Transfer of Land Use Rights in China: Results from a Survey of Rural

Households in 8 Counties of Hebei Province

Wen Yan [*1], K. K. Klein [2]

[*1]Hebei Finance University, Baoding, Hebei, PRC

[2]Department of Economics, University of Lethbridge, Lethbridge, Alberta, Canada

[*1]wassx@126.com; [2] klein@uleth.ca

Abstract

The paper aims to determine why the rate of land use transfers in the selected region Hebei province has been so low compared with the average by the introduction of possible factors that may have an impact on rural households' willingness to transfer land use right and as well to find out the decisive factors so as to propose implications accordingly. Econometric results indicate that the absence of a social security system in rural areas, the lack of freedom of many farm households to negotiate the terms of transfers of their land use rights, and the low proportion of total household income that comes from non-farm sources are the main factors that can explain this phenomenon.

Keywords

Land Use Rights; Household Responsibility System; Land Ownership; China

Introduction

For almost thirty years, an effective system of contracting out agricultural land in China, called the Household Responsibility System (HRS), has helped to transform the countryside. Under this system, the former collective landholdings of a production team were divided more or less equally among the member households (Liu et al., 1998). In the 1980s, the profit incentives introduced by the HRS became a powerful motivator to improve agricultural productivity and brought increased prosperity to the rural regions of China. Between 1979 and 1984, the value of agricultural output (in constant prices) grew at an average of 7.5% per year (Lin, 1988; McMillan et al., 1989). However, with time, shortcomings of the HRS have been exposed. The system hindered the mechanisation and modernisation of agriculture and led to scattered and inefficient use of capital, technology and agricultural labour. In the 1980s, frequent land readjustments among households were imposed by governments. Although these readjustments were designed to adapt to the economic conditions, they also hindered farming investments by rural households. The apparent inability of the HRS to sustain growth in agricultural productivity led to calling for further reform of China's land tenure system (Dong, 1996).

Farmers in China have the rights to use specific parcels of land but do not have full property rights as all rural land is collectively owned. However, "collectively owned" is a vague concept and there is no legal description of the boundary of rights of relevant individuals and organisations, which, in practice, means there is no legal entity that has complete ownership. Farmers cannot sell or buy land, only the rights to use the land. Improving the ability to transfer the rights to use the agricultural land could facilitate improvements in agricultural productivity in China.

China is typical of a dual economy state, characterized by great income disparities between agricultural and urban jobs (both long-term and temporary). This has led to a vast transfer of the labour force from low to high wage sectors. Because of low productivity and low incomes in agriculture, as well as much better living conditions in urban areas, an estimated 18 million people have migrated from rural areas to cities annually in recent years (UNPFA, 2007). China's urban population has outnumbered rural population for first time since the end of 2011 (NBS, 2012). This rapid urbanisation of China's population has resulted in large areas of rural land cultivated by the less productive elderly. Much of the land cultivated by households is underutilized. At the same time, there are some larger and more efficient farmers, including some agricultural corporations, who wish to enlarge their farming scale and produce more output. With improved ability and willingness to transfer the rights to use farm land, scattered land that is not farmed efficiently could be matched with other productive resources to improve agricultural productivity.

The Chinese government has attempted to find ways to encourage greater productivity in agriculture by making changes on laws that have affected agricultural land property and transference rights. By the end of 2009, the amount of agricultural land transferred from one user to another in China was reported to be 8,239,700 hectares, which accounted for 10.12% of the total agricultural land base in China (Yan, 2010). However, farmers in Hebei, a large province in northern China, have been slow to transfer rights to use their land. By the end of 2009, the total area transferred accounted for only 4.8% of the arable land in that province (Yan, 2010). Moreover, most of the transfers of land use rights in Hebei have been informal verbal agreements with few formal contracts signed.

The purpose of this study is to investigate the reasons why the transfers of land use rights in Hebei have occurred at such a low rate. This study seeks to determine the importance of such factors as the absence of a social security system in rural areas, the freedom of farm households to negotiate their own transfers, family size, net returns obtained from their farming operations, educational level, and the proportion of total household income that comes from non-farm sources on the willingness of Hebei farmers to transfer their agricultural land use rights.

Results from this study should be useful to policy makers in Hebei who are concerned about increasing the transference of agricultural land use rights. The results also have implications for the transference of agricultural land in other provinces in China.

The paper is organized as follows. The second section provides additional background materials, including a short history of rural land reform in China and the current legal framework for land policy in China. The third section reviews and analyzes relevant literature on property rights in China. The fourth section presents details of a survey conducted in eight counties of Hebei and provides an analysis of household responses to questions on their willingness to transfer their land use rights. Implications and conclusions from the study are presented in the final section.

History of Rural Land Reform in China

After the foundation of the People's Republic of China (PRC) in 1949, radical transformation and reforms of the country were carried out, including land reform. During the period 1949–1953, control of land was taken from the large and wealthy landowners and then redistributed to peasant farmers. However, private ownership of all land by the farmers remained in the policy. Beginning in 1953, the private ownership of land was converted to collective ownership where individual peasant farmers worked together as members of a collective. In 1978, a group of farmers in Xiaogang village of Anhui province sparked the reform of rural land policies. At the risk of arrest and imprisonment, they collectively broke the law by secretly contracting out land in their village. This illegal activity then was made legal by the Chinese Communist Party (CCP) and a new system of contracting out land, called the Household Responsibility System (HRS), was extended across the country. Since 1984, farmers typically were given the right to use assigned areas of land for their own use for a period of fifteen years. This was extended to 30 years in 1993. In 1998, the Chinese government passed the Land Management Law, which formalized the farmers' land use rights by providing them with legal written contracts.

In 2003, the law on Land Contracts in Rural Areas came into effect. This law has encouraged and protected the voluntary transfer of land use rights (with compensation)as well has given rural households the right to manage and profit from a specified piece of agricultural land previously owned collectively by the peasants, including arable land, forestlands, grasslands and other lands used for agriculture by the permission of the transfer of the right to use that piece of land wholly or partially to a third party as long as there is no change in the nature of the land use. Under the law, local authorities can neither revise the contract nor reclaim the land awarded during the course of the contract. Further, it empowers farmers to transfer, re-contract, enter into share-holding ventures, and exchange land use rights with each other.

The Administrative Regulations Governing, the Circulation of the Right to Land Contractual Management in rural areas went into effect in March 2005. These are special regulations that prescribe all aspects of land use transfers, including the subject eligible to purchase land use rights, guidelines for prices, types of land use transfer (such as sub-contracting, leasing, exchanging and joint share-holding), transfer period, and specific registration procedures.

The Real Right Law, which came into effect on 1

October, 2007, confirms that the holder of land use rights can transfer those rights. Any contracted land cannot be used for non-agricultural purposes without approval.

In 2008, the Third Plenum of the 17th Central Committee of the CCP sought to further reform and develop the countryside by encouraging Chinese farmers to transfer their agricultural land use rights under the HRS. To smooth this process, farmers were granted land use contracts of indeterminate length. This political encouragement is likely to guide land use decisions of many farmers in the future and indicates that further reforms, including subsidies that reduce the transfer costs, might be expected in the future.

Issues Related to Tenure of Rural Land in China

The most significant characteristic of China's land system is its binary urban and rural arrangement. According to the Chinese Constitution (as revised in 1982), urban land is owned by the state and rural land by the rural collectives. This constitutional arrangement sometimes has been called "two-tier" land ownership (Dong, 1996; Piotrowski, 2008). This resulted in the insecurity and vagueness of China's rural land tenure, which is attributed to the collective nature of land ownership (Kung, 1995). The insecure land tenure system in rural China has long been criticized in the following two major aspects.

First, because the central government has repeatedly tinkered with the scale, scope and structure of village administration, it has been unclear who actually owns rural land (Ho, 2001). Collective ownership has been increasingly problematic to villagers because individuals have no demonstrable rights to the management and transfer of their collective property, nor do they have voice in the distribution of land-related compensation. These ambiguities with respect to collective ownership create loopholes in which local political elites can manoeuvre (Po, 2008).

The second criticism relates to how insecure land tenure affects agriculture productivity. A 1990 World Bank working paper showed that insecurity of land tenure did not appear to affect investment before 1989. However, investment in the years past the mid-point of the term of the contract may be more sensitive to perceptions regarding land reallocation (Feder et al., 1990). Prosterman et al. (1996) also observed that tenure insecurity discouraged investment in agriculture and lowered economic growth. Brandt et al. (2002) found that property rights affect farmers' investment incentives and productivity. However, arguments also have been made that insecure tenure did not constrain household's investment on farming. Instead, frequent adjustments of farm holdings among peasant families or insecure tenure have undermined their incentive to invest in stable crop production (Kung, 1995).

It has been shown that the household responsibility system brought about a rapid increase in agricultural production (Dong, 1996; Xu, 2007; Brandt et al., 2002; Liu et al., 1998). However, the administrative land allocation based on the household responsibility system tends to equalize the land-to-labour ratio but disregards contributions of other resources such as farm implements, financial capital and human skills. This results in mismatching of land with other productive resources and reductions in agricultural output and economic efficiency (Zhang et al., 2004). Criticisms focus on two main aspects. One is the dwindling farm size and excessive fragmentation of farmland (Dong, 1996; Tang, 2008), and the other is the observation of a noticeable decline in investment in farmland. In the absence of a stable set of long-term land use rights and security of tenure, farmers tend to reduce their investments in land, thereby adversely affecting long-term growth of agricultural output (Kung, 2000; Tang, 2008).

Development of a market system for allocating rural land use in China has been the focus of recent research in rural development during China's economic reform. It was found that strong market demand, as a result of industrialisation and urbanisation, has stimulated the emergence of a new market in rural collectively-held land (Ho and Lin, 2003; Jiang and Liu, 2003). This market for land transfer (sometimes called the land rental market) can enhance productivity by transferring land to more productive users at low cost and facilitate less skilled producers' participation in the nonfarm economy (Deininger and Jin, 2005). Jin and Deininger(2009), using data of a large household level panel, further estimated gains in productivity of land use via land rental in a growing economy that can amount to productivity increases of some 60%.

Although some worry has been expressed that a market system for allocating rural land might lead to income inequalities, Zhang et al. (2004), in a study in the rural areas of Zhejiang province, found that widening disparities in land use rights and farm income did not constitute a retreat from equality. Rather, voluntary land exchanges had compensatory effects on overall inequality as the exchanges in land led to more optimal matches between land and other productive resources, thereby raising agricultural productivity. Ho and Lin (2003) also argued that developing the land market helped to allocate land more efficiently. Studies on the land lease market came to the same conclusion where land leases were found to be an effective way to bring about efficient resource allocation (Yao, 2000a). Zhang et al. (2004) found that the sorting mechanism of the land market increased efficiency.

However, only a relatively small number of market transactions have occurred for rural land in China. A survey conducted in eight counties across China in 1998 showed that only 3-4% of the rural land was leased (Li et al., 1998). The percentage was higher in Zhejiang province, but still only 7-8% (Li et al., 1998). Yao (2000a) conducted a survey in a semi-industrialized region of Zhejiang province and found that although agricultural income consisted only of a minimal percentage of a household's total income, only a few households had stopped farming their land.

Because of the slowly developing market for farmland, arguments have been made that the farmland lease market is restricted by the existing land tenure system (Song et al., 2008; Prosterman et al., 1999). Piotrowski (2008) concluded that the land rental market fell short of its potential efficiency because of numerous imperfections in the land use rights transfer mechanism. Li (2003) argued that continuing administrative land readjustments have been a substantial obstacle to the development of markets for rural land use rights in China because transferees cannot be certain that the land they obtain through transfer will not be subject to administrative readjustment during the transfer term.

Many studies haves been conducted on the relationship between the land market and off-farm employment opportunities. Kung (2002) explained the development of a land rental market in China as a response to the development of another factor market, the off-farm labour market, and found that as the labour market develops, the benefits accruing to land rental transactions rise. Other studies also have found that land transfers were correlated positively with the occupational movement of farmers away from agriculture (Do and Huang, 2005; Feng et al., 2008). As an increasing number of villagers have obtained off-farm employment, the amount of land potentially available for leasing has increased correspondingly (Yao, 2000a). Zhang et al. (2004) found that having off-farm jobs was the major cause of farmers transferring their land use rights to other users. Piotrowski (2008) also found that the rural land rental market in Shandong was driven mainly by the off-farm labour market. A 2001 survey in Guangyang town in Chongqing City showed that farmers would be more likely to transfer their land use rights to professional farmers or agricultural companies if they had stable non-agricultural jobs. Temporary off-farm jobs resulted in fewer transfers of land use rights. Therefore, increased opportunities for stable full-time off-farm employment seem to be a prerequisite for increased transfers of land use rights and large-scale farming (Song et al., 2008).

Another criticism of China's rural land situation is the misuse of arable land (Ho and Lin, 2003; Tang and Chung, 2002). Despite national efforts to constrain the loss of farmland, these losses have continued (Brown, 1995). Since 1987, arable land in China has suffered an annual net loss of 0.3% and this has accelerated to 1% since 2000 (Heerink et al., 2009).

Empirical Study

Survey of Rural Households in Hebei Province

Hebei province, located in northern China (Figure 1), with a population of 70.34 million: about 62% rural and 38% urban, whose gross domestic product per capita reached 16,570 RMB in 2006 (HPBS, 2007), is a typical agricultural province with arable land of 5,901,400 hectares and one of the three main wheat growing areas in China. In an effort to understand why land transfers in Hebei have developed slowly compared with the average in China, a survey was conducted in eight counties of the province (Figure 1) from May to September 2009. The counties have different levels of economic development and include mountainous areas where arable land is more scattered (Xuanhua, Mancheng and Yixian counties), plain areas where cultivation is easier (Renqiu, Luquan and Xingtaixian counties) and coastal areas where the economic conditions are better (Shanhaiguan and Laoting). The main characteristics of the eight counties are shown in Table 1.

Graduate student assistants at Hebei Agricultural University conducted the interviews in several villages in each county. Six hundred questionnaires were distributed in a number of villages in each of the eight counties. Semi-structured interviews were conducted with those identified and complete responses obtained from 539 respondents. Though not systematically random, the large number of respondents from a number of villages in each of the carefully selected counties provided some assurance of meaningful results. Questionnaires were designed to solicit data on farmers' willingness to transfer their land use rights along with their personal and households' characteristics and income status. The survey data were entered into an EXCEL spread sheet to produce frequency and descriptive statistics.

Following a descriptive analysis of the data, a logistic analysis was conducted to determine the probability that a given household would be willing to transfer their land use rights. The willingness to transfer their land use rights is a binary outcome because a single household either is or is not willing to transfer the rights. The empirical model was specified as:

$$\text{logit}(P) = b0 + b1\ X1 + \ldots + b6\ X6 + \varepsilon \quad (1)$$

where P represents the household's probability of transferring (taking on a value of 1 if the farmer is willing to transfer and 0 if not willing). The independent variables (Xi, I =1, …, 6) were chosen to represent factors that were hypothesized to affect the decision whether or not the household was willing to transfer their land use rights. The coefficients of the logistic model were estimated with E-views software. The variables were used and their hypothesized effects have been described below.

FIGURE 1 MAP OF CHINA AND HEBEI PROVINCE WITH THE LOCATIONS OF THE EIGHT COUNTIES MARKED

TABLE 1 SOCIOECONOMIC CHARACTERISTICS OF THE EIGHT COUNTIES

Selected County	Total Arable land (Ha)	Rural Population (Thousand)	Per Capita Annual Income (RMB)	Sample Size
Shanhaiguan	3,087	314	4942	21
Laoting	62,86	432	5910	97
Xuanhuaxian	41,02	256	3101	57
Luquan	28,60	314	5866	40
Xingtaixian	36,96	375	4102	102
Renqiu	61,00	575	4719	39
Yixian	41,33	505	2846	70
Mancheng	3280	347	4140	113

Sources: Data in the first, fourth, fifth and sixth columns were retrieved from 2006 Hebei Agricultural Statistical Yearbook, Hebei Provincial Bureau of Statistics. Data in the second and the third columns were retrieved from 2006 Hebei Land Statistical Yearbook (HMLR, 2007)

On the basis of results found by earlier researchers, preliminary assessments of the questionnaires, and comments by government officials, six factors were hypothesized to affect the willingness of farmers to transfer their land use rights: (1) their assessment of adequacy of social security, (2) their assessment of freedom to transfer their land use rights at a reasonable price, (3) number of individuals living in the household, (4) net farm income after deducting variable costs of production, (5) educational level of head of household, and (6) ratio of off-farm income to total household income.

The first hypothesized factor is the adequacy of the social security system. In an empirical study of land transfer willingness in six suburban areas of Beijing, He and Xu (2007) found that the availability of social security increased farmers' willingness to transfer their land use rights. It was expected that the better the social security system is, the less dependence farmers would have on the land and therefore the more willingness to transfer their land use rights.

The second factor was the farmer's level of freedom to transfer the land use rights to any qualified individuals or entities both within and outside the collective organisation at a reasonable price. In a well-known article that analyzed the Chinese rural land system, Yao (2000b) pointed out that incomplete rights to transfer land use can limit farmers' investments on their land and decrease the land transference efficiency. Therefore, it was hypothesized that a high level of freedom to transfer, meaning less intervention from local cadres or other political powers, would have a positive effect on their willingness to transfer their land use rights. If they felt they had complete control over transfers of their land use rights, including when, how, to whom and where, they were coded as having full freedom. If they were totally directed by the collective organisation, they were considered as having no freedom. For example, in some circumstances, they can not oppose the decision of "collecting land and leasing out at one time" made by the collective organisation. If they were guided or induced by the collective organisation when deciding to whom they can transfer their land use rights or if they were forced to transfer them at a fixed price, they were coded as having partial freedom.

The third factor was family size. Han (2003) found that the higher the per capita land in the household was, the more willing it was to transfer its land use right. Thus, it was hypothesized that larger family size would be negatively correlated with willingness to transfer land use rights.

The fourth factor was net farm income after deducting variable costs of production. In an empirical study in Nanjing, Jiangsu province, Du and Huang (2005) found that the influence of net farming returns on farmers' willingness to transfer land use rights was complicated. Higher farming returns may lead to less willingness since there would be higher opportunity cost. However, smaller farming returns may lead to both more willingness (since farmers have lost hope of profiting from farming) and less willingness (since farmers having no other abilities may depend heavily on farming income, even if that is small). Although the factor is considered important, no hypothesis about expected sign is offered.

The fifth factor was level of education of the head of the household. Based on a willingness to transfer land study in Bishan county of Chongqing City, Feng et al. (2008) found that farmer's education is closely related to their willingness to transfer land use rights since well-educated farmers can more easily find off-farm jobs and adapt to changes. Therefore, it was hypothesized in this study that farmers with more education would be more willing to transfer their land use rights.

The sixth factor was the ratio of non-farm income to total household income. Kung (2002) and Brandt et al. (2004) found that land use transfers correlate positively with the occupational movement of farmers away from agriculture. Zhang et al. (2004) also found that having off-farm jobs was the major determinant of farmers' willingness to transfer their land use rights to other users. Huang et al. (2011) pointed out that it is transfer-out activities rather than the transfer-in activities that are encouraged by off-farm jobs significantly. Thus, it was hypothesized that households having earned a higher proportion of their total family income from off-farm jobs would be more inclined to transfer their land use rights.

Results

Among the 539 households in the survey, only 30 households had ever engaged in transfer of land use rights, reflecting the low rate of activity in Hebei province. When asked about their willingness to transfer their land use rights, 196 households gave a positive response (36.36% of the sample). The remainder (343 households) indicated that they had no desire to transfer their land use right at the current time.

TABLE 2 CHARACTERISTICS OF SAMPLE HOUSEHOLDS

Variable	S	L	Xu	L	Xi	R	Y	M
Social Security[1]								
1	10%	9%	12%	15%	6%	17%	13%	12%
2	57%	77%	63%	75%	52%	63%	76%	82%
3	33%	14%	25%	10%	42%	20%	11%	6%
Freedom[2]								
1	15%	6%	19%	14%	16%	19%	15%	20%
2	31%	67%	63%	57%	52%	53%	63%	61%
3	54%	27%	18%	29%	32%	28%	22%	19%
Family Size								
≤2	18%	17%	9%	22%	15%	16%	8%	16%
3	32%	40%	35%	35%	24%	42%	22%	31%
4	29%	33%	41%	37%	40%	35%	25%	33%
≥5	21%	10%	15%	6%	21%	7%	45%	20%
Farming Returns (RMB)								
＜2000	15%	7%	21%	3%	20%	14%	18%	3%
2000-5000	21%	20%	22%	18%	44%	13%	48%	39%
5000-7000	42%	57%	49%	63%	30%	55%	17%	46%
＞7000	22%	16%	8%	16%	6%	18%	17%	12%
Education[3]								
1	21%	17%	28%	29%	21%	39%	27%	17%
2	25%	57%	41%	39%	52%	47%	56%	50%
3	36%	22%	20%	17%	10%	11%	13%	21%
4	18%	4%	11%	15%	17%	3%	4%	12%
Income Ratio[4]								
≤20	4%	10%	46%	27%	35%	26%	18%	33%
20%-40%	6%	42%	36%	40%	41%	36%	47%	38%
40%-60%	32%	25%	15%	21%	16%	24%	31%	21%
≥60	58%	23%	3%	12%	8%	14%	4%	8%

1 Household assessment of adequacy of social security: none = 1, some but insufficient = 2, and adequate = 3

2 Household assessment of freedom to transfer land use rights at a reasonable price: no freedom = 1, partial freedom = 2 and full freedom = 3

3 Primary education or under = 1, completed junior middle school = 2, completed senior middle school = 3, and some post-secondary education = 4

4 Ratio of non-farm income to total household income

A statistical description of the data collected is shown in Table 2. Of the 539 respondents, only 58 (10.8%) felt their household had adequate social security. Most of those who felt they did not have adequate social security indicated that they would not be willing to transfer their land use rights. Less than half of the respondents indicated that they had full freedom when choosing terms of any potential transfer of land use rights. About 65% of the families in the survey (349 out of 539) were of a medium size, i.e., three or four persons in the household. A slightly higher proportion of the small families indicated a willingness to transfer their land use rights. The average income from farming activities was 6232 RMB; (it should be noted that average household income in Hebei was 19,911 RMB in 2007) (HLSS, 2008b). A majority of the household heads surveyed had completed junior middle school, secondary middle school or had some postsecondary training. The average ratio of off-farm income to total income was 37%. In the survey, it was found that as the ratio increased, a higher proportion of households indicated a willingness to transfer their land use rights.

The mean levels of the variables used in the logistic estimation are in Table 3 and the results of the estimation are in Table 4. The explanatory power of the estimated model (as revealed by the McFadden R-squared) is 0.627. Since the estimation was based on cross-sectional data collected under very difficult circumstances where most people have low levels of education, and many are somewhat dismayed at their inadequate levels of social security and freedom to transfer their land use rights, this can be considered quite a strong result. Three of the six explanatory variables were statistically significant at the 5% level and most of the others, though not statistically significant, had the hypothesized sign.

TABLE 3 MEAN LEVELS OF VARIABLES IN LOGISTIC MODEL

Variable	Mean
Social Security	2.21
Freedom	2.39
Family Size	3.7
Farming Returns (RMB)	6232
Education	2.00
Income Ratio	37%

Three explanatory variables in the logistic regression analysis were statistically significant at the 5% level: freedom to transfer land use rights at a reasonable price, ratio of off-farm income to total income, and social security (Table 4).

TABLE 4 REGRESSION ESTIMATES FOR FACTORS AFFECTING FARMER'S WILLINGNESS TO TRANSFER THEIR LAND USE RIGHTS IN HEBEI

Variable	Coefficient	Std. Error
Social Security*	3.621	1.701
Freedom*	1.524	0.576
Family Size	0.268	0.399
Farming Returns	-0.147	0.566
Educational Level	0.146	0.453
Non-farm/total income*	3.755	1.693
Constant	-11.528	2.628

* indicates significance at the 5% level
McFadden R-squared = 0.627

As expected, the estimated coefficient on social security was positive and statistically significant. The social security system in China has treated rural residents differently compared to urban residents and often has been criticized for enlarging the rural and urban income gap. In rural areas of Hebei, farmers receive very little government assistance for their medical needs. Most have very small pension eligibility. For example, the average pension for each beneficiary in rural Hebei was only 695 RMB per year, only 6.71% of the 10,325 RMB received by urban residents of Hebei (Ma, 2009). Also, the level of subsidy available for rural residents below the poverty line (known as the minimum living standard) was only 398 RMB per year in rural areas in 2006 as compared to 995 RMB in urban areas (Ma, 2009). In the survey carried out in this study, only 10% of households interviewed responded that they had sufficient social security. Most rural households generally felt uncertain about their future lives and a high majority of them indicated that they would not be willing to transfer their land use rights. Even in some cases where the land is no longer cultivated, many households indicated that they would not transfer their land use rights. When the government fails to provide adequate social security for farm households, agricultural land acts as a kind of social insurance and functions as both employment insurance and pension supplier. Therefore, the absence of a social security system in rural areas has hindered the transfer of farmers' land use rights.

Farmer's perceptions of their freedom to transfer land use rights at a reasonable price had a statistically significant, positive effect on their willingness to transfer their land use rights, consistent with prior expectations and results of previous studies. When farmers are under duress and lack freedom to make their own decisions regarding price and other terms of transfer of land use rights, they often are unwilling to engage in the process. This frustrates the objectives of land use laws that have been designed to encourage the transfer process. Village cadres and others who impose conditions on the transfer of land use rights undermine the development of the transfer market and make a fair pricing mechanism hard to achieve. Where intervention occurs, farmers are unable to obtain reliable information on demand for their land use rights, and also make those who have low social status or weak political power more vulnerable.

The estimated coefficient for the ratio of off-farm income to total income also had a statistically significant positive sign. Farmers count on the use of their farmland to provide their families with a living when they do not have available access to off-farm employment. Access to non-farm employment opportunities, a substitute for land in terms of securing farmers' livelihoods, promotes farmers' willingness to transfer their land use rights. In Hebei, where the economy is largely based on agriculture, farm income still accounts for a big proportion of the income structure of most farm households. This means that farmers who dispose of their land use rights face risks for supporting their families in the future.

The estimated coefficients for the other three variables (family size, farming returns and educational level) were not statistically significant.

Conclusions

In rural China, where the social security system has long been inadequate to meet the needs of rural households, and the access to agricultural land acts as a form of social insurance by providing employment, food for the family and some form of pension for the old age. This study provides further convincing evidence that an inadequate social security system in rural China inhibits the transfer of land use rights. Improvement in the social security system for rural residents is a prerequisite to increasing the rate of transfers of land use rights in rural areas.

The collective ownership of farm land results in tenure insecurity (Ho, 2005). Insecure land tenure has not only led to behaviours that frustrate farmers' interests, but also has caused additional externalities and transaction costs, such as the costs of dealing with disputes, law suits and social unrest. In China, privatisation is incompatible with the socialist ideology. To guarantee farmers with more secure land tenure, the Law on Land Contracts in Rural Areas which was enacted on March 1, 2003 to formulate the "principle of participation voluntariness". "Farmers transact in the cultivated land market on a voluntary basis; local authorities can neither revise the contract nor reclaim the land awarded during the course of the contract." However, current laws do not involve specific regulations on how to execute the principle of voluntariness. Therefore, emphasis on the principle of voluntary participation should be strengthened by formulating further regulations on the responsibilities and rights of rural households, village, and township, respectively, and any kind of political intervention to farmers' land use rights transfer in terms of price, means, and profit distribution should be affirmed to be illegal.

Where members of farm households have easy access to off-farm employment, such opportunities can serve as a substitute for land in terms of securing farmers' livelihood. With off-farm earning possibilities, rural households are much more inclined to transfer their land use rights. In areas where off-farm employment opportunities are few and far between, governments can be helpful by encouraging the development and growth of off-farm employment opportunities. These types of developments would increase farmers' willingness to transfer their land use rights.

ACKNOWLEDGMENT

I would like to express my deepest appreciation to all those who have provided me with the oppotunity to complete this study. My special gratitude goes to the other author and my supervisor, Mr.Kurt Klein, whose suggestions and endless support and encouragement helped me with the whole process of the study. Furthermore, I have to appreciate the guidance given by other supervisor that has improved my survey skills and offered me contributive comments and advices.

REFERENCES

Brandt, Loren, Jikun Huang, Guo Li and Scott Rozelle. "Land Rights in Rural China: Facts, Fictions and Issues." The China Journal 47 (2002): 67–97.

Brandt, Loren, Scott Rozelle, Matthew A. Turner. "Local Government Behavior and Property Right Formation in Rural China." Journal of Institutional and Theoretical Economics 160 (2004): 627–662.

Brown, George P. "Arable Land Loss in Rural China: Policy and Implementation in Jiangsu Province." Asian Survey 35 (1995): 922–940.

Cangzhou Government. "Tentative Regulation of Minimum

Living Standard for Rural Residents." Accessed July 19, 2005. http://www.cangzhou.gov.cn/zhengwu/zfwenjian/48424.shtml.

Deininger, Klaus and Songqing Jin. "The potential of land rental markets in the process of economic development: Evidence from China." Journal of Development Economics 78(2005): 241–270.

Dong, Xiaoyuan. "Two-Tier Land Tenure System and Sustained Economic Growth in Post-1978 Rural China." World Development 24(1996): 915–928.

Du, Wenxing and Xianjin Huang. "Study on Regional Differences of Rural Land Transfer Willingness and Its Driving Force: Based on Case Study in Shanghai, Nanjing, Taizhou and Yangzhou." (In Chinese) Resources Science 27(2005): 90–94.

Feder, Gershon, Lawrance J Lau, Justin Yifu Lin and Xiaopeng Luo. "The Determinants of Farm Investment and Residential Construction in Post-reform China." World Bank Working paper. WPS471, World Bank, Washington, 1990.

Feng, Lingling, Daochi Qiu, Yaping Zhao and Yongming Shi. "Study on Households" Willingness of Rural Land Transfer in Bishan County of Chongqing Province." (In Chinese) Guangxi Agricultural Sciences 39(2008):551–556.

Han, Changbin "On Circulation of Right to Land Contract Management." (In Chinese) CCP official Online Qiushi Journal, Vol.7(4).2003. Accessed April 2, 2003. www.qsjournal.com.cn/qs/20030401/gb/qs%5E356%5E0%5E11.htm.

He, Guojun and Chong Xu. "Analysis of Farmers' Land Transfer Willingness in the Suburban Areas: Based on an Empirical Study in Six Villages of Suburban Beijing." (In Chinese) Economy Science 5(2007): 111–124.

Hebei Labour and Social Security Ministry (HLSS) "Document on adjustment of the Minimum Salary Regulation for Hebei." 2008a. Accessed 8 July 2008. http://www.he.lss.gov.cn/zcfg/index.shtml?id=69305776.

Hebei Labour and Social Security Ministry (HLSS). "Report on Average Salary and Average Pension of Hebei in 2007." 2008b. Accessed 11 April 2008. http://www.he.lss.gov.cn/zfxxgk/index.shtml?lawId=69305805.

Hebei Ministry of Land and Resource (HMLR). 2006 Hebei Land Statistical Yearbook. Shijiazhuang, Hebei, 2007.

Hebei Provincial Bureau of Statistics (HPBS). "Statistical Communiqué on the 2006 National Economic and Social Development." Shijiazhuang, Hebei, 2007.

Heerink, Nico, Xiaoping Shi, Xiao Yi and Futian Qu. "Land Property Rights and Land Acquisition Reforms-Two Case Studies in Jiangxi and Jiangsu Province". Paper presented at The Ninth European Conference on Agriculture and Rural Development in China. University of Leeds, Leeds, UK. April 3–5 2009.

Ho, Peter. "Who Owns China's Land? Policies, Property Rights and Deliberate Institutional Ambiguity." The China Quarterly 166(2001): 394-421.

Ho, Peter. "The Chicken of the Institutions or the Egg of Reforms?" Developmental Dilemmas: Land Reform and Institutional Change in China, 1–33. London: Routledge, 2005.

Ho, Peter and Max Spoor. "Whose Land? The Political Economy of Land Titling in Transitional Economies." Land Use Policy 23(2006):580-587.

Ho, Samuel. P S. and George C. S. Lin. "Emerging Land Markets in Rural and Urban China: Policies and Practices." The China Quarterly 175(2003): 681-707.

Huang, Jikun, liangliang Gao, Scott Rozelle. "The Effect of Off-Farm Employment on Household's Decisions to Rent out and Rent in Cultivated Land in China." China Agricultural Economic Review 4 (2011).

Jiang, Shengsan and Shouying Liu. "Let Peasants Participate in Industrialization With Their Land Rights: Understanding the Nanhai Model." (In Chinese) Policy 79(2003): 54–56.

K. Deininger and S. Jin. "The potential of land rental markets in the process of economic development: Evidence from China." Economic Development 78(2005): 241–270.

Kung, James Kai-Sing. "Equal Entitlement Versus Tenure Security Under a Regime of Collective Property Rights: Peasants' Preference for Institutions in Post-Reform Chinese Agriculture." Journal of Comparative Economics 21(1995): 82–111.

Kung, James Kai-Sing. "Common Property Rights and Land Reallocations in Rural China: Evidence from a Village Survey." World Development 28(2000): 701–719.

Kung, James Kai-Sing. "Off-Farm Labour Markets and the Emergence of Land Rental Markets in Rural China." Journal of Comparative Economics 30(2002): 395–414.

Li, Guo, Scott Rozelle and Loren Brandt. "Tenure, land rights, and farmer investment incentives in China." Agricultural Economics 19(1998): 63–71.

Li, J. D. Ping. "Rural Land Tenure Reforms in China: Issues, Regulations and Prospects for Additional Reform". FAO Land Settlement and Cooperatives Special Edition on Land Reform, 2003. Accessed 11 November 2008. www.rdiland.org/PDF/PDF_Publications/LP-RuralLandTenureReforms.pdf

Lin, Justin Yifu. "The Household Responsibility System in China's Agriculture Reform: A Theoretical and Empirical Study." Economic Development and Cultural Change 36(1988): 199-224.

Liu, Shouying, Michael Carter and Yang Yao. "Dimensions and Diversity of Property Rights in Rural China: Dilemmas on the Road to Further Reform." World Development 26(1998): 1789–1806.

Jin, Songqing, and Klaus Deininger. "Land rental markets in the process of rural structural transformation: Productivity and equity impacts from China." Journal of Comparative Economics 37 (2009): 629–646.

Ma, Yimin. "Study on Balancing Urban and Rural Social Security System"(in Chinese), 2009. Accessed 21 January 2009. http://www.hebei.gov.cn/article/20090121/1140662.htm.

McMillan, John, John Whalley and Lijing Zhu. "The Impact of China's Economic Reforms on Agricultural Productivity Growth." Journal of Political Economy 97(1989): 781–807.

NBS (National Bureau of Statistics). The National Economy Maintained Steady and Rapid Development in 2011, 2012. Accessed 19 September 2012. http://www.stats.gov.cn/tjfx/jdfx/t20120117_402779443.htm

Piotrowski, Stephan. Land Property Rights and Natural Resource Use-An Analysis of Household Behavior in Rural China, p.v. Frankfurt: Peter Lang, 2008.

Po, Lanchih. "Rural Shareholding Co-operatives Redefining Rural Collectives in China: Land Conversion and the Emergence." Urban Studies 45(2008): 1603–1623.

Prosterman, Roy, Tim Hanstad, Brian Schwarzwalder and Ping Li. "Rural Land Reform in China and the 1998 Land Management Law." Working Paper in the CIRD-UNDP co-sponsored International Symposium. Hainan, China. 11–13 January 1999.

Prosterman, Roy, Tim Hanstad and Ping Li. "Can China Feed Itself?" Scientific American 275(1996): 90–96.

Song, Min, Yangui Chen and Lijun Liu. Economic Analysis of Land Institute in China, (in Chinese), 217. Beijing: China Agriculture Press, 2008.

Tang, Wenjin. A study on the Farmers' Will and Behavior of Farmland Circulation, (in Chinese), 75–77. Beijing: China Economic Publishing House, 2008.

Tang, Wingshing and Him Chung. "Rural–Urban Transition in China: Illegal Land Use and Construction." Asia Pacific Viewpoint 43(2002): 43–62.

United Nations Population Fund (UNPFA). "State of World Population 2007". Accessed 24 March 2009. http://web.unfpa.org/swp/2007/english/introduction.html.

Xu, Yueming. Performance of the Household Contract Responsibility System and the Innovation, (In Chinese) 5-25. Beijing: China Agriculture Press, 2007.

Yan, Wen. "Study on Transfer of Agricultural Land Contract Right Based on the Empirical Study in Hebei Province." PhD diss., Agricultural University of Hebei, 2010.

Yao, Yang. "The Development of the Land Lease Market in Rural China." Land Economics 76(2000a): 252-266.

Yao, Yang. "Chinese Land Tenure: An Analytical Framework." (in Chinese) Social Sciences in China 2(2000b): 54-65.

Zhang, Qian Forrest, Qingguo Ma and Xu xu. "Development of Land Rental Markets in Rural Zhejiang: Growth of Off-Farm Jobs and Institution Building." The China Quarterly 180 (2004): 1050-1072.

Running Title: Four *Rosa* Species Section *Caninae* from Tunisia Characterised by Their Rose Hips

Pomological Description and Chemical Composition of Rose Hips Gathered on Four *Rosa* Species Section *Caninae* Growing Wild in Tunisia

Zohra Ben Cheikh-Affene*[1], Faouzi Haouala[2], Najla Trabelsi[3], Monther Boulaaba[3], Riadh Ksouri[3], Fethia Harzallah-Skhiri[4]

[1]Corresponding author, Department of Horticultural Science and Landscape, Higher Agronomic Institute University of Sousse, 4042 Chott Mariem, Sousse, Tunisia

[2]Department of Agronomy and Plant Biotechnology, National Agronomic Institute of Tunisia, University of Carthage, 43 avenue Charles Nicolle, 1082 Tunis Mahrajène, Tunisia

[3]Laboratory of Extremophiles Plants, Center of Biotechnology, Ecopark of Borj Cedria (CBBC), BP 901, 2050 Hammam–Lif, Tunisia

[4]Laboratory of Genetic, Biodiversity and Valorisation of Bioresources (LR11SE14), Higher Institute of Biotechnology of Monastir, University of Monastir, Rue Tahar Haddad, 5000 Monastir, Tunisia

[1]ben-chiekh.zohra@laposte.net; [2] faouzi.haouala@laposte.net; [4] fethiaprosopis @yahoo.fr

Abstract

This study was carried out to determine pomological and chemical characteristics of rose hips from four *Rosa* species belonging to *Caninae* section growing wild in the North and the Centre of Tunisia. Fourteen parameters related to pomological and chemical characteristics were recorded. Mean rose hip weight ranged from 0.9±0.2 to 1.9±0.3 g, the height from 15.1±1.8 to 18.9±1.6 mm and the width from 10.5±0.7 to 14.1±0.8 mm. The vitamin C, carotenoid and total polyphenol contents varied between 372.4±4.7 and 737.8±10.6 mg/100 g fresh weight (F.W.), 206.4±4.0-667.5±9.7 μg. g^{-1} F.W., 25.2±3.0-69.6±2.1 mg gallic acid per gram of dry weight (D.W.), respectively. The result of the present study showed a large morphological variation among rose hips of the Tunisian rose species of section *Caninae* and a high content in bioactive substances. The phenotypic divergent genotypes identified in this study would be of much utilization in future breeding programs.

Keywords

Ascorbic Acid; Carotenoïds; Polyphenols; Pomological Parameters; Principal Components Analysis; Rosa

Introduction

Roses, one of the most popular groups of ornamental plants, have enjoyed much reputation since antiquity. A large number of cultivars have been developed either as gardening plants, or for the rose market or as indoor pot plant. They have been grown also for production of rose oil and rose water as well as for cosmetic and medicinal purposes (Warholm *et al.*, 2003). The genus *Rosa (Rosaceae* family) includes about 200 species subdivided into 10 Sections (Rehder, 1940) dispersed in the temperate and subtropical zones of the Northern hemisphere. The sections *Caninae* and *Cinnamomeae*, the largest ones, include about 50 and 80 species, respectively (Wisseman, 2003). *Rosa* species produce rose hips, a pseudocarp or a false fruit, consisting of a fleshy wall surrounding achenes with one seed.

Rose hips of some species, especially *Rosa canina* (dog rose) (Section *Caninae*) are used in many countries as raw material in different products such as jam, juice, tea and syrup. In Sweden, in the mid-eighties, a program for domestication of wild roses (Section *Caninae*) for rose hips production was initiated in Balsgård and in 1993, about 130 hectares of dog roses have been established (Uggla and Martinsson, 2005). As well, in Chile, since 1991, an annual average amount of 6800 metric tons of dried fruit flesh has been exported to the European countries, mainly Germany (Joublan and Rios, 2005).

Interest in utilization of rose hips is slowly growing, and research is now conducted on the role of these fruits in human nutrition. The fresh rose hips are the richest fruits in terms of vitamin C (Uggla and Martinsson, 2005) and valuable because of their high antioxidant capacity and the high amount of carotenoid and phenolic compounds (Gao *et al.*, 2005). Recent clinical studies have shown that dried rose hips induced a reduction in symptoms in patients previously diagnosed with osteoarthritis (Warholm *et al.*, 2003). Also anti-inflammatory (Winther *et al.*, 1999), anti-ulcerogenic (Gürbüz *et al.*, 2003) and anti-mutagenic (Karakaya and Kavas, 1999) activities have been demonstrated.

Within Tunisian flora, there are known 10 spontaneous species and subspecies of roses (Le Floc'h, Boulos and Vella, 2010) growing wild in ravines, with bushes, and in the forests from the North to the Tunisian Dorsal (Pottier-Alapetite, 1979). This study was designed to explore Tunisian wild rose populations belonging to *Caninae* section, in which rose hips were known for their high value for bioactive substances (Gao *et al.*, 2005), to describe and characterize their false fruits in the aim to select the most promising rose accessions for future breeding efforts or for their use in medicinal or food field.

Materials and Methods

Plant Material

Rosa genotypes from the section *Caninae* were collected from altitudes between 228-942 m from the North, the Northeast, the Centre and the South of the Tunisian dorsal area. According to taxonomic criteria (Bailey, 1963) four species were identified i.e.; *Rosa canina* L. from Zaghouan region, *R. pomifera* L. and *R. rubiginosa* L. from Kairouan region, *R. dumetorum* Thuiller and *R. rubiginosa* L. from Beja region and *R. dumetorum* from Seliana region. All accessions are upright shrubs with thorny branches.

All voucher specimens are deposited in the Higher Institute of Agronomy of Chott-Meriem, Horticultural Laboratory Herbarium and were assigned for each one a corresponding number (codes RR110-RR115).

The rose hips were harvested at the fully ripe mature stage (late September and October 2007). Flesh part was extracted from 500g of other rose hips, mixed in a mortar with liquid nitrogen, and the half of the tissue was stored at 4°C until the analysis of ascorbic acid and carotenoids. The other part was lyophilized and stored at 8°C until the analysis for total phenols (Table 1).

Parameters Studied

1) Biometrical Characteristics

Thirty rose hips were randomly chosen for each accession and used to measure the pomological parameters. It was also noted that personally we enjoyed the aroma of rose hips (qualitative and quantitative parameters) (Table 1).

2) Determination of Ascorbic Acid Content

Free ascorbic acid was extracted from 0.2 mg of the frozen flesh tissue mixed with 0.8 ml of frozen trichloroacetic acid (6%). The mixture was centrifuged at 15,600 g (4°C) for 5 min, and the upper layer (0.2 ml) was placed on ice and mixed with 0.2 ml of dithiothreitol (10 mM), 0.4 ml of buffer phosphate (pH 7.4) and 0.2 ml of N-ethylmaleimide (0.5%). The mixture was incubated for 15 min at 42°C in darkness. After that, 1 ml of trichloroacetic acid (10%), 0.8 ml of phosphoric acid (42%), 0.8 ml of 2.2 dipycridyl (4%) and 0.4 ml of ferric chloride (3%) were added, then shaken vigorously and kept at room temperature 42°C for 40 min. Absorbance of the solution was then measured spectrophotometrically at 525 nm (model Anthelie Advanced II, Secomam) according to Kampfenkel *et al.*, (1995) with some modifications. Analyses were conducted in triplicates. The concentration of total ascorbic acid content was calculated using a standard curve. Results were expressed as mg of ascorbic acid per 100 g fresh weight (F.W.).

3) Determination of Carotenoïd Content

Flesh frozen tissue (0.2 mg) was homogenised 24 h with 200 ml of acetone 80% (v/v). After incubation at ambient temperature during 3 days, the mixture was centrifuged at 14,000 g for 5 min. The absorbance of the extract was measured at 470 (A_{470}), 647 (A_{647}) and 663 nm (A_{663}) using a spectrophotometer according to Nonier *et al.*, (2004). Contents of total carotenoïds were calculated according to the following equation: total carotenoïds (μg.ml^{-1}) = $[(5{*}A_{470}) + (2.846{*}A_{663}) - (14.876{*}A_{647})]$. Analyses were run in triplicates. Total carotenoïd content was expressed as μg carotene per g fresh weight.

4) Assay of Total Phenol Contents

Lyophilised fruit tissue (1 g) was extracted by stirring with 10 ml of absolute methanol at room temperature for 30 min. Extracts were kept for 24 h at +4°C, and then filtered through Whatman filter paper. Extracts were evaporated under vacuum to dryness and stored at +4°C until analysis. The content of total polyphenols was measured according to the method of Dewanto *et al.,* (2002) using Folin-Ciocalteu reagent. 25 µL of suitable diluted sample extract was dissolved in 500 µL of distilled water and 125 µL of the Folin-Ciocalteu reagent. The mixture was shaken, before the adding of 1250 µL Na_2CO_3 (7%), adjustment with distilled water to a final volume of 3 mL, and mixed thoroughly. After incubation for 90 min at 23°C in darkness, the absorbance versus a prepared blank was read at 760 nm. A standard curve of gallic acid was used. Total polyphenols content of the flesh was expressed as mg gallic acid equivalent per gram of dry weight (mg GAE.g^{-1} D.W.) through the calibration curve with gallic acid. The calibration curve range was 0-400 µg mL^{-1} (R^2 = 0.99). All samples were analyzed in three replications.

Statistics Analysis

A two-way analysis of variance (ANOVA) and Duncan's multiple range test were carried out to investigate any significant differences between parameters at $p < 0.05$. All the data were subjected to Principal Components Analysis (PCA) (Cottin, 1988) using the SPSS 16 software (Statistical Package for the Social Science) (Inc. Chicago, IL, USA) and Excel 2007 softwares, allowing the classification of *Rosa* genotypes into similar groups. Correlation between pomological and chemical parameters was calculated using Pearson coefficient.

Results and Discussion

Biometrical Characteristics

The dimension of the rose hips varied in the height range of 15.1±1.8 - 18.9±1.6 mm, from *R. rubiginosa* Beja region to *R. pomifera* Kairouan region and the width between 10.5±0.7 - 14.1±0.8 mm from region *R. dumetorum* Beja region to *R. rubiginosa* Kairouan (Table 2). *R. rubiginosa* Kairouan region has the heaviest rose hip (Rh.W.) and flesh fruit weights (Fl.F.W.) (Rh.W. = 1.9±0.3 g; Fl.F.W. = 1.2±0.2 g) and a medium flesh rose hip ratio (Fl/Rh. = 65.4±4.0%). *R. canina* Zaghouan region has the highest flesh rose hip ratio (Fl/Rh. = 73.7±8.2%) and the highest dry weight of flesh (Fl.D.W. = 0.40±0.08 g). The achene number (A.Nb) varied in a range of 6.1±3.0-18.6±3.5, from *R. pomifera* Kairouan region to *R. canina* Zaghouan region and achene weight (A.W.) varied between 0.016±0.001-0.034±0.001 g from *R. dumetorum* Seliana region to *R. rubiginosa* Kairouan region. *R. canina* Zaghouan region has high achene weight (0.033±0.002 g) but the lowest achene number (6.1±3.0).

It was also noted that when the flesh fresh weight (Fl.F.W.) was as lower as the rose hip weight (Rh.W.), the highest ratio (Fl/Rh) and a moderate number of achenes were gained as for the two *R. dumetorum* accessions and for *R. canina*. Nevertheless, when the flesh fresh weight was at least 1.5 time than Rh.W., the ratio was moderately important as for *R. pomifera* and the two *R. rubiginosa* accessions and the number of achenes was the highest.

TABLE 1 POMOLOGICAL AND CHEMICAL PARAMETERS, CODES AND UNIT

N	Parameters	Code	Unit
	Quantitative parameters		
1	Rose hip height	Rh.H	mm
2	Rose hip width	Rh.Wi	mm
3	Rose hip weight	Rh.W	g
4	Flesh fresh weight	Fl .F.W	g
5	Flesh rose hip ratio (based on F.W.).	Fl/Rh	-
6	Flesh dry weight	Fl.D.W	g
7	Moisture content in flesh	Mo	%
8	Achene number	A.Nb	-
9	Achene weight	A.W	g
10	Total polyphenol in flesh	T.po	mg GAE.g^{-1} D.W.
11	Total ascorbic acid in flesh	As.A	mg/100 g F.W.
12	Total carotenoid in flesh	Car	µg.g^{-1} F.W.
	Qualitative parameters		
13	Rose hip color (dark red 1; red 2; scarlet orange 3)	Rh.C	(1,2,3)
14	Fruit aroma (good 1; medium 2)	Rh.A	(1,2)

* GAE/Gallic Acid Equivalent

TABLE 2 POMOLOGICAL CHARACTERISTICS OF ROSE HIPS FROM THE SIX *ROSA* ACCESSIONS

Parameters	***R. pomifera*** **Kairouan**	***R. rubiginosa*** **Kairouan**	***R. rubiginosa*** **Beja**	***R. dumetorum*** **Beja**	***R. dumetorum*** **Seliana**	***R. canina*** **Zaghouan**
*Rh.H (mm)	18.9 ± 1.6d*	17.7± 1.5 c	15.1± 1.8 a	16.9±1.8bc	16.8± 1.8 bc	16.1±1.1 b
Rh.Wi (mm)	11.7±1.1 b	14.1± 0.8 d	12.3±1.1 c	10.5±0.7a	10.5± 0.8 a3	10.8± 0.7 a
Rh.W. (g)	1.4±0.3 c	1.9±0.3d	1.2±0.3 b	0.9±0.2a	0.9±0.2 a	1.0±0.17 a
Fl.F.W.(g)	0.9±0.2 b	1.2±0.19c	0.79± 0.2 a	0.7±0.15 a	0.69±0.15 a	0.77±0.1 a
Fl/Rh	63.6± 3.2 a	65.4±4 a	66.3± 6.0 a	72.0±3.8 b	72.0± 3.8 b	73.7±8.2 c
Fl.D.W. (g)	0.37±0.07 bc	0.39±0.07 c	0.30± 0.1 a	0.30±0.06 a	0.30±0.06 a	0.40± 0.08 c
Mo. (%)	59.3±4.7c	69.1±7.4 d	57.2±4.5bc	54.1±6.5 b	54.1±6.5b	47.1±5.6 a
A.Nb	18.6±3.5 d	14.2± 2.9 c	13.4± 4.6 c	10.8± 3.2 b	10.8± 3.2 b	6.1± 3.0 a
A.W. (g)	0.020±0.001b	0.034±0.001e	0.026±0.001d	0.023±0.001c	0.016±0.001a	0.033±0.002e
Rh.C	Dark red	Dark red	Dark red	Scarlet orange	Scarlet orange	Red
Rh.A	Good	Good	Good	Medium	Medium	Medium

* For Abbreviation See Table 1. means±SD (n=30). Values with the Same Letter were not Different at $p < 0.05$

Rose hip color varied from dark red (*R. pomifera* Kairouan region and *R. rubiginosa* Kairouan and Beja regions) to red (*R. canina* Zaghouan region) and to scarlet-orange (*R. dumetorum* Beja and Seliana regions). Rose hips of *R. pomifera* Kairouan region and *R. rubiginosa* Kairouan and Beja regions have a good aroma (Table 2).

Similarly, studies in pomological description of various *Caninae* species from different regions of Turkey showed a great variability. In fact, Kasankaya *et al.*, (2005) reported that rose hip weight ranged between 2.0 and 5.8 g which greatly exceeded the values found in our analysis. The Fl/Rh. ratio of the Tunisian genotypes was in general in the limits of previous studies (63.6±3.2-73.7±8.2%). Indeed, in the work of Celik *et al.*, (2009) the proportion of flesh for rose hips varied between 46.7-86.6%, in contrary, the number of achene by hip was higher than our results and varied between 18 and 41.

In some of the previous studies conducted in Turkey, it was demonstrated that the flesh rose hip ratio can also vary depending on species and that the differences in ecological conditions and cultural practices are important factors affecting flesh rose hip ratio. Particularly, irrigation caused a significant increase in rose hip weight and flesh rate (Günes, 2010). In fact, for the same species *R. rubiginosa*, great differences have been reported between the accession from Kairouan region and that from Beja region, two localities with very different soil and ecological conditions.

Total Ascorbic Acid Content

Total ascorbic acid contents varied with rose accessions. The highest total content of ascorbic acid was found for *R. pomifera* (737.8±10.6 mg/100 g F.W.). Nevertheless, rose hips from *R. rubiginosa* Beja region showed also a high value for this acid; 545.0±13.9 mg/100 g F.W. (Table 3). The lowest values were assayed in *R. rubiginosa* Kairouan region and *R. dumetorum* Seliana region (384.0±11.9 and 372.4±4.7 mg/100 g F.W., respectively).

In previous comparative studies, a great variability in vitamin C content of rose hips was also found. Kazankaya *et al.*, (2005) found that the ascorbic acid content of various species of *Rosa* section *Caninae* from different regions of Turkey ranged between 301 and 1183 mg/100 g F.W. Demir and Özcan (2001) found a content of 2712 mg/100 g F.W. in *R. canina* rose hips.

Based on comparison of the amount of ascorbic acid of Tunisian dog roses and other *Rosaceae* species known for their high content of vitamin C, it is indicated that the amount of ascorbic acid in fruits of wild roses was higher than that in blackberry, (*Rubus caesius* L.) (33.85 mg/100 g F.W.) blackthorn (*Prunus spinosa* L.) (21.94 mg/100 g F.W.), rowan (*Sorbus aucuparia* L.) (68.18 mg/100 g F.W.) and wild strawberry (*Fragaria vesca* L.) (80.84 mg/100 g F.W) growing wild in Poland (Jablonska-Rys *et al.*, 2009). As well, this amount is much higher than that for Tunisian *Crataegus azarolus* genotypes (35.9 mg/100 g) (Bahri-Sahloul *et al.*, 2009). The content of vitamin C is determined by numerous factors, including species, variety, cultivation, climate, wheather conditions, ripeness, region and storage time (Pantelidis *et al.*, 2007). Günes (2010) mentioned that the variation in vitamin C content was clearly related to genotypes.

Total Carotenoid Content

Total carotenoid content varied more than three fold, in the range of 206.4±4.0 and 667.5±9.7 μg.g^{-1} F.W. *Rosa pomifera* rose hips have the highest content and those from *R. canina* the lowest (Table 3). A high content was found by Olsson *et al.,* (2005) (1192 μg.g^{-1} F.W.) in *R. dumalis* Bechst. from Sweden. According to our results, the total carotenoïds in rose hips are much better than that in carrot (95.08 μg.g^{-1} F.W.), papaya (46.38 μg.g^{-1} F.W.), guava (42.58 μg.g^{-1} F.W.) and tomato (32.81 μg.g^{-1} F.W.) (Mélo *et al.,* 2006). Razungles *et al.,* (1989) reported in their study that rose hips contained the highest concentration of total carotenoids compared to black chokeberry (*Aronia melanocarpa* Michx.).

Total Polyphenol Content

The variation in total polyphenol contents was relatively low with values ranging from 25.2±3.0 to 69.6±2.1 mg GAE.g^{-1} D.W. (Table 3). *R. pomifera* has the highest content whereas *R. rubiginosa* and *R. dumetorum* from Beja region have the lowest content.

The literature mentioned a great variability in total polyphenol contents of rose hips. Barros *et al.,* (2010) found a content of 143.1 mg GAE.g^{-1} D.W. in *R. canina*. A moderate content was observed in *R. dumalis* (84 mg GAE.g^{-1} D.W.) (Olsson *et al.,* 2005). According to our results, total phenolic contents of rose hips are higher than wild *Rosaceae* edible fruits. Indeed, Jablonska-Rys *et al.,* (2009) found a high content in *R. canina* (32.17 mg GAE.g^{-1} F.W.), whereas a lower content was observed in *Prunus spinosa* L. (4.02 mg GAE.g^{-1} F.W.), *Rubus caesius* (2.47 mg GAE.g^{-1} F.W.), *Sorbus aucuparia* L. (2.26 mg GAE.g^{-1} F.W.) and *Fragaria vesca* L. (1.65 mg GAE.g^{-1} F.W.).

According to Bravo (1998), the presence of polyphenols in plants was greatly influenced by genetic factors, environmental conditions, degree of ripeness, variety, etc.. Phenolic compounds, the secondary metabolites of plant, are needed in terms of normal growth and developmentas protection of the species against adverse factors which threaten its survival in unfavourable environment, such as drought, UV radiation, infections or physiclal damage (Asami *et al.,* 2003).

Indeed, the differences in the environment conditions between regions of collection explain the less amount of polyphenols in the two accessions of *R. rubiginosa* and *R. dumetorum* from Beja region which has been growing in the river bank, compared to the accessions from Kairouan region which have been grown in arid climate. In another way, Shahidi and Naczk (2004) mentioned that phenolic compounds protect the easily oxidizable food compounds and inhibit the oxidation of vitamin C, carotenoids and unsatured fatty acids. We can highlight the *R. pomifera* from Kairouan region presenting the highest amount of polyphenols, ascorbic acids and carotenoids.

Analysis of the Relationship between Pomological and Chemical Parameters

The table 4 shows the highest positive correlation between Rh.Wi and Rh.W. (R^2 = 90%), Rh.Wi and Fl.F.W. (R^2= 88%), and between Rh.W. and F.Fl.W. (R^2 =96%). The highest negative correlation was reported between Fl/Rh and A.Nb (R^2 = -71%). For the nutrional value, only the positive correlation was highlighted between the contents of ascorbic acid (As.A) and of total polyphenols (T.Po) (R^2= 59%).

Principal Component Analysis

To identify whether the morphological and chemical parameters may be useful in reflecting the dissimilarity or the group of species and to characterize each one of them, the 14 morphological and chemical parameters related to rose hips listed in table 1 were assessed for the PCA. The horizontal and vertical axes of the PCA explained 39.42 and 21.63% of the total variance (Figure 1). Rose hip width (Rh.Wi), rose hip weight (Rh.W.), flesh fresh weight (Fl.F.W.), rose hip height (R.H), moister content (Mo), achene number (A.Nb) and rose hip aroma (Rh.A) were highly and positively correlated with PC1; while the flesh rose hip ratio was negatively correlated with this axis. Moreover, caretenoids content (Car.) was the value with the largest contribution on the positive plan of PC2, while achene weight (AW.) was the parameter with the largest contribution in the negative plan of this axis. Other parameters studied were moderately coorrelated with axes 1 and 2. The PCA identified 2 groups. The two species *R. pomifera* Kairouan region and *R. rubiginosa* Kairouan region with a dark red colored, and good aroma, clearly stand out forming a separate group A in the PCA. Those two species were correlated with most of the morphometric parameters for rose hips; weight (Rh.W.), width (Rh.Wi), high (Rh.H), a flesh fresh weight (Fl.F.W.), with a high number of achenes (A.Nb) and high moisture (Mo). Between those two species, *R. pomifera* Kairouan region was different from *R. rubiginosa* Kairouan region by its highest contents in Car., As.A, T.po, but *R. rubiginosa* Kairouan region by its highest achene weight (A.W.).

TABLE 3 TOTAL POLYPHENOL, ASCOBIC ACID AND CAROTENOID CONTENT IN ROSE HIPS FROM SIX *ROSA* ACCESSION

Species	Total contents		
	Polyphenol (mg GAE.g-1 D.W.)	Ascorbic Acid (mg/100 g F.W.)	Carotenoid (µg.g-1 F.W.)
R. pomifera Kairouan	*69.6±2.1c	737.8±10.6e	667.5±9.7e
R. rubiginosa Kairouan	33.0±1.1b	384.0±11.9a	373.3±6.9b
R. rubiginosa Beja	25.2±3.0a	545.0±13.9d	608.8±7.8d
R. dumetorum Beja	25.4±2.3a	433.8±7.8b	590.9±3.7d
R. dumetorum Siliana	37.0±1.3b	372.4±4.7a	446.9±4.9c
R. canina Zaghouan	34.6±1.1b	468.3±3.8c	206.4±4.0a

* Means ±SD, n=3. In the Same Column, Values with Different Letters are Significantly Different at $p < 0.05$

TABLE 4 MATRIX CORRELATION OF POMOLOGICAL AND CHEMICAL STUDIED PARAMETERS (TOTAL POLYPHENOL, ASCORBIC ACID, CAROTENOID CONTENT) FOR ROSE HIPS FROM SIX *ROSA* ACCESSIONS

	Rh.H	Rh.Wi	Rh.W.	Fl.F.W.	Fl./Rh	Fl.D.W.	Mo	A.Nb	A.W.	T.Po	As.A	Car	Rh.C	Rh.A
Rh.H	1													
Rh.Wi	0.33	1												
Rh.W.	0.61	0.90	1											
Fl.F.W.	0.62	0.88	0.96	1										
Fl./Rh	-0.20	-0.46	-0.52	-0.29	1									
Fl.D.W.	0.45	0.53	0.61	0.68	-0.01	1								
Mo	0.39	0.62	0.63	0.59	-0.42	-0.10	1							
A.Nb	0.48	0.55	0.62	0.49	-0.71	0.23	0.45	1						
A.W.	-0.11	0.40	0.32	0.37	0.02	0.29	0.13	-0.23	1					
T.po	0.36	0.01	0.15	0.10	-0.23	0.08	0.08	0.39	-0.29	1				
As.A	0.21	-0.01	0.06	-0.01	-0.32	0.04	0.00	0.40	-0.19	0.59	1			
Car.	0.12	-0.02	-0.01	-0.11	-0.32	-0.26	0.17	0.53	-0.56	0.18	0.51	1		
Rh.C	0.08	-0.23	-0.20	-0.21	0.05	-0.40	0.11	0.14	-0.65	-0.13	-0.27	0.51	1	
Rh.A	0.48	0.57	0.69	0.63	-0.48	0.21	0.59	0.57	0.15	0.56	0.37	0.16	-0.17	1

* For Abbreviation See Table 1

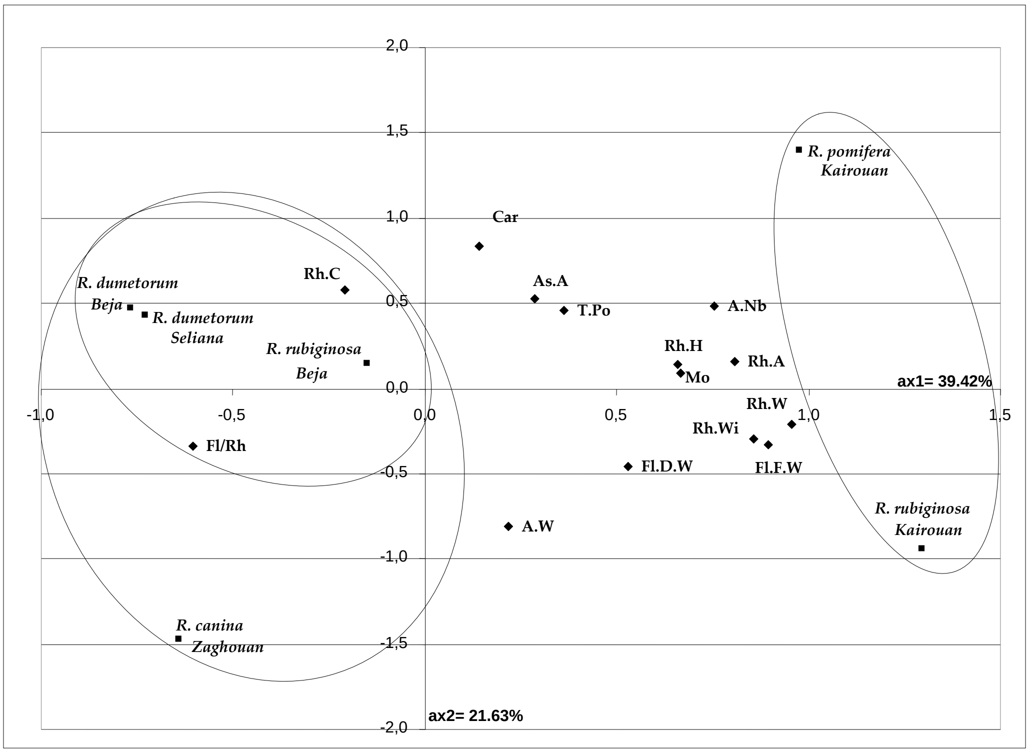

FIG. 1 PRINCIPAL COMPONENT ANALYSIS OF POMOLOGICAL PARAMETERS AND NUTRITIONAL VALUES OF ROSE HIPS FROM SIX *Rosa* ACCESSIONS. ROSE GENETYPES (■), PARAMETERS (♦)

Group B with the 4 other accessions correlated with the proportion of flesh for rose hip (Fl/Rh), whose values were the highest.

Group B1 formed by 3 accessions; *R. dumetorum* Beja region, *R. dumetorum* Siliana region and *R. rubiginosa* Beja region correlated with carotenoid contents having high values, and slightly with A.Nb, Rh.C, As.A, T.Po, whose values were moderatly high. *R. dumetrum* has scarlet orange rose hip color but dark red for *R. rubiginosa*.

Group B2 formed with *R. canina* Zaghouan region was characterized by A.W. and Fl.D.W.

Accessions were grouped on the base of their pomological parameters related to species, nevertheless, the environmental variation between regions of collection influenced the distribution of *R. rubiginosa* species. Indeed, *R. rubiginosa* from Beja region distanced *R. rubiginosa* from Kairouan region which is an arid site. Certainly, the environemental variations affected polyphenols and ascorbic acid content in rose hips, which can explain the separation of the two *R. rubiginosa* accessions.

Conclusion

Rose hip fruits gathered from the six *Rosa* accessions identified in the Tunisian Dorsal area revealed a great variability for the studied pomological and chemical parameters. *R. rubiginosa* Kairouan region had the heaviest rose hip while *R. canina* Zaghouan region had the highest proportion of flesh fruit ratio, the heaviest dry weight of flesh and the lowest achene number per fruit. The best source for active components was *R. pomifera* collected from Kairouan region (264 m altitude).

In conclusion, this study contributed to the characterization of rose germplasm native in the North and Centre of Tunisia. These results were of particular interest because they permited to identify rose genotypes producing rose hips with a potential to be exploited as a new food and a source of valuable natural compounds and their derivatives.

REFERENCES

Asami, D. K., Hong, Y-J., Barrett, D. M., and A.E. Mitchell. "Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional organic, and sustainable agricultural practices. Journal of Agriculture and Food Chemistry". 51:1237-1241, 2003.

Bahri-Sahloul, R., Ammar, S., Grec, S., and F., Harzallah-Skhiri. "Chemical characterisation of *Crataegus azarolus* L. fruit from 14 genotypes found in Tunisia". Journal of Horticultural Science and Biotechnology 84 (1):23-28, 2009.

Bailey, L.H. "The standard cyclopedia of horticulture". Macmillan Co., New York, 1963

Barros, L., Carvalho, A. M., Morais, J. S., and I. C. Ferreira. "Strawberry-tree, blackthorn and rose fruits: Detailed Characterisation in nutrients and phytochemicals with antioxidant properties". Food Chemistry, 120 (1): 247-254, 2010.

Bravo, L. "Polyphenols: chemistry, dietary sources, metabolism and nutritional significance". Nutrition Reviews, *56* (11): 317-333, 1998.

Celik, F., Kazankaya, A., and S. Ercisli. "Fruit characteristics of some selected promising rose hip (*Rosa* spp.) genotypes from Van region of Turkey". African Journal of Agricultural Research, *4* (3): 236-240, 2009.

Cottin, R. "La taxonomie numérique, application aux agrumes". Fruits, 43: 721-733, 1988.

Demir, F., and M. Özcan. "Chemical and technological properties of rose (*Rosa canina* L.) fruits grown wild in Turkey". Journal of Food Engineering 47: 333-336, 2001.

Dewanto, V., Wu, X., Adom, K.K., and R.H. Liu. "Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agriculture and Food Chemistry 50: 3010-3014, 2002.

Gao, X., Uggla, M., and K. Rumpunen. "Antioxidant activity of dried and boiled rose hips". In Proceeding of the first international rose hip conference edited by, H. Nybom and K. Rumpunen. Acta horticulturae, 690. ISHS, 2005.

Günes, M. "Pomological and phenological characteristics of promising rose hip (*Rosa*) genotypes". African Journal of Biotechnology 9 (38): 6301-6306, 2010.

Gürbüz, I., Üstün, O., Yesilada, E., Sezik, E., and O. Kutsal. "Anti-ulcerogenic activity of some plants used as folk remedy in Turkey". Journal of Ethnopharmacology 88 (1): 93-97, 2003.

Jablonska-Rys, E., Zalewska-Korona, M., and J. Kalbarczyk. "Antioxidant capacity, ascorbic acid and phenolics content in wild edible fuits". Journal of Fruit and Ornamental Plant Research 17 (2): 115-120, 2009.

Joublan, J.P., and D. Rios. "Rose culture and industry in Chile". In Proceeding of the first international rose hip conference edited by, H. Nybom and K. Rumpunen. Acta horticulturae. 690. ISHS, 2005.

Kampfenkel, K., Van Montau, M., and D. Inze. "Extraction and determination of ascorbate and dehydrascorbate from plant tissue". Annal of Biochemistry 225: 165-167, 1995.

Karakaya, S., and A. Kavas. Antimutagenic activities of some foods. Journal of the Science of Food and Agriculture 79: 237-242, 1999.

Kasankaya , A., Turkoglu, N., Yilmas, M., and M. F. Balta. "Pomological description of *Rosa canina* from Eastern Anatolia, Turkey". International Journal of Botany 1: 100-102, 2005.

Le Floc'h E., Boulos, L., and E. Vella. "Catalogue synonymique commenté de la Flore de Tunisie". Publication Scientifique tunisiennes, pp. 302-303, 2010.

Mélo, E.A., Galvào de Lima, V.L., and M.I. Sucupira Maciel. "Polyphenol, ascorbic acid and total carotenoid contents in common fruits and vegetables". Brazilian Journal of Food Technology 9: 82-94, 2006.

Nonier, M.F., De Gaulejac , N.V., Vivas, N., and C. Vitry. "Characterization of carotenoids and their degradation products in oak wood. Incidence on the flavour of wood". Comptes Rendus Chimie 7: 689-698, 2004.

Olsson, M.E., Andersson, S., Werlemark, G., Uggla M., and K.-E. Gustavsson. "Carotenoids and phenolics in rose hips". In Proceeding of the first international rose hip conference edited by, H. Nybom and K. Rumpunen. Acta Horticulturae. ISHS, 690: 249-252, 2005.

Pantelidis, G.E., Vasilakakis, M., Manganaris, G.A., and G. Diamantidis. "Antioxidant capacity, phenol, anthocyanin, and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and cornelian cherries". Food Chemistry 102: 777-783, 2007.

Pottier-Alapetite, G. Flore de la Tunisie Angiospermes-Dicotylédones, Apétales Dialypétales. Publications Scientifiques tunisiennes. 1979.

Razungles, A., Oszmianski, J., and J-C. Sapis. "Determination of carotenoids in fruits of *Rosa* sp. (*Rosa Canina* and *Rosa Rugosa*) and of Chokeberry (*Aronia Melanocarpa*)". Journal of Food Sciences 54 (3):774-775, 1989.

Rehder, A. Manuel of cultivated trees and shrubs (2nd Edn), Collier Macmillan Ltd., New York. 1940.

Shahidi, F., and M. Nacsk. "Phenolic compounds in fruits and vegetables. In, Phenolics in food and nutraceutical, edited by F. Shahidi, and M. Nacsk, CRC LLC. 2004.

Uggla, M., and M. Martinsson. "Cultivate the wild roses-experiences from rose hip production in Sweden". In Proceeding of the first international rose hip conference edited by, H. Nybom and K. Rumpunen. Acta horticulturae. 690. ISHS, 2005.

Warholm, O., Skaar, S., and E. Hedman. "The effects of standardized herbal remedy made from subtype of *Rosa canina* in patients with osteoarthritis: A double-blind, randomized, placebo-controlled clinical trial". Current Therapeutic Research 64 (1): 21-31,2003.

Winther, K., Rein, E., and A. Kharazmi. "Anti-inflammatory properties of rose-hip". Inflammopharmacology *7*: 63-68, 1999.

Wisseman, V. (2003). "Classification/ conventional taxonomy (Wild roses)". In: A.V. Roberts, T. Debener, & S. Gudin (Eds.), Elsevier.

Encyclopedia of rose science (pp. 111-117). Oxford.

Rapid Method for the Detection of Fake Protein Addition in Milk

Rao Muhammad Imran[1], *Anam Siddique, Zahid Kaleem[2], Muhammad Naeem[3]

Research and Development Department; Haleeb Foods Limited, Pakistan

[1]rao.imran@haleebfoods.com;
*Anam.Siddique@haleebfoods.com; [2]zahid.kaleem@haleebfoods.com; [3]muhammad.naeem@haleebfoods.com

Abstract

The purpose and aim of this research work is rapid detection of fake protein or added protein in milk, In 3rd world, especially in Pakistan , where total protein criteria for milk acceptance norm exists at Processor end, chemicals containing NH2 or N are added in fresh milk to top up the total protein by middle man to mask the adulteration .When milk is coagulated the casein protein form the coagulant while all other proteins including the Whey protein and chemicals they get separate in the form of filtrate. Naturally, whey proteins (Globulin & Albumin) have a specific ratio to casein protein. Whey proteins are 20% of the total protein .To detects the presence or absence of any fake chemical that produces its impact on milk protein, a rapid and easy method has been established by simple filtration and titration method. To set this method as standard and make it as a benchmark, different commercial milk samples of reliable selected sources were chosen (including Farm Milk samples, Prema, Gourmet milk AA milk, Nestle Milk) and did the comparative study of total and whey protein against the adulterated samples.

Keywords

Total Protein; Whey Protein; Haleeb Milk; Competitors

Introduction

Milk is the complex composition of Fats, Carbohydrates, proteins and solid contents. The main role of milk is to provide the proper nourishment and healthy body. From all of this milk protein contains more essential amino acids than any other natural food (*Jud Heinrichs* 1855). Milk protein consists of two parts namely (i) Total protein that contains casein and (ii) Whey protein (*Frank.K* 2003). The percentage of casein and whey protein varies in cow and buffaloes milk. In case of cow's milk 80% is casein and remaining 20% is whey protein whereas in buffaloes 87.50% casein and 12.50% whey (*A.Hethmankova* 2012).

Concentration of protein in milk is 33 g/L in which casein have its part 26g/L (79.5% of total protein) (*F. Lara* 2005). Furthermore casein is composed of alpha s1, alpha s2, beta casein and kappa casein which is respectively 30.6, 8.0, 28.4 and 10.1 % of total protein. While total whey protein in milk is 6.38g/L (19.5% of total protein).Whey protein is composed of alpha lactalbumin, BSA, immunoglobulin and protease peptone which is 9.8, 1.2, 2.1 and 2.4% of total whey protein respectively (*H.V.Petit* 2001).

Experiments show that addition of ammonium sulphate, ammonium hydroxide and ammonium carbonate increases total protein %age. This increases proportion in whey protein not in casein protein. The addition of these chemicals causes to increase the whey protein level in milk which fortunately raised the total protein percentage (*Rashmi. A* 2013). Physiochemical result shows that due to the addition of these fake chemicals in milk produce the impact in appearance of milk as well, that resembles with the proper whey protein color and it takes much less time in filtration process as compare to pure protein (*Ahmed* 2013).

Material and Method

The purposed study will be conducted for determination of fake protein in milk.

Material

Titration flask100 ml, Beaker 100 ml. Beaker 500ml, Burette, pH Meter, filter paper, Hot plate

Reagents

Sodium Hydroxide 0.1N (4.0gm NaoH n 1000ml Distill water),

Phenolphthalein Indicator 1% (1.0 gm in 100ml Ethanol),

Formalin (37% Formaldehyde)

Citric Acid.

Ammonium sulphate.

Procedure

1. Take 10ml of thoroughly mixed sample in a titration flask.

2. Neutralize the sample with 0.1N NaOH using phenolphthalein (1%) as Indicator up to 8.20 pH Value.
3. Add 2ml of Formalin solution (37%).
4. Wait for 5 minutes.
5. Again Titrate with 0.1N NaOH up to 8.20 pH Value. Note the volume (V1).
6. Also repeat above for blank reading without sample (V2)
7. Now again take sample volume 100 ml. from same sample
8. Heat sample up to 90-95 ºC.
9. Add 1g citric acid to clot milk.
10. Stir clock wise slowly with glass rod.
11. Now filter clotted milk through filter paper whatman No 2 or common filter paper sheet.
12. Take 10 ml sample from filtrate.
13. Titrate with NaOH 0.1N to Neutralize up to 8.20 pH Value.
14. Add 2ml of Formalin solution (37%).
15. Wait for 5 minutes.
16. Repeat titration up to 8.20 pH Value for Whey protein %
17. Perform calculation as below

Observation and Calculations

Total Protein%= (V1-V2) x1.94

Whey Protein = (V1-V2) x1.94

$$\text{Whey \% of total protein} = \frac{\text{Whey Protein \%}}{\text{Total Protein \%}} \times 100$$

Whereas: 1.94 is the Factor of formalin

V2 = Blank reading of Formalin Solution

Results

TABLE 1 PRODUCT: FRESH AND FARM MILK

Sr No.	Product	ANIMAL NO.	DOP	TOTAL PROTEIN (%)	WHEY PROTEIN (%)	Whey % of Total
1.	FARM MILK	383	14-12-11	3.1	0.38	13%
2.	FARM MILK	412	14-12-11	3.7	0.8	21%
3.	FARM MILK	125	14-12-11	3.3	0.8	23%
4.	FARM MILK	405	14-12-11	3.5	0.8	22%
5.	FARM MILK	451	14-12-11	3.3	0.7	20%
6.	FARM MILK	432	14-12-11	2.5	0.4	15%
7.	FARM MILK	363	14-12-11	3.3	0.6	18%
8.	FARM MILK	452	14-12-11	3.1	0.6	19%
9.	FARM MILK	418	14-12-11	3.1	0.4	13%
10.	FARM MILK	1024	14-12-11	3.8	0.8	20%
11.	FARM MILK	__	01-12-11	3.1	0.58	18.7%
12.	FARM MILK	__	13-12-11	2.91	0.58	20%
13.	FARM MILK (HFL)	__	01-12-11	2.52	0.50	20%

TABLE 2 REFERENCE SAMPLES (ADULTERATED)

SR No.	PRODUCT	DOP	DOE	BATCH	TOTAL PROTEIN (%)	WHEY PROTEIN (%)	WHEY % OF T.P
1.	Reference sample	16-11-11	16-02-12	269	2.91	1.16	40%
2.	Reference sample	17-11-11	17-02-12	275	2.52	1.16	46%
3.	Reference sample	17-11-11	17-02-12	276	2.91	1.16	40%
4.	Reference sample	18-11-11	18-02-12	277	2.71	0.97	35%
5.	Reference sample	19-11-11	19-02-12	278	2.32	1.16	50%
6.	Reference sample	19-11-11	19-02-12	280	3.1	1.16	37%
7.	Reference sample	16-11-11	16-02-12	281	3.1	0.97	31%
8.	Reference sample	16-11-11	16-02-12	282	3.1	1.16	37%
9.	Reference sample	16-11-11	16-02-12	283	3.1	0.97	31%
10.	Reference sample	16-11-11	16-02-12	284	2.91	0.97	33%
11.	Reference sample	06-12-11	06-03-12	316	2.91	1.35	47%
12.	Reference sample	08-12-11	08-03-12	317	3.1	1.35	44%
13.	Reference sample	08-12-11	08-03-12	318	3.1	0.97	31%
14.	Reference sample	09-12-11	09-03-12	319	2.91	0.97	33%
15.	Reference sample	09-12-11	09-03-12	320	3.1	0.58	19%
16.	Reference sample	28-11-11	28-02-12	301	3.1	0.97	31%
17.	Reference sample	29-11-11	29-02-12	302	3.1	1.35	44%
18.	Reference sample	29-11-11	29-02-12	303	2.91	0.97	33%

19.	Reference sample	29-11-11	29-02-12	304	3.1	1.55	50%
20.	Reference sample	30-11-11	30-02-12	305	3.1	1.35	44%
21.	Reference sample	30-11-11	30-02-12	306	3.29	0.58	31%
22.	Reference sample	30-11-11	30-02-12	305	3.1	1.35	44%
23.	Reference sample	30-11-11	30-02-12	306	3.29	0.58	31%
24.	Reference sample	01-12-11	01-03-12	307	3.1	0.77	25%
25.	Reference sample	02-12-11	02-03-12	308	3.1	0.77	25%
26.	Reference sample	02-12-11	02-03-12	309	3.1	0.97	31%
27.	Reference sample	03-12-11	03-03-12	310	3.1	0.97	31%
28.	Reference sample	03-12-11	03-03-12	311	3.29	0.38	13%
29.	Reference sample	04-12-11	04-03-12	312	3.1	0.97	31%
30.	Reference sample	04-12-11	04-03-12	313	3.1	0.77	25%
31.	Reference sample	05-12-11	05-03-12	314	3.29	0.97	29%
32.	Reference sample	05-12-11	05-03-12	315	2.9	1.16	40%
33.	Reference sample	24-11-11	24-02-12	291	2.91	0.97	335
34.	Reference sample	23-11-11	23-02-12	290	2.91	1.35	46%
35.	Reference sample	24-11-11	24-02-12	292	3.1	1.16	37%
36.	Reference sample	24-11-11	24-02-12	293	3.1	1.35	44%
37.	Reference sample	26-11-11	26-02-12	296	3.1	1.35	44%
38.	Reference sample	23-10-11	23-01-12	250	3.3	1.16	35%
39.	Reference sample	29-10-11	29-02-12	256	3.1	0.388	13%
40.	Reference sample	31-10-11	31-02-12	257	3.1	1.6	50%
41.	Reference sample	31-10-11	31-01-12	258	3.1	1.6	50%
42.	Reference sample	05-11-11	05-02-12	262	3.1	1.6	50%
43.	Reference sample	03-11-11	03-02-12	259	2.9	1.6	55%
44.	Reference sample	24-10-11	24-02-12	252	2.9	1.4	48%

TABLE 3 PRODUCT: COMPETITOR UHT MILK

Sr No.	Product	DOP	DOE	Batch No.	Total Protein	Whey Protein	Whey % of Total
1.	NESTLE	20-10-11	12-01-12	129315802	3.1	0.39	13%
2.	NESTLE	03-12-11	25-02-12	13371580114	2.91	0.39	14%
3.	NESTLE	11-11-11	03-02-12	13151581	3.1	0.58	19%
4.	NESTLE	10-11-11	02-02-12	131415802	3.1	0.48	16%
5.	NESTLE	11-11-11	03-02-12	131515802	3.1	0.58	19%
6.	NESTLE	01-12-11	23-02-12	133515801	3.1	0.58	19%
7.	NESTLE	01-11-11	14-01-12	130515801p	3.1	0.58	18.7%
8.	NESTLE	14-10-11	14-01-12	1226158121L	2.9	0.38	13%
9.	NESTLE	30-11-11	22-02-12	133415801	2.9	0.38	13%
10.	NESTLE	25-11-11	17-02-12	132915802	2.9	0.38	14%
11.	NESTLE	06-11-11	19-01-12	131015801	3.1	0.58	19%
12.	NESTLE	04-12-11	26-02-12	133815801	3.1	0.58	19%
13.	NESTLE	12-12-11	05-03-12	134615801	3.1	0.47	16%
14.	NESTLE	18-11-11	10-02-12	132215801	2.9	0.56	19%
15.	NESTLE	17-11-11	09-02-12	132115801	3.2	0.49	15%
16.	GOOD MILK	24-11-11	24-02-12	1898	2.13	0.97	46%
17.	GOOG MILK	24-09-12	24-12-11	1620	2.32	0.97	41%
18.	OLPER	16-09-11	15-12-11	5585HO	2.91	1.16	40%
19.	OLPER	13-11-11	11-02-12	5858	3.1	0.39	13%
20.	OLPER	15-11-11	15-02-12	5873	2.9	0.39	14%
21.	OLPER	03-11-11	01-02-12	5816	2.8	0.8	28%
22.	OLPER	29-11-11	27-02-12	5926	2.9	0.582	20%
23.	OLPER	20-09-11	18-12-11	5735	2.9	0.38	14%
24.	OLPER	23-10-11	21-01-12	5775	3.1	1.358	44%
25.	OLPER	02-12-11	01-03-12	5941	2.73	0.54	20%
26.	OLPER	10-12-11	09-03-12	5976	2.73	0.51	19%
27.	OLPER	29-11-11	27-02-12	5930	2.64	0.39	15%
28.	OLPER	12-12-11	11-03-12	5983	2.8	0.47	17%
29.	OLPER	27-11-11	25-02-12	5914	2.8	0.52	19%
30.	OLPER	13-12-11	12-03-12	5985	2.7	0.58	21%
31.	OLPER	14-12-11	12-03-12	5949	2.61	0.46	18%
32.	OLPER	03-12-11	02-03-12	5943	2.74	0.58	21%

33.	OLPER	16-12-11	14-03-12	5953	2.8	0.49	18%
34.	NOOR PUR	25-11-11	23-02-12	322	1.6	0.19	13%
35.	NOOR PUR	11-11-11	04-2-12	310	1.7	0.58	34%
36.	NOOR PUR	09-11-11	07-01-12	282	1.7	0.77	45%
37.	NOOR PUR	06-11-11	05-03-12	340	1.6	0.19	13%
38.	DAIRY PURE	13-11-11	13-02-12	C	1.6	0.8	50%
39.	Nestle by Marketing	16.12.11	09.03.12	1350158010	3.20	0.60	19%
40.	Olper by marketing	6.12.11	05.03.12	5951	3.16	0.83	26%

TABLE 4 PRODUCT: COMPETITOR PASTEURIZED MILK SAMPLE

Sr No.	Product	DOP	DOE	Batch No.	Total Protein	Whey Protein	Whey % of Total
1	PREMA	15-12-11	21-12-11	__	3.298	0.388	12%
2	GOURMET	15-12-11	18-12-11	__	2.716	0.388	15%
3	NURPUR	15-12-11	21-12-11	__	2.134	0.582	28%
4	PREMA	13-12-11	19-12-11	__	3.1	0.58	19%
5	GOURMET	14-12-11	17-12-11	__	2.9	0.582	20%

Discussions

When whey protein or artificial protein is added to raw milk, the ratio between total protein and whey protein gets disturb which is indicator of tempering of milk. Whey proteins and fake proteins gets pass into filtrate once milk protein (casein) is coagulated.

We have done this study on farm collected milk and do the comparison between different milk samples available in market (shown in table 1-4). From all these we observed the following below results.

Total protein of farm milk by Rapid Method: 3.10% (100%). Whey protein after acidification: 0.58% (18%). After addition of Ammonium sulphate 0.50% in above farm milk. Total protein by Rapid Method was 14.74% (which is more 375% More than actual protein) and Whey protein after acidification was 16.49% (114%). Similarly after addition of Ammonium sulphate 0.10% in above farm milk. The results found were, total protein by Rapid Method 4.65% (which is 48% more than actual) whereas whey protein after acidification 3.88% (83 %).

From the above result in the tablet form we can concluded that Farm milk Doodah meets the pure milk protein proportions according to the standard values standards. If we added 0.50% Fake chemical (Aluminum Sulphate) in Farm milk then almost 12% total protein increased and it will be also increased in whey protein 114% with same rapid test method. Same activity performed with reduce dosage of Aluminum sulphate 0.1% in above Farm milk and 1.55% protein increased and also increase whey protein 83% with same method.

By summarizing all the result we can conclude that if we added whey powder to re-constituted milk it is detectable through above mentioned testing protocol.

ACKNOWLEDGEMENT

Mr. Faisal Imran Hussain Malik, the CEO of Haleeb Foods Limited, joined with the aim of turnaround of the company (HFL), which was in severe crises due to bad quality of incoming fresh milk, caused by adulteration. Mr. Malik introduced the concept of "Sell Right" through emphasizing on development of adulteration detection in milk.

The above research, "detection of fake protein addition by rapid method", not only helped to improve the quality of Haleeb foods products but also impacted on overall business results enabling the fastest turnaround in cooperate sector.

REFERENCES

Ahmad(1), S. F. M. Anjum(2), N. Huma(3), A. Sameen(4) and T. Zahoor(5). Composition and physio-chemical characterization of buffalo milk with particular emphasis on lipids, proteins, minerals, enzymes and vitamins. *The Journal of Animal and Plant Sciences*. (2013) 23, Pp: 62-74.

A. Hethmankova(1), V. PivecE.Trankova(2) and H. Dragounounova(3). Differences in the composition of whey protein and total protein in goat milk and ewe milk and their changes through the lactation period. *Czech Journal of Animal Sciences* (2012) 57 (7) pp 323-331.

F. Lara-Villoslada(1), M. Olivares(2), and J. Xaus(3). The Balance between Caseins and Whey Proteins in Cow's Milk determines its Allergenicity. *American journal of Dairy Science* (2005):88 pp 1654–1660.

Frank Kjeldsen (1), Kim F. Haselmann(2) ,Bogdan A. Budnik(3), Esben S. Sorensen(4) and Roman A. Zubarev(5). Complete Characterization of Posttranslational Modification Sites in the Bovine Milk Protein PP3 by Tandem Mass Spectrometry with Electron Capture Dissociation as the Last Stage. *Journal of Analytical Chemistry* (2003), 75 (10), pp 2355–2361.

H. V. Petit[1], R. J. Dewhurst[2], J. G. Proulx1 M. Khalid[3], W. Haresign[4] and H. Twagiramungu[5]. Milk production, milk composition, and reproductive function of dairy cows fed different fats. *Canadian Journal of Animal Science*. (2001) pp 263-271.

Jud Heinrichs [1], Coleen Jones[2], and Ken Baile[3]y. Milk Components: Understanding the Causes and Importance of Milk Fat and Protein Variation in Your Dairy Herd. PENNSTATE. *Journal of Dairy and Animal science* (1855) pp 2-10

Rashmi Arora[1], N Bhojak[2], Rajani Joshi[3]. Comparative Aspects of Goat and Cow Milk. *International Journal of Engineering Science Invention.* (2013):2 pp.07-10