Global Advanced Research Journal of Agricultural Science (ISSN: 2315-5094) Vol. 2(6) pp. 153-159, June, 2013.
Available online http://garj.org/garjas/index.htm

Full Length Research Paper

Lupinus mutabilis Sweet, a traditional Ecuadorian grain: Fatty acid composition, use in the Ecuadorian food system, and potential for reducing malnutrition.

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Accepted 05 June, 2013

Past studies show that in the rural area of the Ecuadorian highlands total fat intake is low and therefore are at risk for deficiency of essential fatty acids (EFAs). The emphasis on the consumption of foods rich in carbohydrates and poor quality fats raises concerns about low intake of fat, especially in children and suggests that the promotion of legumes with high fat can produce health benefits. *Lupinus mutabilis* Sweet ("lupine") is a legume grown in Ecuador and throughout the Andes. The improved variety, INIAP-450, has high yields, high viability, can be grown on marginal soils and is early maturing (six months compared to 12 months of the native varieties). Analyses of its fat content were conducted, and, consistent with previous studies, fat levels were high (22% fat (dry weight)) and of high quality, with essential omega-3 and omega-6 fatty acids comprising over three-quarters of the total fat. A successful lupine promotion program in rural Ecuador was able to increase lupine intakes to achieve 10 to 15% of the recommended EFA intake. Further intervention (with more lupine, processed lupine products, such as oils, or other healthy fat-rich foods) will be required to meet the fat requirements in Andean populations.

Keywords: *Lupinus mutabilis,* traditional Ecuadorian grain, Fatty acids, malnutrition

INTRODUCTION

Dietary fat is essential in the human diet. Insufficient dietary fat may cause deficiencies of essential fatty acids (EFAs), may prevent adequate absorption of fat soluble vitamins, and contribute to an insufficiently energy-dense diet to allow energy needs to be met (especially an issue in infants and young children) (Prentice and Paul 2000). Exclusively breastfeed infants, fed by well-nourished mothers, would usually have sufficient quantity of fat, supplying 40% to 55% of dietary energy, and sufficient intakes of all EFAs for at least the first four months of age. However, the fat content of breastmilk is often too low in developing countries, and complementary foods are introduced to infants earlier than the recommended four to six months, and these introduced foods are often of low energy density/low fat content (Beare-Rogers, Ghafoorunissa et al. 1998). Of particular concern are the n-3 and n-6 fatty acids, as low intakes are associated with various adverse health outcomes (see Discussion). Previously conducted studies recognize rural highland Ecuador as having low total fat intakes (Berti and Leonard 1998;

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Moreano Barragán 2001; Berti, Krasevec et al. 2004) and thus being at risk for EFA deficiencies.

Ecuador is located in northeast South America, bordered by Colombia to the north, Peru to the south and east, and the Pacific Ocean to the west. It is divided in three along its north-south axis into the coastal zone, the highland zone and the Amazon zone. Ecuador's population comprises indigenous groups (52%, primarily the highland-dwelling Quechua), mestizos (40%, mixed European-indigenous ancestry) and other groups, primarily of Spanish and African descent (Dachs 2002). The indigenous groups are at highest risk of malnutrition, with more than 20% of the children in indigenous households malnourished, and these risks are highest in the rural highland areas.

The Legume Program of the National Institute of Agricultural Research (INIAP) in Ecuador has been working since 2001 in the *canton* of Saquisilí, in the province of Cotopaxi, a rural zone 80 km south of the capital city Quito. The principal crop grown in Saquisilí is potato. It is estimated that 86% of the households live in poverty in Saquisilí (Comité de Desarrollo Social Camino al Progreso 2003) and the mortality rate of children under 5 years of age is 77 per 1000, approximately three-fold the national average (Instituto Nacional de Estadísticas y Censos de Ecuador 1997). While malnutrition in children is common in Saquisilí, hunger is not often reported with the families reporting eating three meals and two snacks per day. However the food choices may be somewhat limited with the barley and potato focus common throughout the highlands and pasta, bread, and rice also being commonly consumed (Comité de Desarrollo Social Camino al Progreso 2003). While there are also some legumes and animal foods consumed, the emphasis on carbohydrate-rich, fat-poor foods raises concerns about low fat intakes, especially in children, and suggests that promotion of legumes high in dietary fat would yield health benefits. A 24-hour recall on 55 households in Saquisilí at baseline in 2006 estimated that dietary fat contributed approximately 10% of dietary energy (unpublished data), much less than the 20-25% that is suggested as healthy for developing country children (Prentice and Paul 2000).

Lupinus mutabilis sweet is a locally cultivated legume, known in Ecuador as "chocho", in Peru and Bolivia as "tarwi" or "tarui", and lupine or lupine bean in common English. While it has long been considered a "peasant food", in recent years, the status of lupine bean has changed and is now a premium food served in the better restaurants of Quito. While there are over 550 cultivars of lupine, there are only a few commonly grown. In 2004 INIAP released the improved variety INIAP 450, chosen for its high yields, viability in marginal soils and precocity, maturing in approximately six months compared to 12 months for traditional varieties (Peralta 2006). The bean produced has high levels of alkaloids, which require lengthy washing in order to debitter the bean and make it palatable (Caicedo 2001). Preliminary analyses on different varieties of *Lupinus mutabilis* Sweet (Gross 1982; Allauca 2005; Montatixe 2005) and related species such as *Lupinus albus (United States Department of Agriculture)* suggested that it has a high level of high quality fat. In this paper we present a comprehensive analysis of fat levels in lupine, and demonstrate its potential to improve the diet of rural farmers in a small scale intervention.

METHODS

Fatty Acid Analysis

Samples and reagents

Thirteen samples of lupine beans were collected at maturity from different plant populations in different locations near Quito, Ecuador, as outlined in Table 1. All reagents, chemicals and solvents used were from Merck (Darmstadt, Germany). Fatty acid methyl esters standards were purchased from Aldrich (St. Louis, USA).

Sample preparation.

Six of the samples were debittered; seven were left in their bitter, as harvested, state. To debitter the samples, they were soaked for 12 hours, boiled for 40 min and washed in water for 72 hours. The debittered bean was then dried in a stove at 50ºC, with forced air, until the moisture content was 10%. Then the beans (bitter and debittered) were milled in a disc mill with a mesh opening of 1 mm and stored in sealed glass jars until analysis.

The oil was extracted from a 50 g sample of milled bean (20 mesh) by refluxing for 6 h with 125 mL n-hexane (ACS) in a Soxhlet extractor. The solvent was evaporated under reduced pressure, yielding the bitter oil from the beans processed as harvested and debittered oil from debittered beans. Esterfication of the oil was carried out on 50 mg samples of the extracted oil, to which was added 1 ml of 0.5 M KOH in methanol. This mixture was placed in sealed test tubes was brought to a boil in a water bath for 30 min and cooled. To this was added 0.5 ml of HCl (37% GR):methanol (in 4:1 ratio). The mixture was brought to a boil for 25 minutes, cooled and mixed with 2 ml of doubly distilled water. The esters were extracted through three successive hexane washings. To this solution was added anhydrous sodium sulfate to eliminate residual water. The supernatant was separated and the solvent evaporated with nitrogen gas.

Gas hromatography conditions

The extract was diluted with 2 ml of reactive grade hexane and injected into a gas chromotograph (Shimadzu GC-14B). A thermal column TR-FAME, 3 meters in length x 0.25mm in diameter and 0.25 μm pore size was used for separation of fatty acid methyl esters. The initial temperature of 100 ºC was maintained

Table 1. Description of 13 lupine bean samples.

Sample Number	Variety[1]	Location[2]	Year[3]	Status[4]
1	ANDINO 450	SC	2005	bitter
2	ANDINO 450	SC	2007	bitter
3	ANDINO 450	Rural farm	2007	debittered
4	ANDINO 450	Rural farm	2007	debittered
5	ANDINO 450	SC	2008	debittered
6	ANDINO 450	SR	2006	bitter
7	LPC 09	SR	2006	bitter
8	LPC 13	SR	2007	bitter
9	LPC-015	SR	2006	debittered
10	LPC-03	SR	2006	debittered
11	LPC-04	SC	2006	bitter
12	LPC-05	SC	2006	bitter
13	LPC-06	SC	2006	debittered

Notes:

1. Andino 450 is the most commonly grown variety. LPC are lines or materials showing promise, not yet widely grown
2. Rural farm samples were collected from farmers growing lupine bean in marginal conditions (minimal rain fall, poor soils). SC samples were collected from INIAP's Santa Catalina research station on the northern edge of Quito were conditions were near optimal. SR samples were collected from INIAP's Simón Rodríguez research station in Latacunga, 80 km south of Quito where conditions were intermediate.
3. The 2006 and 2007 growing seasons were both very good; 2005 season was dry.
4. Lupine bean at harvest contain high levels of alkaloids that must be debittered before they are fit for consumptions (see Methods)

for 5 minutes and then increased at 4 ºC/minute until reaching the final temperature of 200 ºC for 2 minutes. A flame ionization detector (air-hydrogen-nitrogen) was used. The split ratio was 1:10, and hydrogen was used as a carrier gas with the flow rate of 0.8 ml/min. The injector and detector temperatures were 250°C and 280°C, respectively.

Peak identification of the fatty acids in the analyzed samples was carried out by comparison with retention times of known standards. Each sample was measured in duplicate and the average of the two samples was reported.

Differences in fatty acid composition between samples were tested with one-way Anovas and with multivariate ANOVAs, assuming status (debittered or not), variety and location (INIAP fields or farmer fields) and year were fixed effects. Year was not expected to have an effect, as the years 2006 and 2007 had similar growing conditions.

Lupine bean promotion and dietary assessment

INIAP agronomists have been conducting participatory research with Saquisilí farmers since 2001, in which the agronomists provide test seed varieties and technical support. In addition to this ongoing agronomical support, starting in October 2006, immediately after the baseline dietary assessment, a nutrition education component was added to the intervention. It included household visits by an INIAP nutritionist, promotion of lupine bean in the local school lunch programs, community recipe-days, in which lupine beans were promoted, and infomercials on local radio. The control communities were exposed to the infomercials but did not receive any of the other intervention components.

Data on consumption of quinoa (not reported here) and lupin in participating households were collected in September 2006, May and October 2007, and May 2008 using a Food Frequency Questionnaire (FFQ) (Gibson 2005) (limited to only quinoa and lupin). The frequency and quantity of consumption were asked at the household level, and consumption was calculated as “grams per day per adult equivalent”. The data were collected by community volunteers and INIAP staff who were trained by the first author in proper interviewing technique. The sampled households included the 45-plus members of the CIALs (Comités de Investigación Agrícola Local – Local Agriculture Research Committees, who collaborate with INIAP in participatory agriculture research), plus other convenience-selected households in the villages. In May 2008 a convenience sample was drawn from two control villages. The sample sizes were 55, 38, 55 and 76 intervention households in September 2006, May and October 2007, and May 2008, respectively and 36 control households in May 2008.

The protocol for the aspects of this research dealing with human subjects was submitted to and approved by the HealthBridge Research Ethics Board.

RESULTS

Fatty Acid Composition

The descriptive statistics of fatty acid levels in lupine bean are presented in Table 2, in both their bitter and

Table 2. Total fat and individual fatty acids levels, g/100 g fat, and percent total fat (proximate composition) in debittered lupine bean samples (n=13).

		mean	SD[1]	min	max	CV[2]	max/min[3]
Capric	C10:0	0.0		0.0	0.0		
Lauric	C12:0	0.6	0.1	0.4	0.7	14.6	1.7
Myristic	C14:0	0.8	0.2	0.5	1.1	21.1	2.0
Palmitic acid	C16:0	10.1	1.4	8.2	12.2	13.5	1.5
Palmitoleic acid	C16:1						
Stearic acid	C18:0	7.9	1.4	6.0	11.3	17.4	1.9
Oleic acid	C18:1	47.5	1.4	45.3	49.8	3.0	1.1
Linoleic acid	C18:2	29.5	1.6	27.6	32.7	5.3	1.2
Linolenic acid	C18:3	2.8	0.4	2.0	3.5	16.1	1.7
Arachidonic acid	C20:0						
Behenic acid	C22:0	0.7	0.2	0.1	0.9	31.1	6.4
Lignoceric	C24:0	0.3	0.1	0.1	0.5	43.9	4.9
	% fat (dry wt)	22.3	2.1	18.8	25.8	9.2	1.4

[1] SD=Standard deviation
[2] Coefficient of variation = SD/mean
[3] maximum value divided by minimum value

Table 3. Comparison of fatty acid composition from different published sources, g/100g fat

		Bitter			Debittered			
Fatty Acid Name	**Chain**	Gross 1982 y Dergal 1988, in Montatixe 2005	Gross, 1982	Parreño C, 2006	Gross 1982 y Dergal 1988,in Montatixe 2005	Allauca, 2005[1]	Parreño C, 2006	current study
Lauric	C12:0							0.6
Myristic	C14:0	0.6	0.3	trace	trace			0.8
Palmitic acid	C16:0	13.4	9.8	10.0	11.3	11.0	11.3	10.1
Palmitoleic acid	C16:1	0.2	0.4	trace	0.2		0.3	
Stearic acid	C18:0	5.7	7.8	8.4	7.3	7.1	8.5	7.9
Oleic acid	C18:1	40.4	53.9	52.2	52.5	51.3	47.9	47.5
Linoleic acid	C18:2	37.1	25.9	26.1	28.4	27.7	28.4	29.5
Linolenic acid	C18:3	2.9	2.6	3.3	3.0	2.9	3.6	2.8
Arachidonic acid	C20:0	0.2	0.6					
Behenic acid	C22:0	0.2	0.5					0.7
Lignoceric	C24:0							0.3
	SUM	100.7	101.9	100.0	102.65	100.0		100.0
Sample size		30	2	7		3	1	13

[1] originally reported on fresh weight basis. Change to g/g fat basis for comparability.

debittered states. In the process of debittering the lupine bean absorbs water so that in the form in which it is consumed it is about 75% water, by weight. Therefore the percent total fat results are presented as fresh weight in the debittered state, as well as dry weight for both bitter and debittered. The averages reported in Table 2 are compared to previously published results in Table 3.

The debittered samples had significantly higher levels of lauric and myristic fatty acids, lower levels of stearic, linoleic and oleic acids, and no difference in the levels of linolenic and palmitic acids. In multivariate analysis, after

Table 4. The Adequate Intake levels (AI) for ω-6 and ω-3 fatty acids, and the percent intake covered by average daily intake of 67g/d of lupin.

	AI		**% AI in 67g/d**	
Infants	**ω-6**	**ω-3**	**ω-6**	**ω-3**
Children				
1–3 y	7	0.7	16%	15%
4–8 y	10	0.9	11%	12%
Males				
9–13 y	12	1.2	10%	9%
14–18 y	16	1.6	7%	7%
19–50 y	17	1.6	7%	7%
> 50 y	14	1.6	8%	7%
Females				
9–13 y	10	1	11%	11%
14–18 y	11	1.1	10%	10%
19–50 y	12	1.1	10%	10%
> 50 y	11	1.1	10%	10%
Pregnancy	13	1.4	9%	8%
Lactation	13	1.3	9%	8%

ω-6 in the form of linoleic acid, 29.5g per 100 g oil, or 6.59 g per 100 g dry weight lupin
ω-6 in the form of linolenic acid, 2.76g per 100 g oil, or 0.62 g per 100 g dry weight lupin

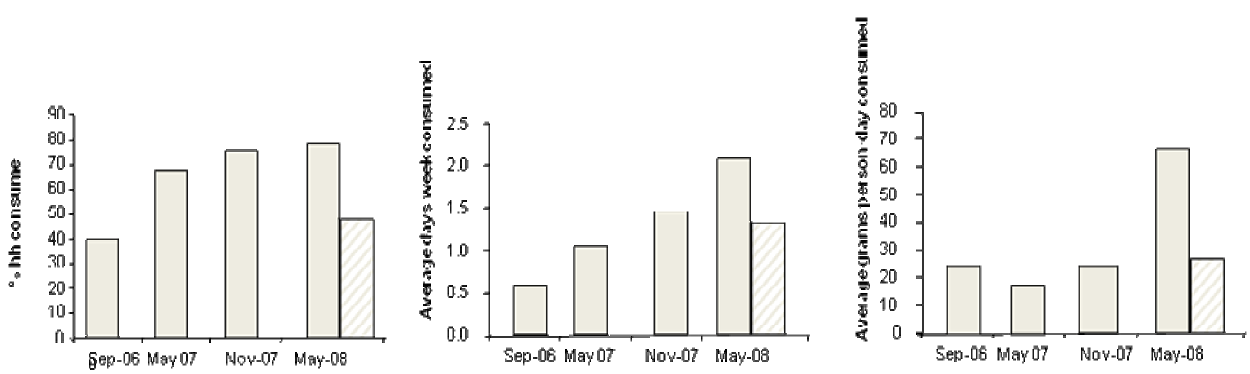

Figure 1. Change in consumption of lupine bean in intervention (solid) and control (striped) communities from September 2006 to May 2008. (a) % of households that consumed lupine at least once in previous week; (b) the average number of days on which lupine was consumed previous week; (c) the average grams of lupine consumed per person per day.

accounting for the variation due to debittering, there were no significant effects of variety, location or year on fatty acid composition.

Table 4 presents the range in values in the debittered samples. The range represents variation due to variety, location, year, measurement error and random error. There is as much as 3-fold differences in the minor fatty acids (C14), but in the fatty acids that form the majority of the fat, maximum levels are less than 2-fold the minimum values.

Change in Lupine Bean Consumption

The number of households that consumed lupine the previous week, the average number of days per week on which it was consumed and the average amount consumed per person per day increased throughout the study period (see Figure 1). For all three variables, the levels in intervention communities were greater than in control communities (one-way ANOVA, p<.05). By the end of the study period, average consumption in intervention communities was 67 grams per person per day, on a fresh weight basis.

DISCUSSION

The intake of dietary fat in the rural highlands of Ecuador is low, and is largely saturated, processed, poor quality fat (Berti and Leonard 1998; Berti, Krasevec et al. 2004; Berti 2006). There are few sources of high quality fat available to the rural poor – the lupine bean may fill an important gap in their diet, as the results presented here indicate it can be a good source of various essential fatty acids. Additionally, lupine bean is reported to be a good source of energy, various micronutrients and protein (Gross 1982). While in the bitter state, it is contains antinutritional factors such as alkaloids, protease inhibitors hemagglutinins, and cyanogenic glycosides, the debittering process eliminates these factors completely, or reduces them to harmless levels (Gross 1982).

The sample used here was drawn from six different varieties, grown in five different locations in three different years. Despite these differences in source, the composition of the beans were relatively constant, particularly with the unsaturated fatty acids for which there was less than a two-fold difference between the maximum and minimum levels (see Table 2). While the debittered lupine beans have a significantly different fatty acid composition then the bitter beans, none of the other factors (variety, location, year) had a significant effect on composition in both one-way ANOVAs and multivariate models. In fact, the fatty acid profile of *Lupinus mutabilis* Sweet is even similar to that reported for a related species, *Lupinus albus (United States Department of Agriculture)* and has a much healthier profile than vegetable lard, the fat most commonly used by the rural poor. Therefore, INIAP and others testing different varieties of lupine bean and promoting its cultivation in the rural highlands may continue to do so focusing on its production properties, without concern for undesirable fatty acid composition – all seem similar and all would be beneficial to the diet of the rural poor. The minimum observed levels in the debittered samples provide 15 and 16% of the “adequate intake” levels (Institute of Medicine 2000) of omega-3 and omega-6 fatty acids for children 4-8 years old, respectively, per 100 grams.

The literature indicates that agriculture interventions which are broad-based and invest in multiple types of capital (physical, social, human (including nutrition education), environmental and financial capital) are more likely to lead to positive changes in the health and nutrition of participating households than agriculture interventions that invest more narrowly (Berti, Krasevec et al. 2004; The World Bank 2007). This intervention invested in physical (seed system development), social (through use of participatory research methods), human (agriculture and nutrition education), and environmental (lupine bean is a green manure which improves soil quality) capital. Consistent with the literature, this broad-based intervention led to a positive nutrition outcome. Lupine bean consumption increased during the study period, both in terms of the number of households consuming and the frequency with which they consume lupine, resulting in a higher per capita intake then the control communities (see Figure 1).

The Adequate Intake levels (AI) for ω-6 and ω-3 fatty acids, the percent intake covered by average daily intake of 67g/d of lupine, and the daily intake required to meet 100% of ω-6 and ω-3 requirements is shown in Table 4. While meeting 8 to 17% of the requirements is encouraging and important, there are few other sources of ω-6 and ω-3 fatty acids in the rural diet, and the levels of deficiency are likely high. Palm-oil based vegetable lard is the main fat added to the rural diet, and it would provide very little ω-6 and ω-3 fatty acids. Many of the leading causes of death in Ecuador (cerebrovascular and cardiovascular disease, hypertension *(Instituto Nacional de Estadísticas y Censos de Ecuador 1997)*) as well as impaired neurocognitive development, impaired immune function, depression, and other adverse health outcomes (Prentice and Paul 2000; Abeywardena 2003; Smit, Muskiet et al. 2004; Su 2008; van der Beek and Kamphuis 2008; Colangelo, He et al. 2009; Eriksson, Mellstrom et al. 2009; Fonolla, Lopez-Huertas et al. 2009; Rees, Austin et al. 2009; Riediger, Othman et al. 2009; Russo 2009) may in part be caused by essential fatty acid deficiencies. While there is still scope for increasing lupine consumption in the rural poor – perhaps an average of 200 grams per day is a reasonable target – it is unlikely that consumption would ever be as high as 500 g in children and 900 g in adults, as would be required to cover all the n-6 and n-3 fatty acid requirements. Other sources of healthy fats are needed in order to increase consumption. Lupine bean can be industrially processed into a liquid fat form or other processed foods (Villacres, Rubio et al. 2008) that retain their healthy dietary fat profile and that could be added during cooking to help meet dietary fat needs. While there are large changes needed in order to reduce malnutrition and to improve the quality of life in Saquisilí and throughout rural Ecuador, increased lupine bean production and consumption can serve as a platform upon which these changes can be made.

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