Physico-Chemical Properties of Immature Pods of Japanese Soybean Cultivars

Article *in* International Journal of Food Properties · April 2006

DOI: 10.1080/10942910500471727

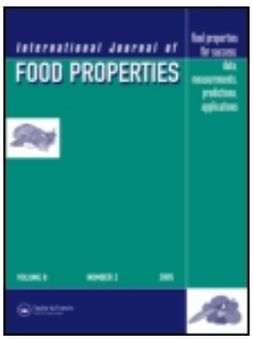
4 authors, including:

Vineet Kumar
Directorate of Soybean Research
152 PUBLICATIONS **543** CITATIONS

SEE PROFILE

Anita Rani
Indian Council of Agricultural Research
165 PUBLICATIONS **910** CITATIONS

SEE PROFILE



International Journal of Food Properties

ISSN: 1094-2912 (Print) 1532-2386 (Online) Journal homepage: https://www.tandfonline.com/loi/ljfp20

Physico-Chemical Properties of Immature Pods of Japanese Soybean Cultivars

Vineet Kumar , Anita Rani , S. D. Billore & G. S. Chauhan

To cite this article: Vineet Kumar , Anita Rani , S. D. Billore & G. S. Chauhan (2006) Physico-Chemical Properties of Immature Pods of Japanese Soybean Cultivars, International Journal of Food Properties, 9:1, 51-59, DOI: 10.1080/10942910500471727

To link to this article: https://doi.org/10.1080/10942910500471727

International Journal of Food Properties, 9: 51–59, 2006

ISSN: 1094-2912 print / 1532-2386 online
DOI: 10.1080/10942910500471727

Taylor & Francis
Taylor & Francis Group

PHYSICO-CHEMICAL PROPERTIES OF IMMATURE PODS OF JAPANESE SOYBEAN CULTIVARS

Vineet Kumar, Anita Rani, S. D. Billore, and G. S. Chauhan
National Research Centre for Soybean, Khandwa Road, Indore, M.P., India

Twelve Japanese cultivars and JS335, the most popular soybean cultivar in India, were grown in the field. Days to arrival of R6 stage (when pods are still green, immature, and tight with fully developed immature green seeds) of these cultivars were recorded. Pods picked at this stage were evaluated for pod yield per plant, pod characteristics (width, presence of hairs) fresh green seed weight and percent moisture content. Fresh green seeds were analyzed for compositional traits viz. protein content, trypsin inhibitor lipoxygenase isozymes, oil content, and polyunsaturated fatty acids (essential fatty acids). Japanese cultivars showed higher fresh green seed weight and pod yield than JS335. On a fresh weight basis, Japanese cultivars exhibited lower protein content but higher oil content than JS335. Most of the Japanese cultivars showed lower trypsin inhibitor content and a varying level of lipoxygenase-I, as well as lipoxygenase-II + III when compared to JS335. Total polyunsaturated fatty acids content in JS335 was higher than some of the Japanese cultivars. The number of pods per plant showed a positive correlation ($r = 0.863$, $p < 0.001$) with pod yield per plant. Some of the Japanese cultivars offer great potential for consumption at the green pod stage or as a source for desirable traits.

Keywords: ***Soybean, Immature pods, Pod yield, Green seed weight, Oil content, Protein content, Lipoxygenase isozymes, Trypsin inhibitor, Polyunsaturated fatty acids.***

INTRODUCTION

Soybean as a "functional food" that reduces the risk of range of hazardous diseases like atherosclerosis, osteoporosis, various types of cancer (breast, uterus cancer, and prostrate)[1,2] has attracted people's attention across the globe. People in India are becoming increasingly aware about the health benefits of consuming soy food. Besides, soybeans being rich in basic nutrients, they can combat diseases arising from malnutrition. However, Indians are not accustomed to soy based preparations, such as soy milk, tofu, and tempeh, as their tastes and flavors do not suit the Indian palate. Soy chunks available in markets are not popular because of their unacceptable flavor and their resemblance to animal protein, which distracts the vegetarian populace. Hence, direct utilization of soybean in food use is less than 1% of the total production (7.0 million tons).

The green seeds shelled from the immature pods of soybeans bear great potential for human consumption in India, like the green pods of other legumes. Immature soybean

Received 13 March 2005; accepted 12 June 2005.
Address correspondence to Dr. Vineet Kumar, Scientist Sr. scale, National Research Centre for Soybean, (ICAR) Khandwa Road, Indore, MP, India 452 017; E-mail: vineetksahni@yahoo.com

pods are soft in texture and can be cooked just like sweet peas (*Pisum sativum*), chickpeas (*Cicer arietinum* L), or lima beans (*Phaseolus limensis* L); their green seeds can be added to stews and soups, boiled in salt water, or roasted like peanut seeds. Furthermore, no other vegetable crop can match the nutritional value of the immature pods of soybeans.[3] Compared to the green seeds of other legumes, green seeds of immature soybean pods are rich in vitamins (B_1, B_2), minerals (iron, calcium, phosphorus), and protein content.[3,4] More importantly, lipoxygenase isozyme, the main culprit responsible for the off-flavor associated with other soy products, is found to be low in the immature soybean pods. Three isozymic forms of lipoxygenase namely, Lox-I, Lox-II, and Lox-III catalyse the hydroperoxidation of polyunsaturated fatty acids (PUFA) containing *cis cis 1,4 pentadiene* moiety leading to the formation of volatile hexanal compounds that contribute to the development of the beany flavor.[5] Mature soybean seeds suffer from the presence of another " . . . undesirable compound—a protease inhibitor [trypsin inhibitor (TI)] that reduces the digestibility of proteins . . ." by inhibiting tryptic activity. This, however, is considered to be low in fresh immature pods. Though trypsin inhibitor is heat labile, the heat treatment insolubilizes the much valued proteins[6] and causes a loss of essential amino acids in soy proteins.[7] With regard to desirable characteristics for consumption at the green pod stage, an early arrival of the R6 stage (suitable picking stage), higher pod yield, more pod width, pods with out hairs, and a large seed size at the picking stage are important physical parameters that determine both the consumer and farmers' acceptability of the cultivar.[8,9]

Consumption of the green seeds of immature pods has been in vogue since early history in far eastern countries. They are known as *edamame* in Japan, as *mao dou* in China, as *poot kong* in Korea, and lately as the beer bean in US. Some soybean cultivars for food uses were procured recently from Japan under the Indo-JICA (Japanese International Cooperative Agency) program on soy-based food and crop improvement. Globally, there are few reports on the physico-chemical properties of immature soybean pods.[10,11,4] It was pertinent to the study to grow these exotic soybean cultivars along with the most popular soybean varieties of India with the purpose to evaluate them at the green pod consumption stage in terms of arrival of the picking stage, 100-seed weight, pod yield, pod characteristics, protein, lipoxygenase isozymes, trypsin inhibitor, and polyunsaturated fatty acids.

MATERIAL AND METHODS

Seeds of twelve Japanese cultivars—Akiyoshi, Akishengoku Boiling-type (commercial seeds available in Japan), Enerei, Fukuyutaka, Hyuuga early, Hougyoku early, Hakucho early, Hatsataka, Kegone, Sappori Midori and Toyoshirome procured from Japanese International Co-operative Agency (JICA) and JS335, the most popular soybean variety of India—were grown in random block designs with three replications at the National Research Centre for Soybean, Indore. Each plot consisted of three rows of 3 m long rows planted 45 cm apart. Plant to plant distance was maintained at 15 cm in such a way that there was an average number of fifteen plants per square meter. The soil of the experimental plot belonged to a typical chromburst—very deep, dark-greyish, brown, clayey, and with low levels of organic carbon (0.45%); it was slightly alkaline pH (7.8). The seeds were grown in rain-fed conditions with an average rainfall of about 1000 mm in normal cropping season; recommended agronomic practices were followed. Pods of all the cultivars were picked when they were completely filled but still green in color, as

described by Shunmughamsundaram et al.[8] Pod width was measured using a vernier calliper, and the presence of hair on pods was recorded visually. Green seeds from the picked immature pods were shelled and observations for 100 fresh green seed weight and moisture content were taken. Subsequently, the green seeds of each cultivar were analyzed for protein content, trypsin inhibitor and lipoxygenase isozymes' activity, oil content, and the fatty acid composition.

Protein Content

Protein content was determined in triplicate according to the method as described in AOAC.[12] A conversion factor of 5.71 was applied to determine the protein content from nitrogen.

Extraction and Estimation of Lipoxygenases

Lipoxygenase isozymes were extracted from fresh green pods with 10 volumes of phosphate buffer (0.2 M, pH 6.8) in a microtissue homogenizer for 20 minutes at 0–4°C using the method of Suda et al.[13] The homogenized solution obtained was centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant so obtained was used as the crude extract for assaying the lipoxygense isozymes following the standard method (Axelrod et al.).[5] The reaction mixture for lipoxygenase-I consisted of a crude extract as an enzyme source, boric acid borax buffer (0.2 M, pH 9.0), and 10 mM sodium linoleate as a substrate. Lipoxygenase- II and III were analyzed collectively with the reaction mixture consisting of crude extract as enzyme source, phosphate buffer (0.2 M, pH 6.8), and 10 mM sodium linoleate as a substrate. The change in absorbance, due to conjugate diene in the enzymatic hydroperoxidation, was recorded with a Shimadzu UV-160 spectrophotometer at 234 nm. One unit of enzyme was taken as equivalent to the amount of enzyme that generated a change in absorbance of 1.0 per min at 234 nm. Values given in Table 1 are mean of triplicate samples.

Extraction and Estimation of Trypsin Inhibitor

Trypsin inhibitor activity was determined in triplicate by using the standard method of Kakade et al.,[14] as modified by Hammerstrand et al.[15] One gram of fresh green seeds was extracted with 50 ml NaOH (.01 N) for 4 hours with constant stirring at 125 rpm in an orbital shaker to keep the samples in suspension. The suspension obtained was appropriately diluted so that 2 ml of the sample extract inhibited 40–60% of the trypsin used as a standard in the analysis. Of the five test tubes taken, 2 ml aliquots of the diluted sample were added to the four test tubes. A fifth test tube was prepared for the trypsin standard by adding 2 ml of distilled water. To three of the four test tubes containing the sample extract, 2 ml of the trypsin solution (prepared by dissolving .004 g of the trypsin in 200 ml of .001 N HCl) was added and were maintained at a constant temperature water bath 37°C for 10 minutes. Five milliliters of benzoyl DL arginine para nitroanilide hydrochloride (prepared by dissolving .08 gm of benzoyl DL arginine paranitroanilide hydrochloride in 2 ml of dimethyl sulfoxide and diluted to 200 ml with 50 mM tris buffer of pH 8.2 containing 20 mM calcium chloride and the contents were warmed to 37°C) was rapidly added into each tube. The contents were stirred immediately on a vortex mixture, and the tubes were placed in a water bath at 37°C. The reaction was terminated after exactly 10 minutes by

Table 1 Percent protein content, lipoxygenase isozymes level, and trypsin inhibitor content of Japanese soybean cultivars and JS335 (Indian cultivar) in fresh green seeds at the R6 stage

Genotype	% Protein	Lipoxygenases (units/g fresh green seeds)[A]		TI mg/g fresh green seeds
		Lox-I	Lox-II + III	
Akiyoshi	9.17^{de} (32.75)	148.8^{c} (531.4)	24.0^{efgh} (85.71)	16.8^{ghif} (60.0)
Akishengoku	9.55^{de} (31.41)	98.8^{ghij} (325)	24.6^{efghi}(80.92)	18.5^{ghi} (60.86)
Boiling type	11.66^{ab} (33.51)	100.5^{gh} (288.79)	18.6^{j} (53.45)	22.3^{def} (64.08)
Enerei	9.12^{dfg} (31.45)	99.0^{ghi}(341.38)	26.8^{df} (95.71)	5.6^{k} (19.31)
Fukuyutaka	8.42^{efh} (30.40)	121.5^{ef} (438.67)	21.0^{h} (75.81)	24.0^{d} (86.64)
Hyuuga early	9.02^{d} (29.67)	126.6^{de} (416.45)	41.0^{a} (134.87)	15.5^{hij} (50.99)
Hougyoku early	11.54^{abc} (32.69)	163.7^{ab} (463.74)	30.9^{cd} (87.54)	24.6^{ab} (69.69)
Hakucho early	9.25^{def} (27.86)	174.4^{a} (525.30)	28.4^{de} (85.54)	22.8^{abc}(68.67)
Hatsataka	6.65^{i} (26.39)	134.8^{d} (534.92)	31.0^{c} (123.02)	25.5^{a} (101.19)
Kegone	7.25^{hi} (30.33)	90.0^{hijk} (376.5)	35.6^{b} (148.95)	16.9^{hij} (70.71)
Sapori Midori	8.44^{efh} (26.88)	110.8^{g} (352.87)	25.8^{efg} (82.17)	18.6^{gh} (59.24)
Toyoshirome	9.79^{d} (32.10)	73.8^{l} (241.97)	23.4^{fgh} (76.72)	16.0^{ghij} (52.46)
JS335	12.5^{a} (38.46)	135.0^{d} (415.38)	24.0^{efghj}(73.85)	24.5^{abc} (75.38)

[A]One unit is the change in OD 1.0 per min at 234 nm. Values given are mean in determination in triplicate samples (values in parenthesis are on dry weight basis).

[a-l]Means superscripted with different alphabets in the same column differ significantly ($p < 0.05$).

the rapid addition of 1 ml of 30% acetic acid. The fourth tube containing sample extract (sample blank) was prepared by the same procedure except that the trypsin solution was added after the reaction was terminated by the addition of 30% glacial acetic acid. The absorbance of each solution was determined at 410 nm against the sample blank. Values obtained from each of the two sample extracts were subtracted from the trypsin standard. These values were averaged and the trypsin content was determined as follows:

$$\text{TI mg/g} = \frac{\text{Differential Absorbance} \times \text{dilution factor}}{.019 \times 1000}$$

$$\text{Percent Inhibition} = \frac{100 \times \text{differential absorbance}}{\text{Absorbance of the standard}}$$

Oil Content

Immature seeds picked at the R6 stage were oven dried and were ground. The ground material (flour) so obtained was then extracted with petroleum ether (b.p. 60–80°C) in Soxhlet unit for 7 hours. Percent oil content was calculated by gravimetric method.

Analysis for Fatty Acid Composition

Oil obtained above was transesterified in methanol with 1 N sodium methoxide as a catalyst using the method of Luddy et al..[16] Fatty acid methyl esters (FAMEs) were separated and analyzed in a gas chromatograph (GLC), Shimadzu GC 17A, using

Table 2 Percent oil (on fresh weight basis) and fatty acid composition of fresh green seeds of Japanese soybean genotypes and JS335 at the R6 stage

		Fatty acid composition (kg/100 kg oil)				
Genotype	Oil kg/100 kg seed	C16:0	C18:0	C18:1	C18:2	C18:3
Akiyoshi	4.90^{bcd} (17.50)	12.4^{cd}	3.04^{c}	25.74^{f}	52.3^{bcd}	6.51^{f}
Akishengoku	5.11^{ac} (16.81)	10.9^{f}	2.42^{g}	41.43^{b}	38.0^{ij}	6.83^{f}
Boiling type	4.78^{cdef} (13.74)	12.9^{bc}	2.78^{df}	30.45^{d}	46.32^{g}	7.78^{cde}
Enerei	4.29^{ghi} (14.79)	8.8^{g}	2.76^{df}	47.71^{a}	30.83^{k}	8.84^{ab}
Fukuyutaka	3.73^{jk} (13.47)	10.9^{f}	2.76^{df}	39.73^{c}	43.21^{gh}	8.96^{a}
Hyuuga early	4.50^{dfg}(14.80)	11.6^{e}	2.84^{ce}	23.76^{g}	53.0^{bc}	8.27^{bcd}
Hougyoku early	4.82^{bcde} (13.65)	12.6	3.82^{a}	18.4^{i}	56.44^{a}	7.86^{cd}
Hakucho early	5.46^{a} (16.45)	13.51^{ab}	3.64^{a}	28.42^{e}	49.65^{df}	7.94^{cde}
Hatsataka	3.83^{j} (15.70)	12.6^{cd}	2.78^{d}	22.03^{h}	54.32^{ab}	8.38^{abc}
Kegone	3.30^{k} (13.81)	12.5^{cd}	2.62^{efg}	22.47^{h}	53.74^{a}	7.80^{cde}
Sappori Midori	5.24^{ab} (16.69)	10.46^{f}	3.35^{b}	41.23^{b}	39.64^{i}	7.13^{f}
Toyoshirome	4.44^{efgh} (14.56)	13.7^{a}	3.32^{b}	25.1^{f}	50.71^{cde}	6.92^{f}
JS335	3.66^{jk} (11.26)	11.0^{ef}	3.0^{cd}	25.6^{f}	53.4^{ab}	7.0^{f}

Values are mean of determination in triplicate samples (values in parenthesis are on dry weight basis).
$^{a–k}$Means superscripted with different alphabets in the same column differ significantly ($p < 0.05$).

a polyethylene glycol packed SGE BP20 capillary column, with a length and diameter of 30 meters and 0.32 millimeters, respectively. Oven temperature of the gas chromatograph was programmed at 140°C for 3.6 minutes, subsequently increased to 170°C at the rate of 13.5°C per minute, and maintained for 3.8 minutes and finally increased to 182°C at the rate of 5°C per minute for the best resolution of methyl esters. The temperatures of flame ionization detector (FID) and injector were maintained at 240°C. Nitrogen, the carrier gas used, was maintained at a flow rate of 15 ml/min. Peaks for individual fatty acid methyl esters were identified by comparing their retention times with those of standard methyl esters (procured from Sigma-Aldrich, India). Data given in Table 2 for different fatty acids are means of triplicate samples.

RESULTS AND DISCUSSION

Quality properties such as color, texture, and the 100 seed weight of vegetable soybeans are a function of development time.[9] Since these quality parameters do not peak at the same time, it is necessary to compromise with the time of harvest. Shanmughamsundaram et al.[8] reported that the optimum time for harvesting green beans was when the pods are still green, immature, and tight with fully developed immature green seeds. This stage coincides with the R6 stage of soybean development as described by Fehr et al.[17]. Soy based preparations made from mature seeds (e.g., *tofu* and soy milk) are not popular in India due to off-flavor associated with these products, while immature pods can be eaten much like that green pods of other legumes. Hence, in the present study, green pods of all the cultivars were harvested at the R6 stage.

Early arrival of the R6 stage suitable for picking is an important criterion for selecting genotypes for green pod consumption, as this allows the farmer to take more than one crop in a year. Table 3 reveals significant genotypic differences for days to achieve R6 stage when the green pods were suitable for harvesting. The average number of days from

Table 3 Arrival of the R6 stage, fresh green seed weight, moisture percentage, number of pods per plant, pod yield per plant, and pod characteristics of Japanese soybean cultivars and JS335 (Indian cultivar)

Genotype	Arrival of R_6 stage for consumption at green pod stage Days after sowing (DOS)	Green seed fresh weight 100 SW (g)	Moisture %	No of Pods/ plant	Pod Yield/ plant (g)	Pod Characteristics Pod width (mm)
Akiyoshi	77	35.8	72.0	80.6	71.1	8.0, Hairy
Akishengoku	89	30.2	69.6	74.0	70.5	8.7, Hairy
Boiling type	72	35.6	65.2	70.0	75.6	12.2, Hairy
Enerei	60	45.0	71.0	40.1	69.3	12.8, Hairy
Fukuyutaka	93	63.6	72.3	87.0	82.0	12.0, Hairy
Hyuuga early	65	38.0	69.6	80.0	82.6	11.8, Hairy
Hougyoku early	92	39.5	64.7	101.0	140.0	11.6, Hairy
Hakucho early	62	38.0	66.8	72.0	62.6	11.0, Hairy
Hatsataka	72	55.0	74.8	25.0	46.0	12.4, Hairy
Kegone	62	53.2	76.1	36.0	54.4	12.0, Hairy
Sappori Midori	60	55.8	68.6	65.0	56.9	12.2, Hairy
Toyoshirome	77	45.3	69.5	104.0	128.0	9.1, Hairy
JS335	85	27.0	67.5	45.7	46.6	7.6, Glabrous

Values given are mean of triplicate observations.

planting to the R6 stage ranged from 60 for cultivar Sappori Midori and Enerei to 93 days for cultivar Fukuyutaka. In JS335 (Indian cultivar), the R6 stage arrived in 85 days. With regard to pod characteristics, green pods from most of Japanese cultivars have greater width than JS335. Hairless pods are preferred by consumers, especially when the green seeds of immature pods are to be popped directly into the mouth after being boiled in salted water. None of the Japanese cultivars were found to be without hair; however, the pods of JS335 were glabrous. The fresh weight of green seeds was sand to be an important parameter to determine consumer acceptability.[8,9] Generally, a large seed size, at the picking stage, attracts the consumers. Japanese cultivars differed in 100 seed weight and pod yield per plant at the picking stage (R6). At this stage, fresh green seed weight (100 green seed weight) ranged from 30.2 g for Akishengoku to 63.6 g for Fukuyutaka with an average value of 44.5 g per 100 seeds. The fresh weight of green seeds for cultivar Fukuyutaka was two times and about 2.4 times greater than Akishengoku and JS35, respectively. Japanese cultivars exhibited average pod yield/plant of 78.2 g ranging from 46 g for Hatsataka to 140 g for Hougyoku. JS335 cultivar showed 100 green seed weight of 27.0 g and a pod yield of 46.6 g/plant at green pod consumption stage. Moisture content of immature pods is a critical factor that affects organoleptic value.[9] The immature pods of Japanese cultivars used in the analyses showed significant differences for percent moisture content. At the picking stage, Japanese cultivars exhibited a mean percentage moisture content of 70.01, ranging from 64.7% for Hougyoku early to 76.1% for Kegone. JS335 showed a moisture content of 67.5%. Rao et al.,[11] however, reported a moisture content of 55.5% at the R6 stage, which is much lower than the one observed in our study, while an equivalent value (68.52 %) for moisture content, as observed in our study, has been reported by Mohammed et al.[10]

Table 1 exhibits percent protein content, lipoxygenase isozymes, and trypsin inhibitors among Japanese cultivars and JS335 on both a fresh and dry-weight basis.

Mean percent protein content on fresh weight basis among Japanese genotypes at picking stage was 9.17, ranging from 6.65 for Hatsataka to 11.66 for Boiling-type. Percent protein content in JS335 was observed to be 12.5%, which is higher than all of the Japanese cultivars. Furthermore, barring few cultivars, the values of the protein content observed in our studies are much higher when compared with 7.6% for lima beans (*Phaseolous limensis* L) and 5.4% for pea (*Pisum sativum* L) as reported earlier.[18] A slightly lesser mean percent protein (8.4) has been reported in immature pods (the R7 stage) in selected Chinese soybean genotypes[10] than the mean value of protein observed in our study. In another study, higher protein content (16.22%) at R6 stage has been reported,[11] which may be attributed to the much lower moisture content at the R6 stage reported in the study.

With regard to the trypsin inhibitor content (expressed as mg/g of fresh green seeds), among Japanese cultivars, it ranged from 5.6 for cultivar Enerie to 25.5 for Hatsataka with a mean value of 15.85 while it was found to be 24.5 for JS335. Hence, barring Boiling-type, Fukuyutaka, Hougyoku early, Hakucho early, and Hatsataka, all remaining varieties showed trypsin inhibitor values lower than that of JS335.

Though, lipoxygenase activity in immature green seeds of selected Chinese genotypes has been reported in an earlier study[10], however, activities of two categories of lipoxygenase isozymes were not separately given. Class I is characterized by a high pH optima of approximately 9.0 and formation of large amounts of 13-hydroperoxides such as lipoxygenase-I (Lox-I), while Class II designates a pH optima of around 7.0, such as lipoxygenase-II (Lox-II) and lipoxygenase-III (Lox-III). Resultant products from the reaction catalyzed from Class II category lipoxygenase isozymes are more offensive to consumers. Activities of both the classes of lipoxygenase isozymes given in Table 1 are expressed as units/g fresh green seeds. Among Japanese cultivars, at the R6 stage when soybean pods were picked, Lox-I activity ranged from 73.8 for Toyoshirome to 174.7 for Hakucho early with a mean value of 120.22, while Lox-II + III activity ranged from 18.6 for Boiling-type to 41.0 for Hyuuga early with a mean value of 27.59. JS335 exhibited values of 135.0 and 24.0 for Lox-I and Lox II + III respectively. Lox-I activity in all the cultivars, both Japanese as well as the Indian cultivar, was found to be higher than Lox-II + III activity. Barring Akiyoshi, Hougyoku early and Hakucho early, all other Japanese cultivars showed Lox-I activity lower or equivalent to the Lox-I activity observed in JS335. With regard to the Lox-II + III activity, Hyuuga, Hougyoku early, Hakucho early, Hatsataka, and Kegone showed higher values than JS335.

Percent oil content (fresh weight basis) observed in the immature pods of different cultivars within the study were expressed as kg/100kg seed in Table 2. Japanese cultivars exhibited a mean value of 4.53 for oil content, which ranged from 3.30 for cultivar Kegone to 5.46 for cultivar Hakucho early. Oil content in immature seeds of JS335 was found to be 3.66kg/100kg seeds, i.e. of the twelve Japanese cultivars, nine cultivars showed higher values of oil content than JS335. A slightly higher value (6.01kg/100kg seed, 6.14kg/100kg seed) of mean oil content in immature pods at the R6 stage in comparison to the oil content of Japanese cultivars observed in our study was reported earlier.[10,11] Table 2 also exhibits the fatty acid composition of green seeds shelled from the immature pods. All the cultivars at the picking stage showed five major fatty acids: palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3), which is in consonance with an earlier study.[19] Linoleic and linolenic acid containing more than two double bonds are polyunsaturated fatty acid; these are essential fatty acids and are required for normal human growth. Several reports

indicate the importance of the PUFAs in reducing cholesterol levels in human blood and thereby reducing the risk of heart diseases.[20] Fatty acids were expressed as kg/100kg seeds. Japanese cultivars exhibited an average value of 55.88 kg/100kg seeds for total polyunsaturated fatty acids (linoleic and linolenic acid), which ranged from 44.84 for cultivar Akishengoku to 64.30 for cultivar Hougyoku early, while JS335 exhibited a level of 60.4 kg/100kg seeds which is higher than some of the Japanese cultivars (Akishengoku, Boiling–type, Enerie, Fukuyutaka, Hakucho early, and Sappori Midori).

Higher protein content and relatively low fat content were observed in the green seeds of Japanese cultivars, as well as the Indian cultivar. These seeds would be an attractive snack for health conscious people following low fat and high protein diets. Our studies showed that in all the genotypes, Lox-II + III activity was much higher than Lox-I activity both on a fresh as well as a dry weight basis. With regard to trypsin inhibitor activity in green seeds picked at the R6 stage, on the fresh weight basis, all the Japanese cultivars, as well as JS335, the Indian cultivar, showed very low level of trypsin inhibitor activity. However, on a dry weight basis these values appeared to be high. Hence, it is because of the presence of a very high moisture content that the trypsin inhibitor content appeared low. Therefore, the immature pods need to be boiled to inactivate the heat labile trypsin inhibitor prior to consumption.

Number of pods per plant and pod number were found to be highly significant ($p < .001$), which is in consonance with the observation recorded in an earlier study by Rao et al..[11] All biochemical constituents at the R6 stage, however, with regard to the fresh as well as the dry weight basis, were found to be independent (no correlation was observed between the two factors). This is in contrast to an earlier study,[10] wherein a negative correlation between oil (on a fresh weight basis) and moisture content has been reported. The absence of a correlation observed between oil and protein content in our study is in consonance with Mohammed et al.,[10] but it is in contrast to Rao et al.[11] who observed significant a negative correlation between oil and protein content ($p < .001$).

CONCLUSIONS

At the picking stage (R6 stage), higher fresh green seed size and pod yield per plant was observed in Japanese cultivars than JS335 with a varying level of lipoxygenases and trypsin inhibitor content. Oil content in immature green seeds of JS335 was observed to be lower than Japanese cultivars, while a higher level of total polyunsaturated fatty acids was observed in JS335. Though, no Japanese cultivar was found to bear pods without hairs—unlike the Indian cultivar. Crossing programs may be initiated involving JS335 and those Japanese cultivars that exhibited not only a higher pod yield, higher seed weight, early arrival of the R6 stage, and a comparatively higher protein and polyunsaturated fatty acid; they also possess low trypsin inhibitor and lipoxygenase isozymes' activities.

ACKNOWLEDGMENT

The authors thankfully acknowledge the Japanese International Co-operative Agency for providing the seeds of Japanese cultivars under the Indo-JICA program (April 2003—December 2003) on "Soy-based food and crop improvement."

REFERENCES

1. Messina, M.J. Soya foods: their role in disease prevention and treatment. In *Soybean Chemistry, Technology and Utilization*; Liu, K.; Ed.; Chapman and Hall: New York, 1997, 443–447.
2. Messina, M. *Overview of research on the relationship between soya intake and potential mechanism by which soya affects breast cancer risk*. Proceedings of VII World Soybean Conference, Brazil, 2004; 661–673.
3. Shanmughamsudaram, S.; Marr, M.R. *Global expansion of high value vegetable soybean*. Proceedings IV International Soybean Processing and Utilisation, Brazil, 2004; 915–920.
4. Chadha, M.L.; Oluch, M.O. V*egetable soybean research and development in Africa*. Proceedings of VII World Soybean Research Conference, Foz du Igaussu 2004; 921–928.
5. Axelrod, B.; Cheesebrough, T.M.; Laakso, S. Lipoxygenases in soybeans. Methods in Enzymology. **1981**, *71*, 441–451.
6. Anderson, R.L. Effect of heating on soybean proteins and trypsin inhibitors. Journal of American Oil Chemists' Society. **1992**, *69*, 1170–1176.
7. Rios-Iriarte, B.J.; Barnes, R.H. The effect of overheating on certain chemical properties of the proteins of soybean. Food Technology. **1996**, *32*, 836–839.
8. Shanmughamsundaram, S.S.; Cheng, S.T.; Huang, M.T.; Yan, M.R. Varietal improvement of vegetable soybean in Taiwan. In *Vegetable soybean: research needs for production and quality improvement*; Shanmugasundarm, S.; Ed.; Asian Vegetable Research and Development Centre: Taiwan, 1991, 30–42.
9. Mbuvi, S.W.; Litchfield, J.B. Green soybeans as vegetable: Comparing green soybeans with green peas and lima beans; and maximized harvest time determinations using mathematical modeling. Journal of Vegetable Crop Production. **1995**, *1*, 99–121.
10. Mohammed, A.I.; Rangappa, M.; Bhardwaj, H.; Pearson, N. Evaluation of immature green Chinese vegetable soybean for nutrient and antinutritional factors. Soybean Genetics Newsletter. **1992**, *19*, 132–134.
11. Rao, M.S.S.; Bhagsari, A.S.; Mohammed, A.I. Fresh green seed yield and nutritional traits of vegetable soybean genotypes. Crop Science. **2001**, *42*, 1950–1958.
12. Association of Official Analytical Chemists. *Official Methods of Analysis*, 13th Ed.; AOAC: Washington, DC, 1980.
13. Suda, I.; Hajika, M.; Nashiba, Y.; Furuta, S.; Igita, K. Simple and rapid method for the selective detection of individual lipoxygenase enzymes in soybean seeds. Journal of Agricultural and Food Chemistry. **1995**, *43*, 742–747.
14. Kakade, M.L.; Rackis, J.J.; McGee, J.E.; Pushki, G. Determination of trypsin inhibitor activity of soy products: a collaborative analysis of an improved procedure. Cereal Chemistry. **1974**, *51* (3), 376–382.
15. Hammerstrand, G.E.; Black, L.T.; Glover, J.D. Trypsin inhibitor in soy products: Modification of standard analytical procedures. Cereal Chemistry. **1981**, *15*, 215–218.
16. Luddy, F.E.; Barford, R.A.; Herb, S.E.; Magidman, P. A rapid and quantitative Procedure for the preparation of methyl esters of butter oil and other fats. Journal of American Oil Chemists' Society. **1968**, *15*, 549–552.
17. Fehr, W.R.; Caviness, C.E.; Burmood, D.T.; Penington, J.S. Development description of soybean, *Glycine max* (L) Mer. Crop Science. **1971**, *11*, 929–931.
18. Anonymous Intsoy reseach focuses on green soybean as commercial vegetable. Intsoy Newsletter. **1987**, *37*, 1–2.
19. Mebrahtu, T.; Mohammed, A.; Rangappa, M. Evaluation of vegetable soybean genotypes for fatty acid composition. Soybean Genetics Newsletter. **1990**, *17*, 119–121.
20. Lands, W.E. Renewed questions about polyunsaturated fatty acids. Nutritional Review. **1986**, *44*, 189–195.