentrations of Pb and Cd in spring wheat have decreased significantly, while that of Zn especially Cu are barely affected, and heavy metals in aerial parts, especially in grain could be lowered, which lead to a reduction of health risk. In summary, changing planting density of spring wheat could be a feasible method for safer production on the slightly polluted alkaline soil.

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Distribution and Socio-Economic Impacts of Prosopis Juliflora in Gamo Gofa, Segen Area People and South Omo Zones, Southern Ethiopia

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Abstract

This study was conducted in Gamo Gofa, Segen Area People and South Omo Zones of Southern Ethiopia. The study is of practical value and has got significant importance. The purpose of this study was to evaluate the rate of distribution of *Prosopis juliflora* and assess its impacts on community perception. Distribution of *P. juliflora* was recorded with systematic sampling techniques at regular interval of 5km with the help of Global Position System tools. Distribution of *P. juliflora* was determined. Semi-structured questionnaire was used to collect data on socioeconomic impacts of *P. juliflora* and data was analysed using SPSS version 15. Observations of *P. juliflora* were made along the main road sides in Arba Minch Zuria, Mirab Abaya, Gidole, Konso, Hamer and Nyangatom districts. *P. juliflora* was distributed along the road sides, grazing lands, waste lands, and around habitations. It was observed spreading along the roads to Nech Sar National Park. From the distribution data Prosopis was low in Mirab Abaya, Gidole, Konso, and Hamer Districts. The infestation level of Prosopis was high in Arba Minch Town and Nyangatom districts mainly on grazing lands and around urban settings. One hundred and forty-six respondents were interviewed. Questionnaire and observation data showed that Prosopis has taken over large grazing lands and blocks movement routes in Nyangatom district. The current study also investigated that no control activities had been employed. It is concluded that the menace of *P.juliflora* is increasing at alarming rate, thus control methods have to be designed to stop further spreading into Nech Sar and Mago National Parks.

Keywords

Distribution; Socio-Economic; Impact; Prosopis Juliflora

Introduction

Prosopis juliflora is native to arid and semi arid zones of South Americas, Central America, Africa, and Asia. Recently, the plant has been introduced and naturalized in many countries. In America, it has been introduced to Brazil, Hawaii and some Caribbean islands. In Oceania, it has been introduced to Australia, New Guinea, and Marquises islands. In Asia, it has been observed in Jordan, Saudi Arabia, Bahrain and Qattar, United Arab Emirates, Iraq, Iran, Kuwait, India, Pakistan, Srilanka, Thailand, Vietnam, Indonesia, Philippines and in many arid and semiarid African countries (Pasiecznik *et al.*, 2001). According to FAO (2002) and Mwangi & Swallow (2005), in the 1970s and 1980s tropical and subtropical regions experienced increasing shortage of fuel wood and other timber in rural communities, coupled with increasing environmental degradation, soil erosion and desertification. As part of national and international efforts to address these issues exotic woody shrub and, in particular, woody legumes were promoted for use in agricultural systems and to revegetate degraded lands. The main reasons for introducing different species of the genus *Prosopis* around the world have been combating desertification and utilization of a fast growing fuel wood and fodder species that thrives in harsh arid and semi-arid conditions (Mwangi and Swallow, 2005). The first record of *P. juliflora* introduction to West Africa and pacific island was in 1820s, to India and Pakistan was in 1870s, and to Australia and South Africa was before 1900 (Pasiecznik *et al.*, 2003). *P. juliflora* plantation was recommended in Ethiopia to control desertification under the national plan to combat desertification while the forest research recognized it as a threat to biodiversity. The plant has been cultivated for

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shade, timber, forage, food, and firewood (Pasiecznik *et al*., 2001 and Rezene Fessehaie, 2006).

However, contrary to its purpose of introduction, the plant escaped out of control and had been invading farm lands, pasture lands, range lands, irrigation networks and causing many land use/ land cover changes (Rezene Fessehaie, 2006). Although there is no precise written document why, when, where, and who introduced *P. juliflora* to Ethiopia, the local people of Amibara district of the Afar National Regional State, stated that *P. juliflora* was introduced by an English person from Sudan in 1970s through the Middle Awash Irrigation Project (Rezene Fessehaie, 2006; Hailu Shiferaw *et al*., 2004) and was planted over a large area of the Middle Awash rift valley by local people in 1980 as wind break and providing shade and shelter around their villages. The Amibara Woreda of the Afar National Regional State was thought to be the putative starting point of the spread of *P. juliflora* in Ethiopia. It represents degraded semi-arid ecosystem. Since 1980s the plant has spread rapidly in eastern Ethiopia, from the Middle Awash Valley in to the Upper Awash Valley and Eastern Hararghe and some localities of Raya Azebo plains of South Tigray. The invasion has also been reported in the town of Arba Minch and neighboring localities in southern part of the country (Rezene Fessehaie, 2006). Quantitative assessments of the area covered by *P. juliflora*, the land use/land cover change due to the plant and its rate of spread has not been undertaken in Ethiopia, though it has been estimated that the plant covers 15,000 ha of land in the Amibara District. Even in the absence of precise figures, it is clear that the spread of *P. juliflora* in Ethiopia has increased in the last decade, both in terms of area coverage and plant density (Demissew Sertse, 2005).

Prosopis juliflora in Ethiopia is under-recorded and under-reported, and it is likely spreading into the Gamo Gofa, Segen Area people and South Omo Zones and the nearby Park areas. The area is centre of ecotourism site, as a result of their rich biodiversity of flora and fauna. This research was very much relevant to study the extent of distribution of *Prosopis juliflora* and its impact on the local plant species diversity as well as socio-economic settings. Currently Prosopis juliflora is a dominant species in the grasslands, waste lands and roadside vegetation of several major urban, park and grazing areas in Gamo Gofa, Segen Area People and South Omo Zones. So far a nationwide survey of the area invaded by this invasive weed is lacking, however, by a joint effort of Department of Plant and Animal Sciences of Arba Minch University, a preliminary survey of ecological distribution and socioeconomic impacts of *Prosopis juliflora* in Gamo Gofa, Segen Area People and South Omo Zones has been conducted.

The recorded knowledge and understanding will help to know more on the extent of the distribution of the plant and the rate of expansion and the positive and negative impacts of the plant in the study area. Moreover, the study will help to know the effective control mechanisms to diminish some of the negative impacts caused by the plant. This investigation may contribute baseline information for future strategies of Prosopis management. *Prosopis juliflora is* the common invasive species in the study area causing severe negative impacts on both environment and human activities. Accordingly this study was initiated to assess their distribution in the southern part of Ethiopia, specifically around Nechsar and Mago National Parks. Thus, the current study was conducted to evaluate the rate of ecological distribution of *Prosopis juliflora* in the Gamo Gofa, Segen Area People and South Omo Zones and assess impacts of *Prosopis juliflora* based on communities' perception in the study areas.

Materials and Methods

Description of the Study Sites

The study was conducted at Gamo Gofa, Segen Area People and South Omo Zones which are located in the southern part of the country. The study areas are also located in the rift valley areas and characterized by semi arid and arid ecological zones. The study area represents a mixture of crop and livestock farming system with extensive area of communal rangelands. The altitude ranges from 381-2019 m above sea level. Arba Minch has monthly mean minimum and maximum temperature of 18^0C and 34^0C respectively. Rain fall ranges from 1400-1800 mm per year.

Data Collection Methods

Data were collected to characterize the ecological distribution trends and analyse socio-economic impacts on the people living around the study area. The study was utilized a three-stage sampling approach wherein the first stage a reconnaissance visit and background data collection was involved. In the second stage group interview was carried out, wherein key stakeholders such as village leaders, NGO representatives, District Administration, and

Office of Agriculture were participated. The questionnaire for the formal survey was pre-tested in this stage. In the final stage, a formal survey was conducted where data has been gathered through a pre-coded (structured) questionnaire and GPS (Geopositioning system).

A Survey on Distribution of Prosopis Juliflora

The survey was conducted both during cropping and off season; distribution of *Prosopis juliflora* was recorded in all accessible roads with the help of Global Position System. Transect line was the main gravel or asphalt road, from Arba Minch to South Omo Zone and from Arba Minch to Mirab Abaya District. Sample plot was systematically selected from each selected study area and field observations were made at 5 km interval along the main roads in the study areas which can be accessible by car and about 200m walk to both direction form sampling points. The reason for selection of the main road as the main transect line is based on the *Prosopis juliflora* distribution characteristics, usually *Prosopis* is found along the main roads as a result of favourable distribution agents along the main road. During vegetation inventory, site was characterized and *Prosopis juliflora* distribution in different land use type was recorded. The location, latitude and longitude coordinate of the *Prosopis species* were collected and recorded using GPS and simultaneously the presence/absence and the extent was observed and noted on the data collection sheet. The field data collected from each sample plot were aggregated in to kebele/district level. Based on the data collected from the survey presence/absence and distribution was determined at district and Zone level.

Social Survey

Social data were collected systematically to assess the socio-economic impact of *Prosopis* tree species on the nearby communities. Social information was collected from the farmers who are settled near to the main road and accessible villages through focus group discussion and semi-structured questionnaires. The sampling unit for the study was the household. The reason for this is that because a household is: a dominant social and economic unit in rural Ethiopia, the primary unit of resource holding, production, distribution and consumption, and a locus of decision making regarding most economic and social functions. The study considered purposively selected kebles from Gamo Gofa, Segen Area People and south Omo Zones which are very close to the main road.

Data Analysis

The collected data were coded and entered in SPSS software version 15. Descriptive statistics was computed and the output presented in the form of percentages using tables

Results and Discussions

Distribution of Prosopis juliflora in Gamo Gofa, Segen Area People and South Omo Zones

The presence and absence of *Prosopis juliflora* in different districts of the study areas were indicated in (Table 1).

TABLE 1. DISTRIBUTION OF *PROSOPIS JULIFLORA* IN GAMO GOFA, SOUTH OMO AND SEGEN AREA PEOPLE ZONES DURING ROAD SIDE SURVEY (2012/13)

ZONE	WOREDA/DISTRICT	VILLAGES	HABITAT
Gamo Gofa	Arba Minch zuria	Sille and Arba Minch Town	Road side, waste places and park areas
Gamo Gofa	Mirab Abaya	Yahike and Wajifo/Kola Mulat	Road side
Segen Area People	Konso	Arfayide	Road side
Segen Area People	Gidole	Gatto	Road side & around habitation
South Omo	Hamer	Ayer Marefiya	Road side& range land
South Omo	Nyangatom	Nyangatom district and surrounding	Habitation & range lands

Source: From field observation (2012/13)

Three Zones namely Gamo Gofa, Segen Area People and South Omo were covered along the main road and both sides of the road from systematically selected sampling points during the study period in 2012/13. Six districts namely Arba Minch Zuria, Mirab Abaya, Gidole, Hamer, Nyangatom and Konso were surveyed in collaboration with each Districts Agricultural Offices and Development Agents. *Prosopis juliflora* was observed and recorded in Gamo Gofa, Segen Area People and South Omo zones in varied infestation level. During the survey conducted, *Prosopis juliflora* was observed in Arba Minch Zuria and Nyangatom districts in a high infestation level compared to another surveyed areas. This indicates that *Prosopis juliflora* has been causing a serious problem in the nearby Nech Sar and Mago National Parks by displacing the native tree and animal species. It is also a threat to natural grazing lands around the area. The study areas were known by large livestock population, rich biodiversity and free grazing areas. No control measures were taken so far in the study area on *Prosopis juliflora* by the local communities and the concerned offices. This is due to lacking of information about the species threatening onto the biodiversity in general. Furthermore, it has little infestation level in Mirab Abaya, Konso, Gidole, and Hamer districts. The presence only indicated further spreading into free areas unless control measure employed. It is also observed around Kulfo River shores spreading into Arba Minch Natural Forest where forty spring water found. *P. juliflora* was marked in Mirab Abaya at Yahike and Wajifo villages. According to Wajifo and Yahike village residents the infestation level was low and it was newly introduced from the Arba Minch Town into the area. It was intentionally or deliberately introduced into the area as shade, fence and multipurpose trees as mentioned by the interviewed communities. Similar finding was also reported by Hailu and Kasahun *et al*. (2004) who reported that prosopis was intentionally introduced as agro-forestry species in the Awash Basin.

P. juliflora was recorded in Konso district at Arfayide kebele planted for fencing purpose. As respondents mentioned that prosopis was introduced into the area from Ziway (Oromia Region) area purposely. The presence of *P. juliflora* in this kebele/district indicates that there will be a danger of further spread into other areas within a short time, which are threating socio-economic and biodiversity in Konso landscape. Similarly, Shashitu (2008) and Worku *et al*. (2004) reported that the indigenous tree species and grazing pasture were lost due to prosopis invasion. This is because of its high competitiveness with native plants. This study indicated that there is a lack of early detection and rapid response mechanisms in controlling the new introduction of *P. juliflora* in the study area. Hence, prosopis should be removed from the area before it spread further into other areas.

P. juliflora was marked in Hamer district at Ayermarefiya villages. There is no exact time when prosopis was introduced in the Dimeka area or Hamer district. But communities stated that prosopis was introduced by foreign person called 'Father Brain' into Hamer district as fodder plant to the local people.

P. juliflora was known to adapt to Gatto kebeles of Gidole district as multipurpose tree and it was evergreen during dry season that is why the people favor the plant. In line with this study, Senayit *et al*, 2004 reported that *P. juliflora* was used as drought resistant, as shade, fuel wood, animal feed and erosion control in Afar Regional State. *P. juliflora* in the near future may replace the native species and become serious threat to Nech Sar National Park if control measures are not undertaken mainly by the concerned offices. Furthermore, Nyangatom district is border to Kenya, *P. juliflora* was expected to further spread into southern part of the country mainly through the movement of livestock.

Prosopis juliflora was observed in Arba Minch Town in all administrative kebele's. It has been spreading in a fast rate from the town to the nearby Nechsar National Park through gorges. During field survey, *Prosopis juliflora* was observed along road side to Konso district and also to Mirab Abaya both by cattle movement and human being needs. Many cattle's and goats released for grazing purpose from Arba Minch town to nearby available natural grazing sites which is the indication of further spreading of the species. In addition, wild animals in Nech Sar National Park is spreading up on feeding of the prosopis pod. Similar finding was also reported by Rezene Fessehaie (2006) prosopis invasion in the Arba Minch and its neighboring localities.

Assessment of Socio-Economic Impacts of Prosopis Juliflora in the Study Areas

General Background on the Respondent'S Category

Prosopis juliflora has been considered in this study. Purposively selected respondents were interviewed to investigate impacts of prosopis in the study area. The study areas were selected based on the biodiversity importance and accessibility for the study. The sites selected for this study were Gamo Gofa, Segene Area People and South Omo Zones in the Southern Nations Nationalities and Peoples Region. Though there are many commercial farms in the study area. For example, cotton, banana, sesame, sugarcane, and maize farms. One hundred and forty-six (146) respondents were interviewed about perception of prosopis weed in the study area (59 respondents from Gamo Gofa, 40 respondents from Segen Area People and 47 respondents from South Omo

Zones). Among them male and female respondents were 69.2 % and 30.8 % (Table 2) respectively. Table 3 as below indicated that the respondents included in this study were farmers (29.5%), students (9.6%), Administrative worker (15%), Development Agents (21.2%), Guard (4.8%) and town dweller (19.9%). Nyangatom district administrative office revealed that densities of livestock have decreased in areas where dense thicket of prosopis present.

TABLE 2. GENDER OF RESPONDENTS

Sex of responders	Frequency	Percent
Male	101	69.2
Female	45	30.8

Source: own survey (2012/13)

TABLE 3. RESPONDENT'S OCCUPATION

Occupation	Frequency	Percent
Farmer	43	29.5
Administrative worker	22	15
Student	14	9.6
Guard	7	4.8
Development Agent	31	21.2
Town resident	29	19.9

Source: own survey (2012/13)

Respondents Perception on Prosopis Juliflora in the Study Area

From the total respondents, 26.7% respondent had no idea from where prosopis weed came to the study area, but 22% of the respondents mentioned that prosopis weed came from Afar Region to their areas deliberately by human being. In Mirab Abaya Woreda, Gatto kebele, Shelle area and Hamer woreda, about 38.3% of the respondents mentioned that prosopis weed was introduced to the area purposely from Arba Minch Town (Table 4). It also confirmed during survey, prosopis is spreading from Arba Minch to Konso and Mirab Abaya road mainly by livestock movement and prosopis weed was observed growing on the roadsides around Mirab Abaya district.

TABLE 4. RESPONSES OF RESPONDENTS FROM WHERE PROSOPIS WAS INTRODUCED INTO THEIR AREA

Prosopis introduction into the study area	Frequency	Percent
No idea	39	26.7
Ziway area	19	13
Afar	32	22
Arba Minch, Mirab Abaya and Hamer districts	56	38.3

Source: own survey result (2012/13)

About 41.8% of the respondents mentioned that the abundance of prosopis was high in Arba Minch area of Gamo Gofa and Nyangatom district in South Omo Zones (Table 5).

TABLE 5. DISTRIBUTION OF *PROSOPIS JULIFLORA* AS PERCEIVED BY RESPONDENTS

Infestation level of Prosopis	Frequency	Percent
High(Arba Minch Zuria & Nyangatom districts)	61	41.8
Medium (Arba Minch Zuria & Nyangatom districts)	49	33.6
Low(Konso, Gidole, Hamer & Mirab Abaya districts)	36	24.6

Source: Survey result (2012/13)

Livestock in the study area makes a very high contribution to agriculture. It is clear from this assessment that *Prosopis juliflora* pod was palatable to livestock according to respondents interviewed in the study area. But it suspected that prosopis infestation affect livestock production by competing for valuable grass species especially in the Nyangatom district. In general, the negative effects of prosopis were reported by the respondents in the Arba Minch and Nyangatom districts than those in Konso, Hamer and Mirab Abaya districts. This can be explained by the fact that people living in the infested area have experienced more life with prosopis and thus can tell its effects on their livelihood with more confidence than their counterparts living in the less infested area.

Arba Minch Town dwellers believe that *Prosopis juliflora* was introduced into the area following the flood problem in the town. Indeed, the invasion of prosopis in the Town began in 2003. Since then, it is expanding at alarming rate from the town to Nech Sar National Park**.** According to the interviewed farmers this is happening due to road animal movement and other human activities. Respondents mentioned that prosopis was used for fencing land in one village of Mirab Abaya district of Gamo Gofa Zone. This implies that farmers have no awareness about spread and impacts of *Prosopis juliflora*. Therefore, Bureau of Agriculture and Rural Development are expected to create awareness about the impact of *prosopis* in the study area. Some of the respondents indicated that *Prosopis juliflora* is used as shade tree and fodder for livestock in Arba Minch, Nyangatom and Gidole Woredas. The major source of spreading *P. juliflora* from Arba Minch area to other areas particularly in Mirab Abaya Woreda was human being, animals and floods as interviewed respondents indicate and observations were made during the survey period. The preferred infestation habitat for *P. juliflora* is along drainage lines and river-banks, which are the most fertile sites in this District. *P. juliflora* is cultivated for fence to protect livestock and wild animals from crop damage in the Konso and Mirab Abaya Woredas. The additional benefits of prosopis other than fence, shading and animal fodder, children also eat the pod of *P. juliflora* in Arba Minch town because of sweetness. The respondents also mentioned that the mechanisms by which Prosopis spreading from one household to the others saying that the species (*P. juliflora*) were evergreen throughout the year as compared to the native species. During the survey time, it was observed that the seedling and trees of *P. juliflora* was managed by respondent to properly grow and give them benefit. They indicated that prosopis can withstand recurrent drought as compared to native tree species. One of the town dwellers also told that the pods of *P. juliflora* is eaten by livestock as additional feed, which is one of the spreading mechanisms from Arba Minch to the nearby Nech Sar National Park.

Similar to this finding, Mwangi and Swallow (2005) reported that community's perception of prosopis was favourable during the early stages of its introduction. It is clear that it was welcomed tree for their shading. Through time the local people were aware of the negative effect of the tree by its invasion of grazing land and suppression of native tree.

There were severe impacts of prosopis species threatening the livestock production in Nyangatom district as the respondents indicated during the interview time. The respondents also have more awareness about the negative aspects of prosopis in this district. In general, they regarded the species as highly aggressive and invasive; forming impenetrable thickets that choke out other plants and it reduces livestock access to the available forage.

Respondents revealed that prosopis had impacts in the Nyangatom and Arba Minch Zuria districts. This indicates that the communities are well aware of invasive trees/shrubs as compared to invasive alien plant species, which was currently threatening the rangelands and croplands of Gamo Gofa, Segen Area People and South Omo Zones.

Agents of Dispersal of Prosopis to other Sites

Most of the respondents (43.7%) reported that animal is the main prosopis seed dispersal agents followed by human being (26.7%), flood (18%) and wind (11.6%), (Table 6). This indicated that the communities are very well aware of the mechanism of prosopis dispersal from one location to another. Each agent contributes to its wide range dispersal of prosopis. In agreement to this finding, this plant came to arba minch with crop aid. Now it is spreading to nearby arba minch forest by flood and both wild and domestic animals.

TABLE 6. COMMUNITIES' PERCEPTION ABOUT AGENTS OF DISPERSAL OF PROSOPIS TO DIFFERENT AREA

Dispersal agents	Frequency	Percent
Animal	64	43.7
Human	39	26.7
Flood	26	18
Wind	17	11.6

Source: own survey result (2012/13)

According to the communities perception Prosopis juliflora came to Gatto villages in the year of 1990. It came in this area by NGOs that in charge to construct irrigation canal. They used it to strongly hold the soil around the canal. Then onward large numbers of seedlings were raised by providing intensive care and distributed to the nearby farmers for different purposes. The community does not know from where the plant came to their area. The respondents revealed that the plant was distributed to other nearby localities by cattle movement, wind, flood, human being, etc.

Management Practices of Prosopis Juliflora in the Study Area

According to the communities perception *Prosopis juliflora* came to Gatto villages in the year of 1990. It came in this area by NGOs that in charge to construct irrigation canal. They used it to strongly hold the soil around the canal. Then onward large numbers of seedlings were raised by providing intensive care and distributed to the nearby farmers for different purposes. The community does not know from where the plant comes to their area. The respondents revealed that the plant was distributed to other nearby localities by cattle movement, wind, flood, human being, etc. In Gatto villages there is no more information available regarding to prosopis disadvantages rather the village dwellers and the adjacent farmers perceived the plant as important to their livelihood for instance, it used for flood control, as bridge cross, construction material, fodder, shade, and, fuel.

Communities in the study area lack deep knowledge about *Prosopis juliflora*. Little information is available about the plant due to negative nature of it. One of the respondents in Gatto village perceived that since the plant has thorny it cause poison to livestock and human being and also when the children feed the fruit of the plant it cause stomach problem. When this plant is present in the farmers field no other plants grow under it and it also competes with *Moringa stenoptela* which is very important plant to livelihood of localities in general. Pods are eaten by monkey and baboons as indicated by the respondents. As indicated by respondents they used the plant as flood control which is supplied by bureau agricultural offices. In addition, father brain is a person who distributed prosopis species in Dimeka/Hamer district of South Omo Zone as communities revealed that. In Arba Minch area pastoralists and city dwellers control this invasive plant by cutting, uprooting, collecting pods and then burn it. They control by community mobilizing activities but yet no stop its infestations.

TABLE 7. MANAGEMENT PRACTICES USED TO CONTROL PROSOPIS SO FAR IN THE STUDY AREA

Management practice	Frequency	Percent
Digging and burn	47	32.2
Above ground cutting	60	41.1
None	39	26.7

Source: own survey result (2012/13)

The systemic and purposive survey was carried out to assess distribution and socioeconomic impacts of *Prosopis juliflora* in Gamo Gofa, Segen Area People and South Omo Zones. A total of 146 sample respondents were selected for assessing socio-economic impacts of *Prosopis juliflora* in the study area. The information about the *Prosopis juliflora* revealed that this invasive weed is expanding along the road network from Arba Minch to Konso and Mirab Abaya Woredas. It was introduced into Arba Minch area as soil and water conservation to protect erosion in the area. Data was collected by administrating interview and group discussion. Communities revealed that Father Brain a leader of Catholic Church was in charge by distributing prosopis seedling in Hamer Districts of South Omo Zone and also confirmed during field observation in Ayer meda (Dimeka Town). Hamer District dwellers indicated that the infestation level of *Prosopis juliflora* was low. The growth nature of this plant is throughout the year as mentioned by the respondents of Dimeka town and the surrounding pastoral communities. Group discussion and interviewed communities of Hamer District revealed that *Prosopis juliflora* spreads from infested to non-infested area through goat and cattle feces. Lacking of awareness about impacts of *Prosopis juliflora* was observed during field survey in all areas except Arba Minch Town and Nyangatom District due to high infestation of *Prosopis juliflora* in these two study areas. One farmer in Wajifo/kola mulat kebele of Mirab Abaya District purposively planted *Prosopis juliflora* as fence to control damages of wild animals to his farm. The farmer/kebele dwellers were brought the plant from Arba Minch Town, particularly from secha sub-city. It has been observed the plant growing up to 1,550 m.a.s.l (Konso area). In rural setting, this invasive weed is mainly found in roadside but in Arba Minch it is distributed to the whole Town including Nech Sar National Parks. It has been found less frequently in cultivated agriculture lands. However, once the rangeland is abandoned for the coming years, prosopis will become a dominant plant. In most of the places, invasion is considered to be at the early stage.

Respondents also indicated that Prosopis juliflora is used as shade tree and fodder for cattle, bee forage and goat in Gatto and Arba Minch District. The major source of spreading P.juliflora from Arba Minch to Nech Sar National Park, Shele village and Mirab Abaya Districts were men, animals and floods as interviewed respondents indicate and observations were made during the survey period. The preferred infestation habitat for *P. juliflora* is along drainage lines and riverbanks in Arba Minch Town. Similarly, Mwangi and Swallow (2005) reported that community's perception of Prosopis was favorable during the early stages of its introduction.

P. juliflora is cultivated for shade, firewood, and for pods, which are fed to livestock. The additional benefits of the species other than shading and animal feed, children also eat the pod of *P. juliflora* because of sweetness. The respondents also mentioned that the mechanisms by which Prosopis spreading from one household to the others saying that the species (*P. juliflora*) was evergreen throughout the year as compared to the native species. During the survey time, it was observed that the seedling of *P. juliflora* was managed by one of the respondents to have upright position in Konso and Mirab Abaya districts. He indicated that prosopis can withstand recurrent drought as compared to native tree species. One of the village dwellers also told that the pods of *P. juliflora* is eaten by monkey and baboons, which is one of the spreading mechanisms from Arba Minch area to Nech sar National Park.

Level of Infestation

The infestation level of prosopis species is increasing in fast rate around Arba Minch and South Omo Zone specifically in Nyangatom district. In Conso district the plant was planted purposely as fence. The interviewed farmer was brought the plant from Ziway area (Oromia region) due to its green nature throughout the year. However, the infestation level was at an infant stage. Similarly in Mirab Abaya district this plant was brought from Arba Minch town and the infestation level was low.

Conclusion and Recommendation

The spread of *Prosopis juliflora* is now recognized as one of the greatest threats to the ecological and wellbeing of the planet. Pastoralists and agro-pastoralists in Gamo Gofa, Segen Area People and South Omo Zones are affected by *Prosopis juliflora* infestation differently in different habitats. From ecological distribution data *Prosopis juliflora* was found in Mirab Abaya, Arba Minch Zuria, Gidole, Konso, Hamer and Nyangatom districts of the study Zones. Arba Minch Zuria and Nyangatom districts in the study areas have seen an increase in the invasion of *Prosopis juliflora*. Pastoralists in the Nyangatom district have information about *Prosopis juliflora* due to its impact on their livestock production. On the other hand, it was confirmed that *Prosopis juliflora* is found densely in Arba Minch Town and grazing lands of Nyangatom woredas during field observation.

Hence, it was concluded that much has not been done to aware the local people on the negative effects of *Prosopis juliflora* causing impacts on biodiversity, livestock and crop production. Many of the farmers in the study area are not aware of *Prosopis juliflora* and that they pose threat to the loss of biodiversity and low productivity. The endangered plant species include grasses that are economically important for livestock production. Based on the results obtained, the following recommendations were drawn: Findings of this study can be used as part of baseline information in managing the threat of *Prosopis juliflora*; impacts of *Prosopis juliflora* on animal and human health should be investigated in the study area; very little is known about the negative impacts of *Prosopis juliflora* except in Nyangatom woreda and therefore, attention should be given to create awareness and action plan should be devised how to control *Prosopis juliflora*.

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Evaluation of Variability Parameters in Apple Trees – a Statistical Approach

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Abstract

In the present paper, an attempt has been made to study the morphological variations in apple trees with respect to their ages and locations. Among the different variables, maximum coefficient of variation of 119.8% and 82.35% was observed for Volume of the tree at Theog and Kotkhai locations respectively, whereas maximum coefficient of variation for number of fruits per tertiary branch was observed at Shimla location. The minimum value of coefficient of variation was observed for height (24.55%), the number of secondary branches (36.44%), and the number of main branches (21.4%) at Shimla, Kotkhai and Theog respectively. The variability analysis indicated that an increase in girth, height, spread, volume, the number of main branches, the number of secondary branches, length of spurs, and yield was noticed with the increase in age of trees in general at all the three locations. Significant variation was observed in all the tree characteristics among the three sites or locations.

Key words

Coefficient of Variation; Coefficient of Skewness; Coefficient of Kurtosis; Bartlettt's X [2] Test Statistic; Apple; Variability Analysis

Introduction

Apple is a predominant fruit crop of Himachal Pradesh and in recent years it has been the leading cash crop amongst fruit crops. It alone accounts for about 48 percent of total area under fruit crops (2, 11, 295 hectares) and more than 86 percent of the total fruits production (1027.82 thousand tonnes). The area under apple and its production have increased from 88,673 hectares and 49.13 thousand tonnes in 1999-00 to 1,01,485 hectares and 892.11 thousand tonnes in 2010-11 (Anon. 2012), respectively. Shimla, Kullu, Mandi ,Chamba and Kinnaur are apple producing districts of Himachal Pradesh. The fact that Shimla district of Himachal Pradesh is the leading apple producing district with 3, 36, 753 MT which accounts for more than 50 percent of total apple production of the State (Anonymous, 2009) and forms the basis for selecting it as the study area.

Many workers have reported variability in apple vigour characters like tree size, tree girth, tree height and mean tree spread (Chadha and Sharma, 1975). Variability has also been observed for characters like trunk girth (Gautam and Chauhan, 1986), the number of shoots and spurs density (Xin and Shen, 1992). Variations for various plant vigour characteristics have also been reported by Bhat *et al.* (2006) under high – hill conditions of Jammu and Kashmir (tree spread and scion girth); Sharma *et al.* (2004) under mid hill conditions of Himachal Pradesh (tree spread, tree volume and annual shoot growth) and Wazbinska *et al.* (2003) under temperate conditions of Poland (scion girth), in different cultivars of apple. Variations for fruit characters like length, breadth, shape, weight and colour have also been reported by Kumar and Verma (2001) in mid hill conditions of Kullu, Dwivedi *et al.* (2005) in Ladakh region of Jammu and Kashmir and Sharma *et al* (2004) in different cultivars of apple under mid hill conditions of Himachal Pradesh. Keeping in view the significant contribution of morphological characters towards the yield, the present study deals with age - wise and location - wise variability among various morphological tree - growth characters in apple trees.

Material and Methods

Data were recorded from a random sample of 300 apple trees from randomly selected orchards at three different locations of Shimla district of Himachal Pradesh - Shimla, Kotkhai and Theog on different tree growth characteristics viz., X_1=Age (years), X_2=Girth (m), X_3=Height (m), X_4=Spread (m), X_5=Volume (m^3), X_6=Number of

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main branches, X_7=Number of secondary branches, X_8=Number of spurs per tertiary branch, X_9=Length of spurs, X_{10}=Number of flowers per tertiary branch, X_{11}=Number of fruits per tertiary branch, X_{12}=Fruit weight (g) and X_{13}=Yield of tree (kg) under three different age groups viz., A_1: Below 15 years, A_2: 15-25 years, and A_3: 25 years and above to work out the data variability. Density of trees at Shimla (3×3 m^2) was the highest and lowest at Kotkhai (6×6 m^2). Plants at Theog site were planted according to 5× 5 m^2 spacing. The data were analyzed age wise for various statistical measures viz., mean, standard deviation, coefficient of variations, coefficient of skewness and coefficient of kurtosis for all the variables under study. The Bartlett's χ^2 test was applied to test the variation among all the locations for these directly measured and derived variables. The calculated values through Bartlett's χ^2 test when compared with the table value **($\chi^2_{0.05}(2) = 5.99$)** at 5% level of significance which revealed that there was significant variation in all the sites for the tree characteristics under study.

To study the effect of location and age on different growth parameters, data were also subjected to Randomized Block Design considering treatment combinations factorial in nature. The data set is comprised of 15 trees from each age group for three locations. Thus, the analysis was carried out on 135(15x3x3) observations.

Results and Discussion

Age Wise Variation among Different Tree Characters

The data collected from randomly selected 300 trees from Shimla, Kotkhai and Theog sites by taking 100 trees from each location for various tree growth characteristics under different age group categories mentioned above was subjected to variability analysis.

As evident from Table1, in case of Shimla location, trees having age above 25 years (A_3) recorded the maximum mean values for all the variables i.e. girth (0.52m), height (4.22m), spread (2.87m),volume (19.77m^3), the number of main branches (4.66), the number of spurs per tertiary branch (11.64), the number of flowers per tertiary branch (35.93), the number of fruits per tertiary branch (9.59), fruit weight (141.44g)and yield (13.24kg) except the number of secondary branches and length of spurs which was maximum incase of age group A_2. The highest coefficient of variation was shown by the number of fruits in tertiary branch (91.78%) among trees of 25 years and above age group (A_3) followed by number of fruits in tertiary branch (91.22%) among trees ranging from 15 to 25 years age (A_2). In A_1 age group the volume (88.39%) showed the maximum coefficient of variation. CV was observed minimum for length of spurs (3.99%) in A_1 age group. For most of the variables under consideration, standard deviation was higher in A_3. However, the highest standard deviation was shown by the variable depicting number of flowers in tertiary branch (21.33) while the lowest was in length of spurs (0.05cm) in $A_1$and A_3age groups. All characters except the number of secondary branches, length of spurs increased with the increase in age of trees in Shimla location which were maximum in A_2 age group. Number of spurs, flowers and fruits were minimum in A_2 age group.

Similarly, in case of Kotkhai location, trees having age 25 years and above (A_3) had maximum mean values for girth (0.78m), height (25.05m), spread (20.49m),volume (5687.30m^3), the number of secondary branches (15.80), length of spurs (1.61cm), fruit weight (168.43g)and yield (250.98kg). However, the number of main branches (4.09), the number of spurs per tertiary branch (4.70), the number of flowers per tertiary branch (23.48), the number of fruits per tertiary branch (14.27) was maximum incase of age group A_2. The highest coefficient of variation was shown in all age groups by volume (A_1: 90.22%, A_2: 82.70%, A_3: 44.28%) and while the lowest coefficient of variation was shown in A_1 age group by length of spurs (4.95%). The standard deviation was noted to be the highest in volume in A_3 category whereas the lowest in girth and length of spurs in A_1. The girth, height, spread, volume, the number of secondary branches, length of spurs, fruit weight and yield increased with the increase in age of trees in Kotkhai location.

In case of Theog location, the mean values for girth (1.13m), height (12.40m), spread (8.69m), volume (510.99m^3), the number of main branches (4.81), the number of secondary branches (25.25), length of spurs (1.49cm) and yield (180.00kg) were found to be maximum in A_3 age group while the number of spurs (8.10), the number of flowers (40.50), the number of fruits (24.07), and fruit weight (152.87g) were higher in A_2 age group. The CV was found to be the highest in yield (70.61%) in A_1, followed by volume in A_2 and A_3 age groups (59.95%, 41.53%) respectively. It was the lowest in case of length of spurs (4.21%, 4.44%, and 3.52%) in all the age groups. The standard deviation was minimum in girth (0.03m) in A_1 and maximum in volume (212.19m^3) in A_3 age group. An increase in girth, height, spread, volume, the number of main branches, the number of secondary branches, length of spurs, and yield was noticed with increase in age of trees in Theog region. However, trees of 15-25 years had much more spurs, flowers and fruits per tertiary branch.

TABLE 1. MEAN AND COEFFICIENT OF VARIATION OF VARIOUS TREE CHARACTERS FOR DIFFERENT AGE GROUPS.

Variables	Age Groups								
	Shimla (L_1)			Kotkhai (L_2)			Theog(L_3)		
	A_1 21 trees	A_2 41 trees	A_3 38 trees	A_1 26 trees	A_2 23 trees	A_3 51 trees	A_1 27 trees	A_2 57 trees	A_3 16 trees
X_2: Girth (m)	0.29 (22.14)	0.39 (15.56)	0.52 (21.21)	0.24 (27.69)	0.60 (25.97)	0.78 (16.73)	0.30 (9.46)	0.71 (29.05)	1.13 (6.48)
X_3: Height (m)	3.23 (34.44)	3.91 (23.40)	4.22 (16.80)	12.28 (31.06)	16.47 (46.34)	25.05 (16.52)	4.18 (9.01)	6.56 (21.23)	12.40 (23.86)
X_4: Spread (m)	1.69 (30.19)	2.33 (26.24)	2.87 (25.22)	8.62 (37.21)	14.97 (48.66)	20.49 (19.52)	1.91 (18.24)	5.89 (27.27)	8.69 (12.42)
X_5: Volume (m^3)	5.81 (88.39)	12.49 (57.70)	19.77 (60.80)	565.01 (90.22)	2832.15 (82.70)	5687.30 (44.28)	8.21 (36.47)	138.18 (59.95)	510.99 (41.53)
X_6: Number of main branches	2.95 (31.18)	3.59 (36.88)	4.66 (39.70)	2.81 (40.32)	4.09 (44.21)	3.98 (31.58)	3.78 (18.48)	4.72 (19.92)	4.81 (16.78)
X_7: Number of secondary branches	11.29 (21.55)	12.90 (31.76)	12.11 (34.18)	8.04 (22.93)	13.78 (24.06)	15.80 (27.73)	10.37 (15.63)	22.05 (20.00)	25.25 (12.17)
X_8: Number of spurs per tertiary branch	10.20 (63.61)	9.45 (63.46)	11.64 (56.19)	1.41 (41.84)	4.70 (53.02)	4.15 (36.43)	2.35 (22.11)	8.10 (43.10)	6.08 (24.19)
X_9: Length of spurs(cm)	1.32 (3.99)	1.34 (4.49)	1.33 (4.13)	1.32 (4.95)	1.54 (11.65)	1.61 (9.04)	1.48 (4.21)	1.47 (4.44)	1.49 (3.52)
X_{10}: Number of flowers per tertiary branch	34.25 (62.28)	29.62 (63.67)	35.93 (56.65)	7.07 (41.84)	23.48 (53.02)	20.74 (36.43)	11.81 (22.62)	40.50 (43.10)	30.39 (24.19)
X_{11}: Number of fruits per tertiary branch	7.45 (57.85)	6.77 (91.22)	9.59 (91.78)	3.84 (59.21)	14.27 (52.52)	12.57 (36.38)	4.44 (22.52)	24.07 (44.23)	17.27 (32.59)
X_{12}:Fruit weight (kg)	112.30 (35.06)	135.87 (26.61)	141.44 (20.32)	162.09 (13.62)	162.75 (9.27)	168.43 (9.13)	141.75 (9.18)	152.87 (11.33)	147.48 (10.18)
X_{13}: Yield (kg)	7.29 (58.26)	9.66 (70.84)	13.24 (61.20)	16.62 (72.17)	160.43 (38.45)	250.98 (44.15)	7.67 (70.61)	101.75 (47.46)	180.00 (26.06)

Note: Figures in parentheses represent the corresponding Coefficient of Variation in respective age groups

For assessing the normality assumption, coefficients of skewness and kurtosis were also worked out for each variable (Table 2). The skewness value was observed near to zero in girth and length of spurs, but kurtosis value was far from 3. Thus, no variable at location Shimla showed normality assumption (i.e. skewness = 0 and kurtosis = 3). As evident from the table, the skewness value was near to zero in girth and equal to zero in length of spurs, but kurtosis was observed to be quite less than 3 in case of Kotkhai. Hence, no variable at location 2 showed normality assumption. Similarly, the value of coefficient of skewness was found to be closer to zero in girth, spread, number of main branches, and length of spurs. Whereas the coefficient of kurtosis was found to be near 3, only in case of the number of main branches (2.44). This indicates that the variable indicating the number of main branches is followed by normal distribution.

TABLE 2. COEFFICIENTS OF SKEWNESS AND KURTOSIS OF VARIOUS TREE CHARACTERS FOR DIFFERENT AGE GROUPS

Variables	Coefficient of Skewness			Coefficient of Kurtosis		
	Shimla	Kotkhai	Theog	Shimla	Kotkhai	Theog
X_2: Girth (m)	0.09	-0.09	0.07	0.06	0.13	0.16
X_3: Height (m)	-0.29	-2.92	4.29	2.21	135.14	40.26
X_4: Spread (m)	0.42	-1.82	-0.13	2.53	96.09	12.06
X_5: Volume (m^3)	16.48	1797.38	361.45	666.30	24792899.10	259247.74
X_6: Number of main branches	1.60	0.91	0.42	12.62	7.22	2.44
X_7: Number of secondary branches	1.93	0.96	-1.56	38.11	49.08	79.27
X_8: Number of spurs per tertiary branch	5.17	1.90	2.11	129.07	15.11	27.76
X_9: Length of spurs(cm)	-0.01	0.00	-0.01	0.01	0.05	0.01
X_{10}: Number of flowers per tertiary branch	16.21	9.50	10.56	1358.02	377.87	694.01
X_{11}: Number of fruits per tertiary branch	20.31	5.15	5.68	595.93	140.41	277.99
X_{12}:Fruit weight (kg)	9.81	-15.89	-4.35	8243.62	1223.01	937.85
X_{13}: Yield (kg)	64.57	68.40	21.47	18221.93	43881.62	9610.75

Location Wise Variation among Different Tree Characters

TABLE 3. LOCATION WISE VARIATION AMONG VARIOUS TREE CHARACTERS

Tree Characters	L_1: Shimla $n_1 = 100$			L_2: Kotkhai $n_2 = 100$			L_3: Theog $n_3 = 100$			Bartlettt's χ^2 test statistic
	Mean	SD	CV(%)	Mean	SD	CV(%)	Mean	SD	CV(%)	
X_1: Age (Years)	20.23	6.71	33.18	22.88	11.40	49.82	17.94	9.19	51.23	26.76*
X_2: Girth (m)	0.42	0.12	28.79	0.59	0.26	43.15	0.67	0.31	47.05	80.56*
X_3: Height (m)	3.89	0.95	24.55	19.76	7.54	38.17	6.85	3.09	45.07	311.76*
X_4: Spread (m)	2.40	0.77	32.09	16.13	6.88	42.67	5.26	2.62	49.83	343.78*
X_5: Volume (m^3)	13.86	10.41	75.10	3698.82	3045.98	82.35	162.74	193.96	119.18	1352.25*
X_6: Number of main branches	3.86	1.61	41.83	3.70	1.46	39.46	4.48	0.96	21.40	27.14*
X_7: Number of secondary branches	12.26	3.84	31.34	13.32	4.85	36.44	19.41	6.71	34.57	31.32*
X_8: Number of spurs per tertiary branch	10.44	6.32	60.58	3.56	2.08	58.25	6.23	3.67	58.89	112.01*
X_9: Length of spurs(cm)	1.33	0.06	4.26	1.52	0.18	12.11	1.47	0.06	4.25	178.07*
X_{10}: Number of flowers per tertiary branch	32.99	19.97	60.53	17.81	10.38	58.25	31.14	18.32	58.83	42.65*
X_{11}: Number of fruits per tertiary branch	7.99	7.05	88.34	10.69	6.44	60.25	17.68	11.87	67.13	46.29*
X_{12}:Fruit weight (kg)	133.04	35.66	26.80	165.48	17.38	10.51	149.01	16.55	11.11	79.38*
X_{13}: Yield (kg)	10.52	7.24	68.84	169.22	129.07	76.28	88.87	70.13	78.92	444.87*

*Significant at 5% level of significance.

The location wise descriptive statistics (Table 3) showed that average age of trees was 20.23, 22.88 and 17.94 years at L_1, L_2 and L_3 locations. The trees of Theog site were found to have the highest values for tree girth (0.67m), the number of main branches (4.48), the number of secondary branches (19.41) and the number of fruits per tertiary branch (17.68). These parameters were minimum at L_1 as the tree diameter growth is sensitive to the environmental variations. Similarly, the second location i.e. Kotkhai recorded the maximum mean values for height (19.76m), spread (16.13m), volume (3698.82m^3), length of spurs (1.52), fruit weight (165.48), and yield (169.22kg). This might be due to micro climate and soil conditions such as moisture, available nutrients etc of site L_2 which resulted in more tree growth.

Most of the variables recorded minimum values at Shimla except the number of spurs (10.44) and flowers (32.99) per tertiary branch, which were observed to be the highest. The growth in site L_1 might be less due to the least age of trees, less exposure for the light because of direction and aspect of slope of the orchard. Tree height increased with the decreasing plant density. Although the number of spurs and flowers per tertiary branch were the highest in L_1, yet the number of fruits per tertiary branch, fruit weight and yield of tree were found to be minimum here. Similarly, the number of spurs and flowers per tertiary branch were found to be minimum in Kotkhai location, yet the yield recorded was the maximum.

Among the different variables, maximum coefficient of variation of 119.8 and 82.35 was observed for volume of the tree at L_3 and L_2 respectively, whereas L_1 observed maximum coefficient of variation for the number of fruits per tertiary branch. The minimum value of coefficient of variation was observed for height (24.55), the number of secondary branches (36.44), and the number of main branches (21.4) at L_1, L_2 and L_3 respectively.

Effect of Location and Age on Different Tree Characters

To gauge the relationship between age groups and locations with respect to growth characteristics, the data recorded were also subjected to the analysis of variance technique (factorial approach). For this purpose, 15 trees were randomly selected from the study area for measurements. The results are presented in Table 5.

The perusal of results (Table 4) revealed that the trees of A_3 age group were observed to have maximum mean values for girth (0.84m), height (14.37m), spread (11.74m), the number of main branches (4.89), the number of secondary branches (18.58), the number of spurs (8.17), length of spurs (1.51cm), the number of fruits (13.57), fruit weight(158.70g) and yield (196.30kg). These variables increased significantly with ages. However, volume decreased with the increase in age and was observed to be maximum in (2720.00m^3) in trees below 15 years age group (A_1). The results further revealed that the number of spurs, flowers and fruits were the highest in A_2 age group, but statistically at par with the A_3 age group. Although the number of spurs, flowers and fruits, fruit weight were at par in A_2 and A_3 age groups, yet the yield was higher in A_3 age group. This may be due to the fact that more number of branches yielded more number of fruits. Length of spurs was not significantly different from one another in A_2 and A_3 age groups.

While compared with the locations, it can be seen from the data that trees of Shimla had significantly higher number of spurs (12.21) and flowers (38.81) than the other two locations but had the lowest girth, height, volume, number of secondary branches, number of fruits and yield. Trees of Kotkhai location were observed to have significantly more height (19.33m), spread (16.54m), volume (3963.00m^3) and yield (200.00kg). The number of main branches was found to be at par in each location. The girth (0.71m), the number of main branches (4.44) and secondary branches (18.73), and the number of fruits (16.27) were recorded to be the highest in Theog location.

Trees of age 25 years or above at L_3 were thickest (1.13m) and had the maximum number of secondary branches (25.53) while trees between 15-25 years possessed the maximum number of flowers (44.42) and fruits (26.48). Trees of age 25 years or above of Kotkhai were the tallest and had maximum volume and yield. Trees of the same age group at Shimla had the maximum number of main branches and the number of spurs. Here, the number of flowers (A_3) were at par with that of Theog location (A_2).

The characters like height (26.35m), spread (23.62m), volume (7617.00m^3), length of spurs (1.72cm), fruit weight (169.90g) and yield (386.70kg) were found to be statistically significantly different from others and maximum in case of A_3 age group trees at Kotkhai location. The number of main branches was recorded to be maximum in A_3 age group at Shimla, but not significantly different from those in A_2 and A_3 age groups both in Kotkhai and Theog locations. The number of secondary branches was recorded to be maximum and significantly different in Theog location for A_3 age group. The maximum number of spurs and flowers was observed in A_3 age group at Shimla while the highest number of fruits was found in A_2 age group at Theog.

TABLE 4. EFFECT OF LOCATION ON DIFFERENT TREE - GROWTH CHARACTERS

Tree characters	Age Groups	L_1: Shimla	L_2: Kotkhai	L_3: Theog	Mean	Least significant difference (lsd)
X_2: Girth	A_1	0.29	0.26	0.30	0.28	lsd_L= 0.04
	A_2	0.42	0.64	0.69	0.59	lsd_A= 0.04
	A_3	0.52	0.85	1.13	0.84	lsd_{LA}= 0.06
	Mean	0.41	0.59	0.71		
X_3: Height	A_1	3.64	13.91	4.21	7.25	lsd_L= 1.31
	A_2	4.14	17.74	6.48	9.46	lsd_A= 1.31
	A_3	4.27	26.35	12.48	14.37	lsd_{LA}= 2.27
	Mean	4.02	19.33	7.72		
X_4: Spread	A_1	1.82	9.75	1.98	4.52	lsd_L= 1.27
	A_2	2.57	16.26	5.77	8.20	lsd_A= 1.27
	A_3	2.87	23.62	8.75	11.74	lsd_{LA}= 2.20
	Mean	2.42	16.54	5.50		
X_5: Volume	A_1	7.12	768.20	8.87	2720.00	lsd_L= 473.73
	A_2	15.07	3503.00	130.80	1216.00	lsd_A= 473.73
	A_3	20.00	7617.00	521.30	261.4	lsd_{LA}= 820.33
	Mean	14.06	3963.00	220.30		
X_6: Number of Main Branches	A_1	3.00	2.87	3.93	3.27	lsd_L= 0.50
	A_2	3.87	4.53	4.53	4.31	lsd_A= 0.50
	A_3	5.20	4.60	4.87	4.89	lsd_{LA}= 0.87
	Mean	4.02	4.00	4.44		
X_7: Number of Secondary Branches	A_1	11.60	8.67	10.53	10.27	lsd_L= 1.34
	A_2	12.67	14.73	20.13	15.84	lsd_A= 1.34
	A_3	11.33	18.87	25.53	18.58	lsd_{LA}= 2.32
	Mean	11.87	14.09	18.73		
X_8: Number of Spurs	A_1	11.40	1.57	2.50	5.16	lsd_L= 1.67
	A_2	10.95	4.67	8.88	8.16	lsd_A= 1.67
	A_3	14.28	4.00	6.23	8.17	lsd_{LA}= 2.89
	Mean	12.21	3.41	5.87		
X_9: Length of Spurs	A_1	1.31	1.30	1.49	1.37	lsd_L= 0.03
	A_2	1.34	1.67	1.49	1.48	lsd_A= 0.03
	A_3	1.33	1.72	1.49	1.51	lsd_{LA}= 0.06
	Mean	1.33	1.55	1.49		
X_{10}: Number of Flowers	A1	38.38	7.83	12.42	19.54	lsd_L = 5.93
	A_2	33.90	23.33	44.42	33.88	lsd_A= 5.93
	A_3	44.13	20.00	31.17	31.77	lsd_{LA}= 10.27
	Mean	38.81	17.06	29.33		
X_{11}: Number of Fruits	A_1	8.22	4.55	4.67	5.81	lsd_L= 2.83
	A_2	8.80	14.22	26.48	16.50	lsd_A= 2.83
	A_3	10.92	12.15	17.65	13.57	lsd_{LA}= 4.91
	Mean	9.31	10.31	16.27		
X_{12}: Fruit Weight	A_1	134.80	161.20	138.10	144.70	lsd_L= 6.55
	A_2	136.20	164.90	153.90	151.70	lsd_A= 6.55
	A_3	158.30	169.90	146.40	158.70	lsd_{LA}= 11.34
	Mean	143.10	165.30	146.10		
X_{13}: Yield	A_1	7.80	21.47	9.60	12.96	lsd_L= 3.77
	A_2	14.60	192.00	110.00	105.50	lsd_A= 3.77
	A_3	18.23	386.70	184.00	196.30	lsd_{LA}= 6.53
	Mean	13.54	200.00	101.20		

Conclusion

The variability analysis indicated that an increase in girth, height, spread, volume, number of main branches, number of secondary branches, length of spurs, and yield was noticed with increase in age of trees in general at all the three locations. Significant variation was observed in all the tree characteristics among the three sites or locations.

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Effect of Partial Substitution of Wheat Flour by Processed (Germinated, Toasted, Cooked) Chickpea on Bread Quality

Impact of Processed Chickpea Flour on Bread Quality

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Abstract

The effect of partial substitution of wheat flour with either raw and processed chickpea (germinated, toasted and cooked) flour at different levels (10 and 20%) on pasting properties of composite flours and on physical and nutritional parameters of the composite breads were studied. Composite flours of wheat and processed chickpea showed different pasting properties, decreasing the viscosity in all kinds of flour and levels, being more accentuated with the cooked flour. The least affected flour was the toasted that showed similar pasting profile than raw flour. Breads with 10% wheat flour replacement showed minor changes in their quality, but 20% replacement resulted in great detriment of the quality. Crumb hardness was greatly improved when 20% raw or toasted flour was incorporated. Overall, germinated chickpea flours were the most appropriate flour for wheat replacement pertaining bread specific volume, crumb hardness and nutritional composition (higher protein content).

Keywords

Legumes; Chickpea Flour; Bread; Germination; Cooking; Toasting; Quality

Introduction

Chickpea (*Cicerarietinum L.*) is one of the top five important legumes on the basis of whole grain production [1], and the second most important cool season pulse crop in the world. It is grown in at least 33 countries including Central and West Asia, South Europe, Ethiopia, North and South America, Australia and North Africa [2]. In Algeria, chickpeas and broad beans are the most cultivated dry legumes [3], widely consumed and are almost present in all traditional dishes. Various traditional oriental foods are prepared using chickpea floor slurry, both at household and industrial levels [4]. Regarding nutritional value, chickpea seed has high protein digestibility, contains high levels of complex carbohydrates and it is rich in vitamins and minerals [5]. Chickpea seed is processed and cooked in a variety of forms depending upon traditional practices and taste preferences. Different domestic processing methods (decortications, soaking, sprouting, fermentation, boiling, roasting, parching, frying, steaming) remove anti-nutritional factors and increase the protein digestibility of chickpea seed [6]. Although till recently consumers have neglected legumes, currently they are becoming increasingly health mindful. Bread fortification is one of the most successful strategies to mitigate nutrition deficiencies in developing countries [7]. Legumes combined with cereals constitute one of the worldwide staple commodities. Combination of cereal and legumes is effective and interesting; both have limiting essential amino acids that human body is not able to synthesize and it is needed to get them from food intake. Legumes are limited in methionine essential amino acid and rich in lysine, while cereals are lysine limiting amino acid [8]. In fact, several studies have focused on the influence of the addition of legume flours on the functional properties of doughs and final baked products quality [9-11]. In addition, some attentions have been paid to the effect of different types of pulses added to wheat flour to obtain baked goods and processed bread [12-14]. Nevertheless, there is no information about the effect of diverse processed chickpea flours on wheat flour replacement for breadmaking. Since chickpea is considered as an ideal

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complement to cereals in a healthy diet, it is taken into concern the possibility of replacing cereal-based products by the substitution of raw or processed chickpea flour to reduce the dependence of some countries on wheat importations. Therefore, the present work was undertaken to better understand the effects of substitution of wheat flour with raw and treated (germinated, toasted and cooked) chickpea flour at two levels 10 and 20% on the pasting properties of the flour blend, as well as to study the effect of substituting wheat flour by 10 and 20% of chickpea flour on physical, chemical and sensorial parameters of final bread.

Material and Methods

Materials

Chickpea was grown in winter 2013 and harvested in June 2013, in the region of Merj-Ouamane, commune of Amizour, wilaya of Bejaia; Algeria. Wheat flour was supplied by Harinera La Meta (Lleida,Spain), dry baker's yeast (Lesaffre, France) and salt were purchased from the local market.

Preparation of Chickpea's Flour

Four types of chickpea flour were compared: raw chickpea, germinated chickpea, cooked and toasted chickpea. Germinated chickpea flour were obtained by soaking chickpea seeds in tap water (1:10 w/v) for 12 h at room temperature (22 °C ± 2) and kept in the dark, then seeds were germinated between two sheets of wet filter papers for 48 h at room temperature in the dark (22 °C ± 2). Germinated seeds were dried overnight in stove at 60 °C before subjected to milling.

Cooked chickpea flour were obtained by soaking as described before and then samples were cooked for 15 min until reaching soft texture when pressing between the fingers. Cooked seeds were rinsed with tap water, drained and dried overnight at 60 °C before milling.

Toasted chickpea flour was obtained by toasting cleaned chickpea seeds in stove (Bergstr.14D-78532, Tuttlingen) at 180 °C for 20 min.

All the processed chickpea seeds and the raw chickpea seeds were ground into flour with a mortar and a pestle and then with a coffee grinder. The obtained powder was passed through a 0.5 mm screen to remove particle clumps and then flours were stored in air-tight plastic containers and held at 4°C until further analysis.

Flour Composition

The contents of moisture, ash, fat and crude protein (% N × 6.25) of the flour were determined according to AOAC official methods [15]. The percentage of carbohydrates was estimated by difference [100 – (% protein + % fat + % ash + % water)].

Pasting Properties of Flour Mixtures

Flour blends of chickpea flours and wheat flour were prepared at two different ratios 10:90 and 20:80 (g:g). Pasting properties of flour blends were determined using a rapid visco analyzer (RVA) (Newport Scientific Pty Ltd., Australia) by following the AACCI method [16], with some minor modifications. Sample (3 g based on 14 g of moisture per 100 g of flours blend) was added to 25 mL of water. RVA settings during assessment were heating from 50 to 95 ºC in 282 s, holding at 95 ºC for 150 s and then cooling to 50 ºC. Each cycle was initiated by a 10 s, 960 rpm paddle speed for mixing followed by a 160 rpm paddle speed for the rest of the assay. Viscosity was recorded during a heating-cooling cycle using Thermocline software for Windows (Perten, Stockholm, Sweden). Peak viscosity (maximum viscosity during heating), breakdown (decay of viscosity during heating), final viscosity (viscosity at the end of cooling) and setback (difference between final viscosity and peak viscosity) were evaluated. Samples were run in duplicate.

Breadmaking Process

Breads with different levels of chickpea flour were formulated: bread at 100% wheat flour, bread at 20% chickpea flour (raw and treated), and bread at 10% chickpea flour (raw and treated). The bread dough formula consisted of 300 g flour (wheat or blends), 4.5 g salt, 2.1 g dry yeast (Saf-instant, Lesaffre Group, France) and water. Dry ingredients were mixed in a Farinograh (Brabender, Duisburg, Germany) for 30 s at 30 °C and then water was added and mixed again for 7 min at 30 °C. Water content was adjusted to keep constant the dough consistency (172.5 ml for wheat bread, raw 10 and 20%, 180.5 ml for germinated 10%, 185.5 ml for germinated 20%, 181.5 ml for toasted 10%, 189.5 ml for toasted 20%, 187.5 ml for cooked 10%, and 197.5 ml for cooked 20%). Dough was divided into 9 hand-rounded pieces (50 g) that were mechanically molded. Proofing was carried out in a fermentation cabinet (Salva Industrial S.A., Lezo, Guipuzcoa, Spain) for 55 min at 30 °C and baked into an electric oven (Salva Industrial S.A., Lezo, Guipuzcoa, Spain) for 25 min at 180 °C. Loaves were cooled down for 30 min at room temperature and then packed into polyethylene pouches till further analysis. Two sets of breads were made for each flour blends.

Bread samples were coded for wheat (W), raw chickpea (R), germinated chickpea (G), toasted chickpea (T) and cooked chickpea (C), followed with 10% or 20% to indicate the amount of chickpea flour used for making the blends.

Bread Quality Parameters

1) Instrumental Parameters

Technological parameters of bread quality included: volume (rapeseed displacement) [16], specific volume (by dividing volume by weight), moisture content [16], crumb color and crumb texture profile analysis (TPA). The color of the bread crumbs was measured at three different locations. A colorimeter (Chroma Meter CR-400/410, Konica Minolta, Japan) was used to measure the crumb colour parameters (L^*, a^*, b^*). The results were expressed in accordance with the CIELAB system (D65 illuminant and 10° viewing angle). The measurements were made with a 30 mm diameter diaphragm inset with optical glass. The parameters measured were L^* (L^*= 0 [black], L^*=100[white] indicates lightness, a^* indicates hue on a green ($-a^*$) to red ($+a^*$) axis, and b^* indicates hue on a blue ($-b^*$) to yellow ($+b^*$) axis. Chroma (C^*) and hue angle ($h°$) were calculated using the following equation: $(a^{*2} + b^{*2})^{1/2}$ and the arctangent of b^*/a^*, respectively. Data from three slices per bread were averaged for each batch.

Crumb texture analysis was carried out on uniform slices of 10 mm thickness. Four slices from the center of each loaf were taken for evaluation. Texture profile analysis (TPA) was performed using a universal testing machine TA.XTplus (Stable Micro Systems Ltd., Godalming, UK) equipped with a stainless steel cylinder probe (P/25, 25 mm diameter). A double compression test up to 50% penetration of its original height at a crosshead speed of 1 mm/s and a 30 s gap between compressions was carried out. Hardness, cohesiveness, springiness, chewiness and resilience were calculated from the TPA recorded plot using the software "Texture Expert".

2) Chemical Composition of Breads

The chemical composition of bread samples was determined according to AOAC official methods [15]. The percentage of carbohydrates was estimated by difference [100 – (% protein + % fat + % ash + % water)].

3) Sensory Evaluation

A descriptive sensory analysis was preliminary performed for evaluating the sensory characteristics, and then a quantitative descriptive sensory analysis. Bread slices, including crust and crumb, were presented (1 cm thick) on plastic dishes coded and served in randomized order under normal lightening conditions and at room temperature. Twelve trained panelists that had participated in descriptive analysis and scale rating of a wide range of bread products for more than 10 years carried out this evaluation. Preliminary training test was performed, in which they were sat in a round table and after evaluating the sample, an open discussion was initiated for defining and describing the best descriptors for characterizing the product. Evaluation included perception at first glance of the bread slice (crust and crumb included) and mastication with the molar teeth up to swallowing. The attributes assessors finally agree were appearance (by observing the product slice), flavor and taste. The descriptors for each attributes were appearance (visually liking or disliking), flavor (scale goes from high when typical of bread or bakery products to low, uncharacteristic of bakery products), taste (scale goes from high when typical taste of bread or bakery products to low, uncharacteristic of bakery products).

Attribute intensity was scored on a scale varying from 1 (disliked extremely) to 9 (like extremely). Two samples were evaluated during one session. Breads were considered acceptable if their means score for overall acceptance were above 5.

Statistical Analysis

Data were subjected to multifactor analysis of variance (ANOVA) to study the differences in bread quality induced by the kind of treatment and the chickpea flour level incorporated in the blends. A multiple sample comparison was carried out for analyzing bread parameters and pasting properties of flour individually. Fisher's least significant difference (LSD) test was used to describe means with 95% confidence. StatgraphicsPlus Centurion XVI (Statpoint Technologies, Warrenton, USA) was used as the statistical analysis software.

Results and Discussion

The composition of raw and treated chickpea seeds is presented in Table 1. There was a significant ($P<0.05$) increase in the protein content of the chickpea, this increase was also noted by Camacho et al. [17] during germination of beans, chickpea and pea's seeds. Hsu et al. [18] observed that protein content of legumes generally increase during germination as a result of biochemical changes induced by sprouting, leading to an increase in free amino acids. Conversely, toasting slightly decrease the protein content. Seeds that were subjected to soaking; showed lower carbohydrates content due to the losses of the water soluble compounds. Conversely, germination was the only process that decreased the amount of fat content due to the germination process itself, and differences were not significant with the raw seeds. Germinated and cooked chickpea exhibited lower ash content than raw and toasted ones. This significant ($P<0.05$) decrease might be caused by leaching and diffusion of water soluble compounds into soaking and cooking water. These results are in agreement with those observed by Alajaji and El-Adawy [19] in chickpeas.

TABLE 1.PROXIMATE COMPOSITION OF PROCESSED CHICKPEA FLOURS (% AS IS)[A]

Treatment	Protein (%)	Carbohydrates* (%)	Fat (%)	Ash (%)
Raw	21.85±0.18 b	66.42±0.74 a	6.36±0.49 ab	2.92±0.03 a
Germinated	22.27±0.14 a	65.93±0.20 b	6.21±0.29 b	2.66±0.04 b
Toasted	21.44±0.12 c	66.49±0.65 a	6.85±0.11a	3.07±0.50 a
Cooked	21.88±0.11 b	65.90±0.37 b	6.93±0.50 a	2.19±0.39 c

[A] Means in a column with different letters are significantly different ($P< 0.05$).
* Carbohydrate content was calculated by difference.

Pasting Properties of Flour Mixtures

The pasting properties of wheat and flour blends were determined following a heating-cooling cycle (Figure 1) and parameters recorded from the pasting curves are shown in Table 2. Overall, the presence of chickpea flour lowered the wheat viscosity profile and the effect was intensified when increasing the level of wheat replacement. The effect was readily evident when reaching the maximum viscosity and differences were kept along holding at 95 ºC and cooling. The 20% composite slurries peaked earlier during heating than the 10% and control wheat slurry. Considering the type of process, cooked chickpea flour gave the lowest paste viscosities followed by germinated chickpea, and those effects were even more pronounced with 20% replacement. Presumably, the removal of water soluble compounds plus partial gelatinization of starch during cooking would explain the behavior of wheat-cooked chickpea blends. Conversely, pasting profile obtained with germinated flour should be explained by the activation of enzymes during germination, as reported Cornejo and Rosell [20] with germinated rice flour. Toasting was the least severe processing, since wheat blends showed similar plots to wheat flour. Likely toasting inactivated endogenous enzymes present in chickpea having less impact in wheat pasting performance than raw chickpea.

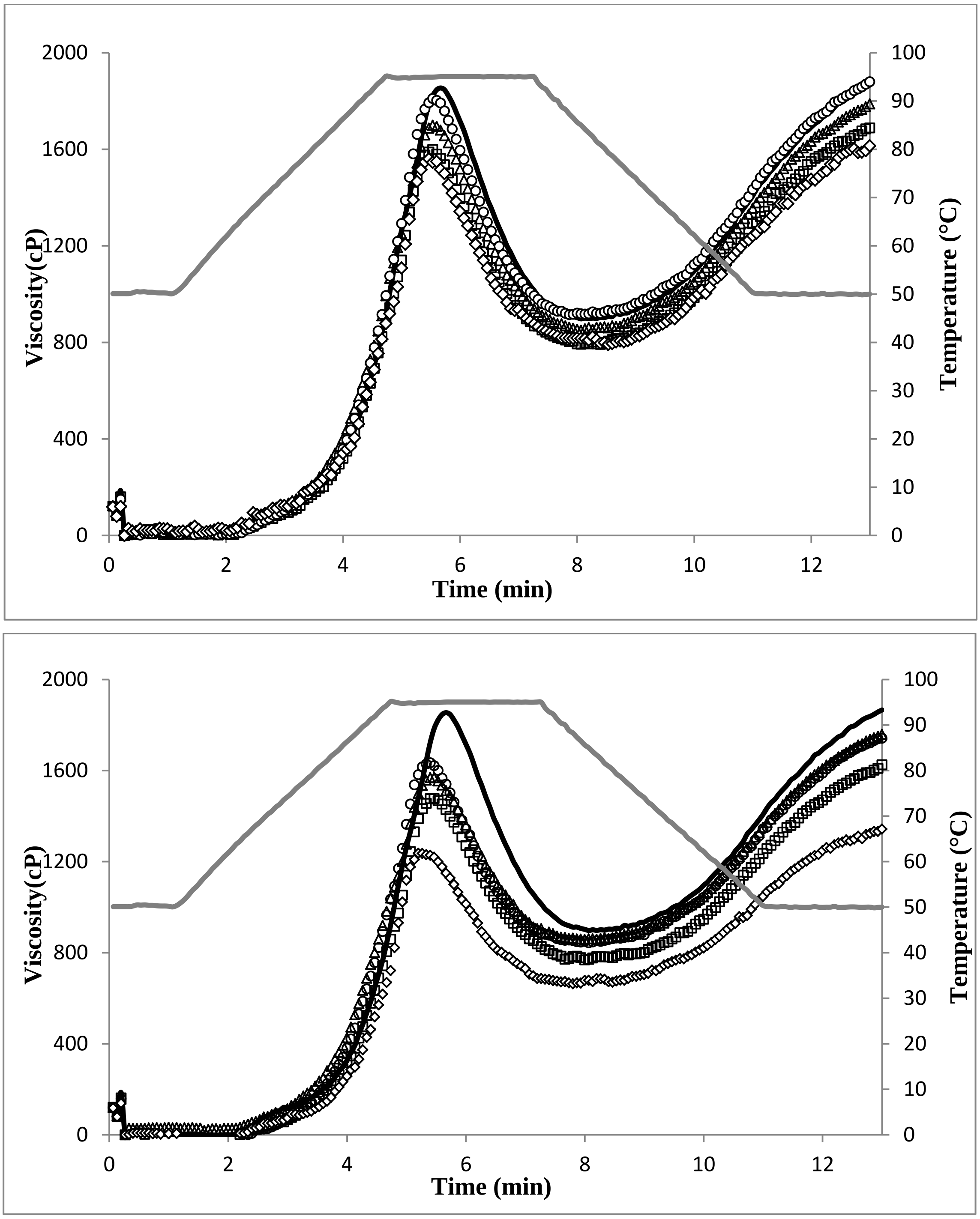

FIGURE 1. RVA PROFILES OF COMPOSITE FLOURS CONTAINING CHICKPEA FLOURS AT 10% (A) OR 20% (B) LEVEL OF WHEAT REPLACEMENT .LEGENDS: WHEAT (SOLID BLACK LINE), RAW CHICKPEA (Δ), GERMINATED CHICKPEA (□), TOASTED CHICKPEA (**O**), COOKED CHICKPEA (◊).

Significant differences were observed in pasting parameters among wheat-chickpea flour blends (Table 2), except for the pasting temperature. Differences were induced by the presence of processed chickpea flour and also by the level of legume flour, as revealed the statistical analysis. The maximum viscosity reached by wheat and composite flours varied from 1240 to 1854 cP, obtaining the lowest value at 20% wheat replacement and particularly in the case of cooked chickpea flour (C20%) due to legume starch pre-gelatinization during cooking. The breakdown that measures how the swollen granules get disintegrated upon shearing decreased significantly in all composite slurries. The highest decrease (40%) was observed in C20% (578 cP), indicating its lowest paste stability. Sandeep et al. [21] pointed out that proteins could provide some protection against the breakdown due to the contribution of denatured proteins to support the structure of the matrix and inhibiting the thixotropic nature of starch in flour, but it seems that the protection effect would be dependent on the proteins nature because it was not observed in the chickpea flours. As the paste was cooled down, the viscosity increased due to the aggregation of the amylose molecules. The composite slurries gave slightly lower final viscosities and lower setback except for the C10% and C20%, which was significantly lower. The slurries of these blends swelled faster and bound more water; however, less retrogradation occurred leading to less viscous gel after cooking and cooling. Presumably, the lowest setback obtained with C10% and C20% might be ascribed to lower amylose content, which leached out during chickpea cooking.

TABLE 2. PASTING PROPERTIES OF COMPOSITE FLOUR CONTAINING CHICKPEA FLOUR AT 10% OR 20% LEVEL OF WHEAT REPLACEMENT

Flours	Pasting Temperature (ºC)	Peak Viscosity (cP)	Breakdown (cP)	Final Viscosity (cP)	Setback (cP)
W	65±1	1854±3f	959±1g	1866±7fg	971±4e
R 10	58±10	1699±8e	852±4e	1788±4ef	941±1e
G 10	67±3	1599±113cd	811±43d	1689±120cd	900±49d
T 10	59±10	1812±2f	899±11f	1880±6g	967±7e
C 10	59±9	1538±32bc	771±2c	1590±34b	823±0b
R 20	65±12	1570±42cd	714±2b	1759±56de	902±16d
G 20	66±17	1480±13b	713±1b	1625±20bc	859±5c
T 20	72±6	1634±18de	791±6cd	1742±41de	899±16d
C 20	57±9	1240±17a	578±37a	1343±16a	680±4a
P-value					
Factor 1	0.580	0.001	0.001	0.001	0.001
Factor 2	0.400	0.000	0.000	0.000	0.000

Mean ± standard deviation values followed by different letters within a column denote significantly different levels (P< 0.05) (n = 3). Factor 1: Flour; Factor 2: Level of substitution.
W: Wheat, R: raw chickpea, G: germinated chickpea, T: toasted chickpea, C: cooked chickpea.

Bread Characterization

Photographs of breads made with different processed chickpea flours are shown in Figure 2. Cross-section of bread slices displayed open and aerated crumb structure, which had even bigger gas cells than the wheat bread.

Results on the physicochemical characteristics (specific volume, moisture and color parameters) of composite bread samples containing 10 and 20% chickpea flours (raw and processed) are shown in Table 3. The substitution of the bread by chickpea flour at 10 and 20% did not significantly (P<0.05) affect the specific volume, with the exception of C20% that showed a significantly reduced specific volume (Figure 2). An increase in the specific volume was envisaged with 10% germinated chickpea (G10%) (3.06 mL/g), likely due to the enhancement in hydrolytic enzymatic activity and soluble materials, as has been reported for rice germinated flour [20]. Composite breads could be obtained with 20% wheat replacement without affecting specific volume and germinated and toasted chickpea flour led higher specific volume, although differences were not significant. Similar trends were reported by Shin et al. [14] when comparing bread made with germinated and cooked soy flour with bread made by untreated soy flour. A decrease in specific volume is usually the effect observed when wheat flour is replaced with legume based ingredients [9, 22]. Gularte et al. [23] found that the incorporation of 50% legume flour (pea, lentil and bean) increased the specific volume of wheat cakes, whereas chickpea did not significantly affect it. Likely, the adjustment of dough hydration during breadmaking minimized the effect of wheat replacement by chickpea flour.

The flour type and the level of substitution affected significantly (P<0.05) the moisture content of bread, which was higher in the presence of cooked chickpea flour (Table 3). This was attributed to the higher amount of water required for breadmaking when wheat flour was substituted with cooked chickpea flour.

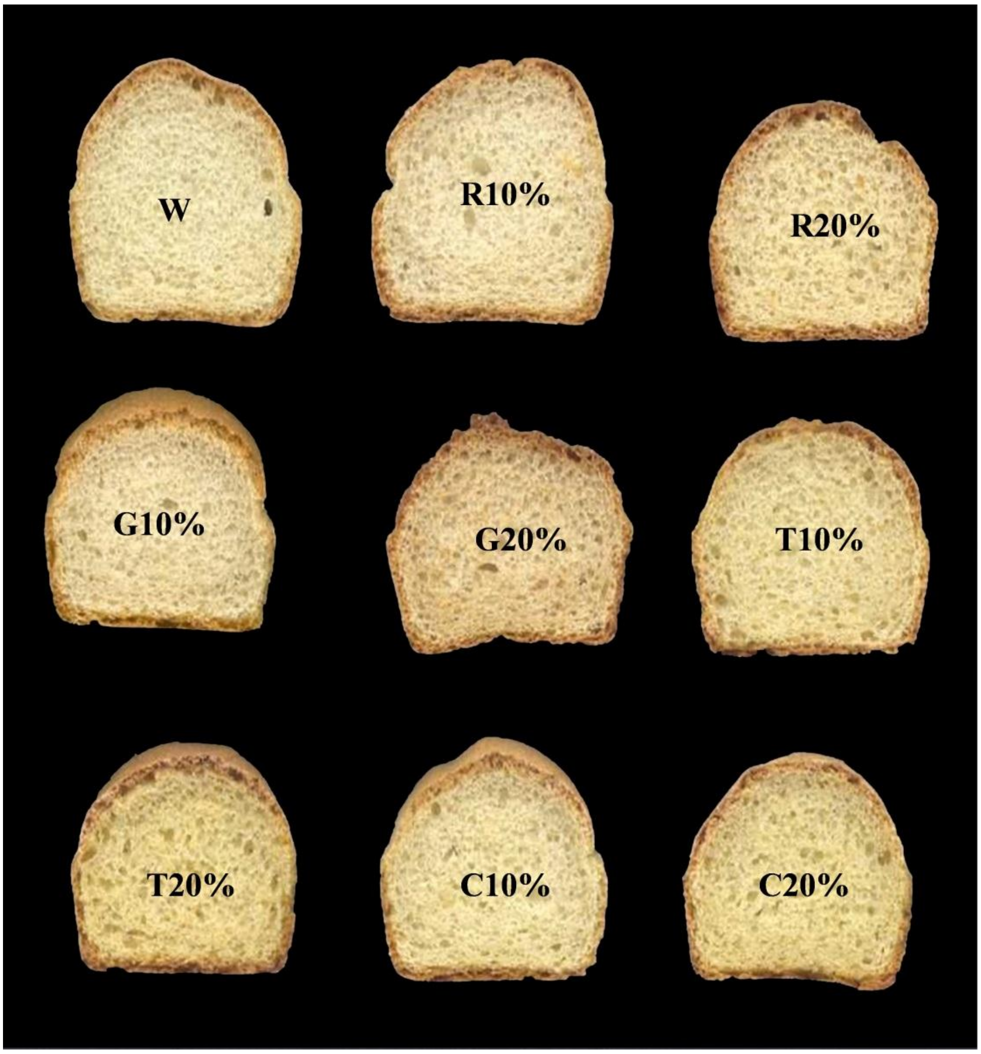

FIGURE 2. PHOTOGRAPH OF BREADS OBTAINED AFTER SUBSTITUTION OF WHEAT FLOUR BY RAW AND PROCESSED CHICKPEA FLOUR AT 10 AND 20%. LETTERS ARE REFERRED TO THE TYPE OF FLOUR AND PERCENTAGE TO THE AMOUNT OF CHICKPEA FLOUR ADDED. W: WHEAT; R: RAW CHICKPEA; G: GERMINATED CHICKPEA; T: TOASTED CHICKPEA; C: COOKED CHICKPEA.

Color in baked goods could come from different sources: intrinsic color imparted by individual ingredients, developed color resulting from the interaction of ingredients, like Maillard or caramelization reactions, besides processing changes associated to chemical or enzymatic reactions [23]. The substitution of wheat flour with raw and processed chickpea flour affect the color properties of composite breads, inducing a decrease in the whiteness in the composite breads especially for the T20%, which showed the most dark crumb. This might be due to the chemical browning reactions during toasting [24]. It has been reported that the addition of legume flours to baked products led to darker crumbs [23] in some legume based products. The hue red (a^*) increased significantly ($P<0.05$) among the composite breads except for the C10% and T10%. The hue yellow (b^*) significantly ($P<0.05$) increased when 20% chickpea flours were present and that effect was also observed with 10% wheat replacement with toasted and cooked chickpea flour. Similar results have been reported when partial substitution of wheat flour was carried out with different levels (up to 30%) of raw chickpea flour [25]. The color of crust and crumb got progressively darker as the level of chickpea flour substitution increased. The saturation (C^*) was not affected when the wheat flour was substituted with 10% of raw and germinated chickpea flour. Conversely, it increased significantly ($P<0.05$) when this replacement was with thermally treated chickpea flour (toasted and cooked). All the breads made with 20% substitution increased significantly ($P<0.05$) the chroma comparing to wheat bread. Regarding the hue angle $h°$, it was significantly increased in G10%, R20% and G20% and no effect was detected with thermally treated chickpea flours (toasted and cooked). In opposition, Shin et al. [14] found that heat treated soy flours (steamed and toasted) decreased the $h°$ value compared to those non heated soy flours (raw and germinated).

The textural properties of composite breads were significantly dependent on the type of flour and level of substitution (Table 4). All the texture parameters tested were statistically (P<0.05) affected by the level of substitution, resulting in an increase of hardness and chewiness when increasing chickpea level and a decrease in springiness and resilience, implying that it will take more time for the structure of the crumb to recover after compression [26]. These results are in concordance with those found by Gomez et al. [9], where resilience decreased though chewiness augmented when increasing chickpea flour percentage in wheat- chickpea cake. Composite breads at 20% level of substitution had higher hardness and chewiness and less springiness, but the extent of the effect was dependent on the type of chickpea flour. Cooked and germinated chickpea flour at 20% level gave softer crumbs with lower chewiness and thus they showed easy chewing.

TABLE 3. EFFECT OF ADDING CHICKPEA FLOUR (10% OR 20%) IN WHEAT BREAD ON PHYSICAL CHARACTERISTICS OF BREAD

Breads	Specific Volume (ml/g)	Moisture (%)	Color				
			*L**	*a**	*b**	*C**	*h°*
Wheat	2.89 ± 0.14 bcd	29.51 ± 0.29 a	69.08 ± 1.74 d	-1.22 ± 0.38 a	17.70 ± 1.80 a	17.75 ± 1.78 a	-1.33 ± 0.72 ab
R 10	2.92 ± 0.08 bcd	29.13 ± 0.33 a	66.40 ± 1.72 bc	-0.19 ± 0.39 d	17.08 ± 1.44 a	17.08 ± 1.44 a	-0.86 ± 1.33 bc
G 10	3.06 ± 0.11 d	29.46 ± 0.58 a	66.94 ± 1.47 cd	0.03 ± 0.31 d	17.96 ± 0.93 a	18.01 ± 0.94 a	-0.09 ± 1.60 d
T 10	2.99 ± 0.12 cd	29.94 ± 0.52 ab	66.49 ± 1.63 bc	-1.14 ± 0.31 ab	21.52 ± 1.38 bc	21.55 ± 1.37 b	-1.52 ± 0.02 a
C 10	2.84 ± 0.16 bc	31.13 ± 1.16 bc	67.54 ± 1.18 d	-1.45 ± 0.27 a	21.00 ± 0.76 b	21.05 ± 0.75 b	-1.50 ± 0.01 a
R 20	2.78 ± 0.27 b	29.40 ± 0.54 a	66.66 ± 1.34 bcd	-0.12 ± 0.50 d	21.95 ± 1.38 c	21.96 ± 1.39 b	-0.52 ± 1.51 cd
G 20	2.87 ± 0.13 bc	30.49 ± 0.89 b	65.64 ± 1.23 b	0.88 ± 0.31 e	21.52 ± 0.81 bc	21.31 ± 1.28 b	1.37 ± 0.71 e
T 20	2.83 ± 0.22 bc	30.93 ± 0.66 b	63.66 ± 1.36 a	-0.52 ± 0.42 c	26.18 ± 1.33 e	26.19 ± 1.32 d	-1.20 ± 1.00 ab
C 20	2.37 ± 0.10 a	32.10 ± 1.34 c	66.17 ± 1.95 bc	-0.91 ± 1.09 b	24.84 ± 1.34 d	24.88 ± 1.34 c	-1.18 ± 0.98 ab
P-value							
Factor-1	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Factor-2	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Mean ± standard deviation values followed by different letters within a column denote significantly different levels (P < 0.05) (n = 4). Factor 1: type of flour; Factor 2: Level of substitution.
R: raw chickpea, G: germinated chickpea, T: toasted chickpea, C: cooked chickpea.

TABLE 4. EFFECT OF PROCESSED CHICKPEA (10% OR 20%) ON WHEAT BREAD TEXTURE AND SENSORY PARAMETERS

Breads	Hardness (g)	Springiness	Cohesiveness	Chewiness (g)	Resilience	Appearance	Flavor	Taste
Wheat	642 ± 81 a	1.04 ± 0.04 b	0.85 ± 0.01 c	590 ± 50 ab	0.45 ± 0.02 d	7 ± 2	9 ± 0 d	9 ± 0
R 10	714 ± 78 a	1.05 ± 0.03 b	0.83 ± 0.01 c	604 ± 43 ab	0.43 ± 0.02 c	7 ± 1	8 ± 1 ab	9 ± 1
G 10	645 ± 37 a	1.04 ± 0.02 b	0.82 ± 0.02 bc	558 ± 16 a	0.43 ± 0.02 c	9 ± 2	8 ± 1 ab	7 ± 0
T 10	642 ± 59 a	1.04 ± 0.03 b	0.83 ± 0.01 c	626 ± 29 abc	0.42 ± 0.05 b	9 ± 2	9 ± 0 d	9 ± 0
C 10	676 ± 50 a	1.05 ± 0.03 b	0.82 ± 0.02 bc	565 ± 21 ab	0.46 ± 0.02 e	9 ± 1	8 ± 1 ab	8 ± 1
R 20	1089 ± 81 c	1.02 ± 0.02 a	0.79 ± 0.02 ab	849 ± 41 d	0.37 ± 0.02 a	8 ± 1	8 ± 1 ab	8 ± 1
G 20	807 ± 54 b	1.00 ± 0.05 a	0.76 ± 0.08 a	651 ± 49 bc	0.42 ± 0.03 b	8 ± 2	7 ± 0 a	6 ± 1
T 20	1037 ± 92 c	1.02 ± 0.03 a	0.80 ± 0.02 b	797 ± 64 d	0.40 ± 0.03 b	9 ± 1	9 ± 0 d	9 ± 1
C 20	831 ± 37 b	1.03 ± 0.02 a	0.83 ± 0.04 c	704 ± 62 c	0.44 ± 0.02 cd	9 ± 2	9 ± 1 cd	8 ± 1
P-value								
Factor-1	0.002	0.576	0.071	0.00 0	0.00 1	0.33 6	0.23 0	0.10 0
Factor-2	0.000	0.013	0.000	0.00 0	0.00 0	0.19 0	0.04 6	0.09 8

Mean ± standard deviation values followed by different letters within a column denote significantly different levels (P< 0.05) (n = 4). Factor 1: type of flour; Factor 2: Level of substitution. R: raw chickpea, G: germinated chickpea, T: toasted chickpea, C: cooked chickpea, W: wheat.

Chemical Characterization of Bread

The wheat replacement by chickpea flour significantly changed the chemical composition of breads, and also the type of flour significantly affected the composition pattern (Table 5). Ash content gradually increased in all composite breads as compared to the control except for the C10%, which agrees with the lower ash content measured in the cooked flour. Similar results were obtained by Saleh et al. [10] and Ndife et al. [13] in breads substituted by soya beans and chickpea.

TABLE 5. EFFECT OF ADDING CHICKPEA FLOUR (10% OR 20%) ON WHEAT BREAD PROXIMATE COMPOSITION (EXPRESSED AS PERCENTAGE AS IS BASIS)

Breads	Protein (%)				Carbohydrate (%)				Fat (%)				Ash (%)			
Wheat	9.26	±	0.00	a	59.51	±	0.03	g	0.16	±	0.01	a	1.64	±	0.03	a
R 10	9.97	±	0.02	d	57.78	±	0.03	de	0.31	±	0.02	c	1.78	±	0.01	bc
G 10	10.06	±	0.01	d	58.90	±	0.03	f	0.31	±	0.01	c	1.80	±	0.01	c
T 10	9.86	±	0.14	c	57.72	±	0.15	d	0.29	±	0.02	b	1.73	±	0.01	b
C 10	9.56	±	0.04	b	55.37	±	0.01	b	0.34	±	0.00	d	1.63	±	0.05	a
R 20	10.75	±	0.02	h	57.88	±	0.06	e	0.48	±	0.01	f	1.98	±	0.05	e
G 20	10.35	±	0.04	f	55.75	±	0.04	c	0.44	±	0.02	e	1.89	±	0.01	d
T 20	10.63	±	0.02	g	55.42	±	0.01	b	0.45	±	0.01	e	1.90	±	0.02	d
C 20	10.24	±	0.01	e	54.14	±	0.03	a	0.57	±	0.00	g	1.73	±	0.02	b
P-value																
Factor-1	0.008				0.003				0.004				0.002			
Factor-2	0.000				0.000				0.000				0.000			

Mean ± standard deviation values followed by different letters within a column denote significantly different levels ($P< 0.05$) (n = 4). Factor 1: type of flour; Factor 2: Level of substitution.
R: raw chickpea, G: germinated chickpea, T: toasted chickpea, C: cooked chickpea, W: wheat.
*Carbohydrate content was calculated by difference.

Fat content was statistically ($P<0.05$) affected by substituting wheat flour by raw and treated chickpea flour, increasing with the level of replacement. Cooked flour was the one that majorly increased the fat content. The high fat content in chickpea flour (~6-7%) must have contributed to increasing fat content in composite bread comparing with control bread made with 100% wheat flour. As expected, the protein content in breads augmented with the level of wheat replacement by chickpea flour. The highest increase in protein content was observed in breads containing germinated chickpea flour. Similar results are found in literature about some legume based products [10, 13]. Carbohydrate content decreased in all composite breads. These results were expected due to the supplementation of wheat flour with legume flour (chickpea) that contains lower amount of carbohydrate. Results are in agreement with those found by Rababah et al. [27] and Ndife et al. [13].

In general, composite breads gave sensory acceptable breads and met sensory standards receiving approval by judges (Table 4). No significant differences were induced in the appearance, flavor and taste due to the type of chickpea flour or the level of replacement, with exception of the effect of level on the flavor. In terms of appearance the panel considered that C10% had the best appearance comparing with other composite breads and control wheat bread (W). Concerning flavor, breads containing toasted flour were highly appreciated due to toasting liberated some volatile compounds making the flour smell good.

Conclusion

Chickpea processing (germination, toasting, and cooking) led to flours with different chemical composition, main effect was the decrease in protein and fat due to germination and the decrease in carbohydrate due to soaking of seeds during germination and cooking. The cooking process induced the greatest effect due to the loss of water soluble compounds. Pasting properties of the wheat-chickpea blends were significantly modified due to wheat flour replacement by 10 and 20%. Cooked flour induced the greatest decrease in viscosity due to starch gelatinization during cooking. Conversely, toasting promoted the least effect due to the inactivation of the chickpea enzymes, and behavior was rather similar to raw seeds flour. Regarding bread quality, wheat replacement by 10% processed chickpea flour led breads with similar technological and sensory characteristics than wheat bread. The increasing replacement (20%) resulted in harder breads, especially in the case of raw and toasted flour (1089 g and 1037 g vs 714 g and 642 g when 10% replacement, respectively). Overall, germinated chickpea flours were the most appropriate flour for wheat replacement pertaining bread specific volume crumb hardness and nutritional composition (higher protein content), but they worsened when 20% replacement. Conversely, although toasted flour impaired hardness, it gave pleasant flavor that was really appreciated.

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