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Title Physical, Chemical and Biological Investigations of the Barcelona and the Du Chilly Filbert Nuts.

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A number of quantitative analyses of the shells and the kernels of Barcelona and Du Chilly varieties have been determined.

The physical and chemical constants of Barcelona and Du Chilly filbert nut oils were determined and compared with other vegetable oils.

The fatty acid components of these oils obtained by expression were established by fractional distillation in vacuum of the methyl esters after a primary separation into solid and liquide acids fractions by Twitchell's method.

The sitosterols from both oils were isolated from the unsaponifiable fraction and identified as similar to those obtained from other vegetable oils.

The influence of storage condition with respect to the chemical and physical changes in Barcelona and Du Chilly oils have been studied. In general, Barcelona oil is more stable than Du Chilly oil. The oils obtained from kernels or whole nuts after one year of storage at 35°C showed no change of taste or flavor whatsoever whereas expressed oils which were stored under the same conditions developed rancidity.

The nutritive value of oil free Barcelona and Du Chilly filbert meals were evaluated by paired-feeding experiments with rats, using vitamin free casein as a reference. Experimental evidence indicated that filbert meal rations promoted growth of young rats but were not as good as the casein ration. It may be greatly improved when supplemented with lysine and slightly improved by supplementing with tryptophan, cystine or both. The rations prepared by using filbert globulin as a sole source of protein did not promoted growth of young rats. These results indicated that the rations were deficient in lysine and methionine.

Sixteen amino acids of these globulins were determined by microbiological assay. A sample of vitamin free casein was determined also and was compared to the literature values in order to judge the accuracy of these determinations. The biological results were confirmed by the microbiological evaluation.

PHYSICAL, CHEMICAL AND BIOLOGICAL INVESTIGATIONS OF THE BARCELONA AND THE DU CHILLY FILBERT NUTS.

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PHYSICAL, CHEMICAL AND BIOLOGICAL INVESTIGATIONS OF THE BARCELONA AND THE DU CHILLY FILBERT NUTS

I. The Filbert Nut.

1. Introduction

The filbert is among the oldest of cultivated plants and many references to it are found in the literature (30, 31, 38, 39). It was cultivated by the Romans and was called Abellinae after their native place, Abellina, supposed to be the valley of Damasus in Asia, from which is derived the specific name, avellana. The term hazel is applied to the native American species and varieties of Corylus, and is derived from the Anglo-Saxon haesel, a hood or bonnet. Filbert is used for all varieties of C. avellena.

Filbert culture is an important industry in parts of Turkey, Spain, and in Kent, England, where they grow to perfection. It has been grown more or less on the Pacific Coast since the region was settled, but only in recent years have large plantings been made. At present the acreage is expanding very rapidly in Oregon and Washington.

A cool moist climate is best for growing filberts. The trees are not particular as to soil types and will probably thrive on the average farm and garden soil.

The soil should be well drained since the filbert is no more tolerant of excessive soil moisture than other fruit trees. Winter injury is severe on a wet soil.

Filberts, like fruit trees, make better growth and produce heavier crops if the soil is fertile, although in overly rich soils growth is vigorous and production low. No exact rules for fertilizing filberts may be laid down, but in general nitrogenous fertilizers may be expected to increase growth.

2. Botany

The genus Corylus which comprises the filberts and hazels, belongs to the family of Betulaceae, which includes the birches, alders, hornbeams and hop-hornbeams. Corylus avellana is the only one cultivated extensively for its nuts. This species is characterized by having the length of the husks equal to or shorter than the nuts.

Two species are common in eastern North America, C. americana, the American hazel, and C. cornuta, (C. rostrata of Gray's "Manual of Botany" (15) and other authors) the horn or beaked hazel; C. californica is the Pacific Coast form of C. cornuta. Both species are bushes which sucker freely and are common along fence rows and waste land. C. cornuta, which is readily identified by

its long tubular bristly husk, is of no value for its nut. C. americana is more promising. The best varieties, however, are inferior to the European filberts.

C. chinensis, is the tree hazel of China and may attain a height of 120 feet. It is described by Rehder (32) as a "handsome tree with large leaves and spreading branches forming a broad oval head".

C. avellana, the European filbert is cultivated extensively for its nuts. Varieties of the species are numerous and are the basis of the rapidly expanding filbert industry in Oregon and Washington. The same varieties are also cultivated extensively in southern and central Europe. The chief varieties grown in this country at present are of European origin.

The filbert is monoecious, that is, the staminate and pistillate flowers are borne separately on the same plant similar to corn. The staminate, or male flowers are in the catkins and produce the pollen. The pistillate or female flowers are borne in small, scaly buds with the stigmas or pollen-receiving surfaces being visible during the flowering season only. They are reddish threadlike, and appear in very small, short bundles. The pistillate flowers usually appear a few days before the catkins begin shedding pollen.

It is now believed that practically all varieties of filbert are self unfruitful, hence, growers should provide for cross-pollination by including two or more varieties in all plantings.

It is much easier to transplant and establish than is the walnut. The planting operation for filberts is the same as for the other fruit trees. The roots should be protected from the time the plants are received until they are in the ground.

3. Varieties

Varieties of filberts are as distinct as varieties of tree fruits and each variety has its faults and merits.

A good variety is characterized by strong, vigorous, hardy, and productive tree of upright spreading growth habit. The catkins must be hardy and preferably abundant. The blooming season must coincide with that of the variety with which it is interplanted. The nut should be large, thinshelled, of good quality, well-filled, and should drop freely from the husk to facilitate havesting.

Barcelona is the commercial variety of the Pacific Northwest. Its merits are the large, vigorous, productive tree which has outyielded all the other

varieties nearly two to one on five-year-old trees. The nut is of good size, and attractive appearance. It was introduced in 1885 by Felix Gillellte, Nevada City, California, as "Grosse Blanche de Angleterre" and by him later renamed Barcelona.

Du Chilly, which is apparently identical with Kentish Cob, is grown extensively in Oregon and Washington as a standard variety and as a pollinizer for Barcelona. It was introduced into that region prior to 1887 also by Felix Gillellte. Its popularity is due to the vigorous, productive tree and the large handsome, thin-shelled, high quality nut. Its late blooming habit makes it an excellent variety to plant with Barcelona for pollinating.

4. Acreage and Production in the Northwest.

The production of filberts has been one of the most profitable and rapid growing farm enterprises in the Pacific Northwest (10, 48). As a whole, this development has been on a sound basis and most growers have made excellent profits from their orchards.

Filbert production in the United States is confined almost exclusively to Western Oregon and Washington. These two areas appear to possess climatic and other conditions especially favorable to filbert production. Satisfactory yields are usually obtained;

nuts of high quality are produced.

Prior to 1927 the commercial tonnage of filberts probably did not exceed 60 tons. Production increased very rapidly since 1937. The Oregon crop average for the five years 1943 to 1947 was 6,280 tons a year and of Washington's crop for those years was 964 tons (46).

The acreage of filbert orchards has been increased from 4,900 acres in 1930 to 16,710 acres in 1945. Data are not at hand for the past two years, but the combined Oregon and Washington acreage in bearing is probably about 20,000 acres with another 6,000 acres not yet producing. (see Table 1)

5. Purpose of this Research

Data on the composition of filbert oil are few and not in agreement (1, 35, 36). Numerous reports on its more common chemical and physical characteristics are available for European filbert nuts but those which have been obtained in the examination of domestic oils are indeed few. No investigations of the oils from Barcelona and Du Chilly filberts from the Parcific Northwest have been made.

There appears to be unanimity of opinion as to the presence of palmitic and stearic acids but no agreement with respect to the presence of arachidic acid.

Table 1

Filbert Acreage, Production and Amounts of Imports (46).

	Acreage			Production, tons			
Year	Oregon	Washington	Total	Oregon	Washington	Total	Import tons
1927				60	-	60	-
1928				200	-	200	-
1929	2000	290	2290	200	-	200	-
1930	2500	380	2880	300	-	300	7877
1931	3100	460	3560	380	40	420	7931
1932	3700	560	4260	400	90	490	5797
1933	4300	650	4950	930	140	1070	6570
1934	4800	740	5540	1000	210	1210	3525
1935	5600	760	6360	1100	140	1240	3544
1936	6300	790	7090	1850	250	2100	3993
1937	7000	1000	8000	2230	340	2570	4798
1938	7700	1250	8950	2060	380	2440	2278
1939	8500	1500	10000	3300	590	3890	2221
1940	9300	1650	10950	2700	510	3210	3492
1941	10000	1800	11800	4900	850	5850	1672
1942	10900	1950	12850	3600	670	4270	92
1943	11700	2100	13800	6200	830	7030	65
1944	12600	2250	14850	5600	920	6520	1174
1945	14400	2310	16710	4500	820	5320	8072
1946				7300	1150	8450	11089
1947				7800	1100	8900	12902

There is, also, no question as to the presence of oleic and linoleic acids, but the amounts of those acids present are not in agreement (36).

The chemical composition of hazel nut (C. americana) has been investigated by Wainios and Forles (47). The composition of shell has also been determined by Phillips and Goss (29) and Helpert and Kruger (17).

Its nutritive properties has been studied by Cajori (11), De Caro and Franceschini (13), Bevilotti (7), and Engels (14).

A protein, "Proto Acid" has been isolated from the oil free meal by extraction with dilute sodium hydroxide by Perov's method (12). However, for all of these investigations, European filbert nuts were used.

This study was undertaken in the hope that the data derived from the investigation may be of value in two ways. First, to gather accurate information on the chemical composition of Oregon-grown filberts and, second, to suggest the possible course in which research on utilization of probable filbert surpluses might most profitably be directed.

II. Chemical Investigation of Filbert Nuts

The problems of isolating and identifying the constituents of any plant are extremely complex. It is far more difficult to ascertain the organic constituents of a plant than its inorganic ones. Moreover, the plant chemist must determine whether the substances obtained were originally present in the plant or whether they are decomposition products arising from the action of heat, atmospheric oxidation, enzyme action or interaction of other substances. At the present time, many methods are available for use in plant studies, but the selection of a procedure depends largely upon the type of compound to be examined.

With full recognition of the rapid growth of the filbert industry in the Northwest and a probable surplus problem in the near future, the investigation was directed first toward the general study of the filbert nut to ascertain whether the filbert nut shell has some uses and if the nut meat could be used for a source of edible oil and the protein for human consumption or industrial uses. A quantitative analysis for substances shown in the following list has been made in this laboratory because no complete data are available on these two varieties.

A. Quantitative investigations.

1. Moisture
2. Total ash
    a. Hot water soluble ash
    b. Hot water insoluble ash
    c. Alkalinity of water soluble ash
    d. Alkalinity of water insoluble ash
3. Ether extract
4. Crude protein
5. Reducing sugars
6. Sucrose
7. Starch
8. Pentosans
9. Crude fiber.

B. Procedures - The samples used here were from the 1947 crop supplied by the Northwestern Nut Growers Association. The shells were ground into a powder with a Wiley mill. The nut kernels were ground in a meat grinder. The samples were well mixed and kept in screw cap bottles at room temperature for analysis.

Moisture was determined by drying a sample in a vacuum oven at 50° C. to constant weight.

The procedures outlined in the A. O. A. C. official methods (5) were followed for the determination of ash, ether extract, crude protein and carbohydrates.

The total nitrogen was determined by the Kjeldahl method. The crude protein was calculated by multiplying the nitrogen value by 6.25. The reducing sugars were determined by the Munsen and Walker method (5). Sucrose was calculated as the difference multiplied by 0.97 between reducing sugars and total invert sugar after hydrolysis with hydrochloric acid at room temperature. For determination of starch, the direct acid hydrolysis method was used in the case of nut kernel and the diastase method with subsequent acid hydrolysis was used with shell samples. The official phloroglucinol method was used for determination of pentosans (5). The crude fiber was determined according to the A. O. A. C. official method (5). The plant material was first extracted with ether, then digested with 1.25% sulfuric acid and 1.25% sodium hydroxide in succession. The difference in weight of residue and the ash after ignition was reported as crude fiber.

C. The analytical results were summerized in Tables 2 and 3.

The analytical data reveal a substantial nutritive value of both Barcelona and Du Chilly filbert nut kernels as judged by the crude protein, fat, sugars, starch and other carbohydrates present.

The shell of both varieties contains over 25

Table 2

Chemical Composition of Filbert Nut Shell

	% Natural Basis	
	Barcelona	Du Chilly
Moisture	7.08	7.81
Total ash	0.997	1.297
Hot water insoluble ash	0.416	0.645
Hot water soluble ash	0.581	0.652
Alkalinity of insoluble ash	0.87*	1.36*
Alkalinity of soluble ash	0.34*	0.92*
Crude protein, N x 6.25	1.35	1.70
Ether extract	nil	nil
Reducing sugars	0.98	1.15
Sucrose	0.20	0.75
Starch	nil	nil
Pentosans	27.0	25.3
Crude fiber	67.8	67.7

Table 3

Chemical Composition of Filbert Nut Kernel

	% Natural Basis	
	Barcelona	Du Chilly
Moisture	3.43	3.58
Total ash	2.53	2.69
Hot water insoluble ash	1.09	1.50
Hot water soluble ash	1.44	1.19
Alkalinity of insoluble ash	2.02*	1.90*
Alkalinity of soluble ash	0.78*	1.29*
Crude protein, N x 6.25	17.1	15.6
Ether extract	65.5	63.1
Reducing sugars	0.12	0.18
Sucrose	4.79	5.57
Starch	3.54	4.16
Crude fiber	2.09	1.94

* The alkalinity of ash was calculated and expressed in terms of number of ml. of normal acid per 100 grams moisture free sample.

per cent of pentosans. It may be a good source of materials for preparing pentose sugars.

III. The Filbert Oils

1. Introduction

The three most important classes of plant compounds are proteins, the carbohydrates, and the fats. Each of these classes of compounds is represented by numerous and varied individual members which are elaborated by nature from a small number of simpler compounds. The proteins, no matter how complex, are all built up from simple amino acids, the carbohydrates from glucose residues or other simple monosaccharides, and the fats from a relatively small number of fatty acids.

The fats differ from the proteins and carbohydrates by the fact that the latter two consist solely of condensation products of similar structural units, whereas in the fats the component acids are attached to a common skeleton, namely, glycerol. The natural fats also differ from proteins and carbohydrates by virtue of the fact that their complexity is due in part to mechanical admixture or mutual solubility of several components of relatively low molecular weight rather than to the existence of highly condensed systems.

The large number and complexity of the natural fats have their origin in two sources, namely, in the number, kind, and mode of arrangement of the individual

fatty acids which are attached to the glycerol skeleton to form specific glycerides, and in the number and relative proportions of such glycerides in the mixture of the solution comprising the fat.

The isolation and identification of component fatty acids comprising a natural fat is generally a difficult process, especially if it is to be carried out in a quantitative rather than a qualitative manner. Fats and oils are of all degrees of complexity and sometimes contain fatty acids of widely different properties, that is they may comprise readily volatile and highly non-volatile acids or completely saturated and highly unsaturated acids. On the other hand, they may comprise a homologous series of acids differing only slightly from member to member in chemical and physical properties and thus affording no ready means of sharp separation or of unequivocally differentiating them from neighboring members of the series.

Owing to the complexity of the mixture of acids present in natural fats, no single method is universally applicable to their separation. The present methods have been developed over a long period and through refinement are now quite reliable.

The available techniques may be divided into three general types involving separation by (1) distillation,

(2) solubility, and (3) absorption of fatty acids, fatty acid esters, or the halogenated derivatives of fatty acids. It is sometimes feasible to effect a partial separation of the glycerides by molecular distillation or by crystallization either with or without solvent, but generally little advantage is gained by such preliminary fractionation.

2. Chemical and Physical Constants of Filbert Oils obtained by Expression and by Extraction.

A. Procedures

a. Preparation of samples

1. By expression - a sample of filbert nuts was ground in a meat grinder. The oil was pressed from the ground nuts by means of a Carver hydraulic press at 5,000 - 10,000 p.s.i. and then filtered. The clarified oil was stored at ca 35° F. in a tightly covered jar.

2. By extraction - the ground nuts were extracted with petroleum ether (Skellysolve F) in a Soxhlet apparatus of two kilo capacity for 48 hours. Most of the petroleum ether was driven off on a steam bath and the remainder was completely removed in a vacuum oven at 50° C.

b. Physical constants - The specific gravity was determined by means of a Westphal balance at 25° C.

The index of refraction was determined by the Abbe refractometer. The procedure outlined in the A. O. A. C. official methods was followed for the determination of titer test (5). The viscosity was determined by an Ostwald apparatus. The Klett-Summerson colorimeter with a No. 42 filter was used to determine the density of color.

c. Chemical constants - the procedures outlined in the A. O. A. C. methods of analysis (5) were followed for the determinations of iodine absorption number, thiocyanogen number, saturated and unsaturated fatty acids, free fatty acids, acetyl value, unsaponifiable residue, soluble and insoluble acids. Carotene and vitamin A were determined by the modified method of Koehn and Sherman (45).

B. Data and Results - The results are summerized and tabulated in Table 4.

An investigation of the physical characters and chemical constituents revealed that filbert oil belongs to the olive oil group which has a specific gravity ranging from 0.911 to 0.923 (1). The oils of this group contain oleic glyceride as the main constituent, with smaller quantities of the saturated fatty acids glycerides. Both Barcelona and Du Chilly oils yielded no soluble bromides on treatment with bromine in ether solution which indicated the absence of linolenic acid. As a whole, the filbert

Table 4

Physical and Chemical Constants of Barcelona and Du Chilly Filbert Oil Obtained by Expression and by Extraction

	Barcelona		Du Chilly	
	By Expression	By Extraction	By Expression	By Extraction
Specific gravity, 25°/4° C.	0.9106	0.9114	0.9116	0.9116
Refractive index, 20° C.	1.4694	1.4698	1.4698	1.4700
Viscosity, 25° C., centipoise	59.1	53.6	58.2	53.0
Optical rotation	inactive	inactive	inactive	inactive
Color, Klett unit	304	690	475	865
Titer test, ° C.	8.5	-	7.7	-
Iodine number (Hanus)	93.2	95.4	97.2	97.5
Thiocyanogen number	79.67	79.94	79.93	79.97
Saponification number	188.1	186.8	188.2	187.2
Soluble acid, %	nil	-	nil	-
Insoluble acid, %	94.74	-	95.31	-
Saturated acid, %	5.5	-	4.1	-
Unsaturated acid, %	89.7	-	90.7	-
Unsaponifiable matter, %	0.40	-	0.35	-
Free fatty acid, % as oleic acid	0.12	0.44	0.10	0.43
Acetyl value	6.3	-	3.7	-
Carotene, γ/1 gm	0.94	-	1.1	-
Vitamin A.	none	-	none	-
Oleic acid glycerides, %	76.83	74.87	72.72	72.50
Linoleic acid glycerides, %	15.60	17.85	19.98	20.24
Saturated acid glycerides, %	7.17	6.88	6.95	6.91

oil is very similar to almond oil in properties. This confirms the reports of Salvatore (35) and Krusser (20) on European varieties.

3. Fatty Acid Components of Barcelona and Du Chilly Filbert Oils.

A. Preparation of the mixed fatty acids from the oil - One hundred parts by weight of oil with a solution of 60 parts by weight of potassium hydroxide in about 500 parts of ethyl alcohol (95%) was refluxed for 6 hours. Most of the alcohol was then removed by distillation. The soaps were dissolved in water and the unsaponifiable matter was removed by extracting the aqueous-alcoholic solution of the soaps with ether in a continuous extractor for 24 hours. They were then converted into the free fatty acids by warming with dilute sulfuric acid. When the acids were completely liberated, they were removed by extraction with ether and dried under a reduced pressure at 50° C.

B. Separation of saturated from unsaturated acids - The separation of 'solid' (or mainly saturated) from 'liquid' (or mainly unsaturated) higher fatty acids was made by extraction of the mixed lead salts with ether. This procedure, introduced by Gusserrow (16) in 1828 and improved by Lewkowitsch (23), involves preliminary

precipitation of the lead soaps from the aqueous alkali soap solutions and subsequent extraction of the washed precipitated lead soap with ether, there-by removing the 'liquid' acids. The use of alcohol instead of ether was first suggested by Twitchell (44). A modification of Twitchell's method described by Hilditch (19) was used here to separate 'solid' acids from the 'liquid' acids. The fatty acids were regenerated from the lead salts. Each group of acids was converted into the methyl ester by refluxing with four times the weight of methyl alcohol in the presence of about 2 per cent of concentrated sulfuric acid. After distilling off about 70 to 80 per cent of the methyl alcohol, the esters were taken up with ether and the free acids were removed by washing with dilute potassium carboate solution.

C. Fractional distillation of the methyl esters - Vacuum distillation of fatty acid esters constitutes one of the most important methods of separation of these products. Under ideal conditions of fractional distillation it should be possible to separate a mixture of components completely by removing successive molecular species at a fixed temperature and pressure. Also, ideally, it might be assumed that one component of a given mixture should distill at a fixed temperature and pressure until all of that component had been removed, following which the

temperature of the condensate vapor should rise to that of the next higher boiling component and again remain constant until it had been removed.

Such ideal conditions almost never prevail and in the case of routine fatty acid ester fractionation, they are seldom even approximated. Distillation is more often carried out over a range of temperatures and pressures without any very exact knowledge of the manner in which these conditions are varying. The resulting fractions cannot, therefore, be expected to be composed of a single component, or if the original mixture is very complex, even of a very small number of components. Many workers have assumed, for example, that the fractions are two components systems if, as in the case of saturated acid fractions, they are found to have an appreciable iodine value. It is generally true that one component predominates in the mixture, but the proportion depends on the percentage composition of the original mixture. The distillation unit must of necessity be a compromise between theory and practice, but it is possible to design and operate such equipment with a relative high degree of efficiency. Such a unit consists of a boiler and a source of heat, a column including the packing and insulation, a still head including a condenser, reflux controller and fraction cutter, and a means of producing the vacuum.

Two electrically heated columns were built according to Laughlin, Nash and Whitmore (21). Both columns were all glass making the entire operation visible. One was 100 cm. in height with an inside diameter of 15mm. The other was 25 cm. in height with an inside diameter of 13 mm. Both columns were packed with the single turn glass helices and had an efficiency of 28 and 6 theoretical plates respectively. The efficiency of both columns was determined with benzene and carbon tetrachloride mixture under total reflux condition. A Duo-Seal pump was used to evacuate the system during the distillation.

'Liquid' acid esters were fractionated by means of the long column while for the 'solid' acid esters the short column was used because of its small holdup. The large still was heated with an electric mantle, the small one by means of an oil bath. For each fraction, the saponification equivalent, iodine number and thiocyanogen number were determined. The composition was then computed accordingly. These data are recorded in Tables 5 and 6.

D. Some features of the calculation of the composition of the individual ester fractions - The most significant analytical constants characterizing mixtures of esters of the fatty acids are the iodine value, the saponification equivalent, percentage of saturated acids and the thiocyanogen value.

Table 5

Complete Fractionation Data for the Component Fatty Acids of Barcelona Filbert Oil

In this analysis, the esters of the 'solid' and of the 'liquid' acids were distilled through the electrically heated and glass helix packed columns.

The original oil had saponification number 188.1, iodine number 93.2, thiocyanogen number 79.67, the mixed acids yield 35.75% 'solid' and 64.25% 'liquid' acids.

A. Fractional Distillation of Methyl Esters of the 'Solid' Acids.
(66.98 grams distilled through helix packed column)

No.	Grams	Temperature of Still °C	Column °C	Head °C	S.E.	I.V.	SCN	Calculated Weight of Ester Fraction Saturated C_{16}	C_{18}	C_{20}	Unsaturated Oleic	Lino-leic	C_{20}
BS1	2.00	202-207	145-145	135-140	283.3	48.45	40.57	0.58	0.39	-	0.92	0.11	-
BS2	3.34	207-208	145-159	140-145	293.3	67.98	59.35	0.34	0.60	-	2.16	0.24	-
BS3	6.41	208-208	159-163	145-150	296.5	79.11	69.87	-	0.95	0.09	4.82	0.55	-
BS4	13.00	208-209	163-167	150-152	297.5	83.72	74.06	-	1.02	0.54	10.34	1.18	-
BS5	21.93	209-215	167-167	152-153	301.9	88.53	78.33	-	-	1.58	14.25	1.62	4.46
BS6	13.86	215-215	167-167	153-154	302.3	90.57	80.68	-	-	-	9.37	1.06	3.43
BS7	3.77	215-220	167-196	154-155	302.8	90.17	80.52	-	-	-	2.50	0.28	0.99
BS8	2.67	Residue			305.1	76.30	57.06	-	-	0.44	1.58	0.18	0.47
	66.98				Weight of Me esters gm.			0.92	2.96	2.65	45.94	5.22	9.35
					Me esters %			1.37	4.42	3.96	68.57	7.79	13.96

Table 5, Continued

B. Fractional Distillation of Methyl Esters of the 'Liquid' Acids.
(128.72 grams distilled through helix packed column)

No.	Grams	Temperature of Still °C	Column °C	Head °C	S.E.	I.V.	SCN	Calculated Weight of Ester Fraction Saturated C_{16}	Unsaturated Oleic	Linole-ic	C_{20}	C_{22}
BL1	1.64	221-224	211-212	109-115	299.8	93.93	75.53	0.16	0.71	0.35	0.42	-
BL2	3.43	224-234	212-216	149-153	299.0	94.95	76.37	0.30	1.65	0.73	0.75	-
BL3	8.57	234-237	216-216	153-156	297.1	95.85	77.23	0.82	4.59	1.85	1.31	-
BL4	8.14	237-237	216-217	156-161	298.6	97.50	78.54	0.55	4.34	1.79	1.46	-
BL5	31.12	237-238	217-218	161-162	300.8	100.24	80.79	1.16	15.72	7.02	7.22	-
BL6	39.70	238-238	218-229	162-163	301.3	101.67	82.77	0.58	21.57	8.70	8.85	-
BL7	7.01	238-241	229-232	163-fall-	303.1	102.42	83.51	0.02	3.51	1.54	1.94	-
BL8	29.17	refractionation		ing								
BL81	4.48	229-229	185-190	157-163	302.8	102.29	83.57	0.01	2.32	0.97	1.18	-
BL82	7.53	229-229	185-185	163-170	302.3	102.62	83.67	0.03	3.98	1.66	1.87	-
BL83	12.64	229-239	185-199	170-173	305.4	101.69	83.52	-	5.55	2.65	4.44	-
BL84	4.52	Residue			321.7	97.90	56.74	-	-	1.00	2.71	0.81
128.72					Weight of Me esters gm.			3.63	63.94	28.26	32.15	0.81
					Me esters %			2.82	49.67	21.95	24.98	0.63

Table 5, Continued

C. Calculated Composition of Total Fatty Acids of Barcelona Filbert Oil

Acids	'Solid' Acids 35.61%	'Liquid' Acids 63.99%	Total %
Saturated			
Palmitic	0.49	1.80	2.29
Stearic	1.57	0.00	1.57
C_{20}	1.41	0.00	1.41
Unsaturated			
Oleic	24.42	31.78	56.20
Linoleic	2.77	14.05	16.82
C_{20} monoethenoid	4.97	15.98	20.95
C_{22} monoethenoid	0.00	0.40	0.40
Unsaponifiable matter			.40
			100.04

Table 6

Complete Fractionation Data for the Component Fatty Acids of Du Chilly Filbert Oil

In this analysis, the esters of the 'solid' acids and of the 'liquid' acids were distilled through the electrically heated and glass helix packed columns.

The original oil has saponification number 188.2, iodine number 97.2, and thiocyanogen number 79.93, the mixed acids yield 63.03% 'solid' acids and 36.62% 'liquid' acids.

A. Fractional Distillation of Methyl Esters of the 'Solid' Acids.
(120.8 grams distilled through helix packed column)

No.	Grams	Temperature of Still °C	Column °C	Head °C	S.E.	I.V.	SCN	Calculated Weight of Ester Fraction Saturated C_{16}	C_{18}	C_{20}	Unsaturated Oleic	Linoleic	C_{20}
DS1	2.60	225-227	205-206	80- 82	291.6	78.2	69.87	0.08	0.36	-	1.90	0.26	-
DS2	2.31	227-235	206-214	82-124	298.9	77.2	69.19	-	0.17	0.25	1.67	0.22	-
DS3	25.54	235-238	214-214	124-126	298.4	82.3	73.54	-	0.94	2.36	19.61	2.63	-
DS4	42.93	238-270	214-217	126-128	299.2	87.2	78.72	-	-	2.91	34.20	3.24	2.58
DS5	8.99	270-273	217-221	128-132	300.2	89.7	80.70	-	-	0.26	6.72	0.76	1.25
DS6	9.25	273-284	221-225	132-134	301.1	90.8	81.15	-	-	0.07	7.38	0.84	2.03
DS7	1.07	284-	225-232	falling									
DS8	28.10	Residue			301.9	90.8	78.25	-	-	-	19.33	2.20	6.57
	120.8				Weight of Me esters gm.			0.08	1.47	5.85	90.81	10.15	12.43
					Me esters %			0.07	1.22	4.84	75.17	8.40	10.29

Table 6, Continued

B. Fractional Distillation of Methyl Esters of the 'Liquid' Acids
(37.60 grams distilled through the helix packed column)

No.	Grams	Temperature of Still °C	Column °C	Head °C	S.E.	I.V.	SCN	Calculated Weight of Ester-Fraction Saturated C_{16}	Unsaturated Oleic	Linoleic	C_{20}
DL1	9.86	200-210	170-190	156-158	298.9	104.0	80.42	0.47	5.00	2.70	1.69
DL2	10.70	210-215	190-190	157-158	301.5	110.3	86.57	-	5.09	3.25	2.35
DL3	7.99	215-215	190-197	158-159	302.6	111.9	86.84	-	3.60	2.57	1.82
DL4	7.24	215-224	197-225	falling	302.6	110.3	86.67	-	4.05	1.55	1.64
DL5	1.81	Residue			318.6	86.3	69.06	-	0.18	0.14	1.49
	37.60				Weight of Me esters gm.			0.47	17.92	10.21	8.99
						Me esters %		1.25	47.66	27.15	23.91

Table 6, Continued

C. Calculated Composition of Total Fatty Acids of Du Chilly Filbert Oil

	'Solid' acids 63.03%	'Liquid' acids 36.62%	Total %
Saturated			
Palmitic	0.04	0.46	0.50
Stearic	0.77	0.00	0.77
C_{20}	3.05	0.00	3.05
Unsaturated			
Oleic	47.38	17.45	64.83
Linoleic	5.29	9.94	15.23
C_{20} monoethenoid	6.49	8.76	15.25
Unsaponifiable matter			0.35
			99.98

It is assumed that the components in a mixture of esters of the fatty acids derived from a simple monohydric alcohol are without appreciable chemical influences on each other. The composition of an ester fraction which contains esters of only two saturated acids with unsaturated components all of the same number of carbon atoms can be deduced from its equivalent and iodine value by comparatively simple calculations. Again, ester fractions which include only unsaturated derivatives of acids of two groups in the homologous series can be evaluated directly from their saponification equivalents. In other cases, however, the computation becomes somewhat more complicated, since the weights of more than three independent components are involved.

Assume S_1, S_2 to be the respective weights of two saturated and x, y, the weights of the two unsaturated acids esters in a fraction of weight w; also ES_1, ES_2, Ex, Ey, Ew to be the corresponding saponification equivalents; Ix, Iy, Iw to be the corresponding iodine values; and Yx, Yy, Yw to be the thiocyanogen value. Therefore:

1. $S_1 + S_2 + x + y = w$
2. $S_1/ES_1 + S_2/ES_2 + x/Ex + y/Ey = w/Ew$
3. $x.Ix + y.Iy = w.Iw$
4. $x.Yx + y.Yy = w.Yw$

(a) Esters of the 'solid' acids - Since the lead salts

of tetradecanoic and hexadecanoic acids are freely soluble in alcohol and the small iodine values can be attributed to methyl oleate, methyl linoleate and possible C_{20} monoethenoid acid ester, the calculation is complicated but straight forward.

(b) Esters of the 'liquid' acids - The only saturated acid component present in the liquid acid fraction by the lead salt separations is methyl palmitate. Stearic or higher saturated acids do not pass appreciably into the liquid acid.

Examples of calculation are illustrated as follows:

(1) Fraction DS1 (2.60 gm., S.E. 291.6, I.V. 78.2, SCN value 69.9 containing methyl esters of palmitic, stearic and unsaturated acids) - If S_1, S_2, x and y represent the four esters, the equations are:

$S_1 + S_2 + x + y = 2.60$ . . . . . .(1)

$S_1/270 + S_2/298 + x/296.3 + y/294.3 = 2.60/291.6$ . . .(2)

$85.6x + 172.4y = 78.2 \times 2.60$ . . . . . .(3)

$85.6x + 86.2y = 69.9 \times 2.60$ . . . . . .(4)

From the above four equations, S_1, S_2, x and y may be calculated.

(2) Fraction DL1 (9.86 gm., S.E. 298.9, IV. 104.0, SCN value 80.4 containing methyl esters of palmitic, oleic, linoleic and C_{20} monoethenoid acids) - Since it is a 'liquid' acid fraction, the only saturated acid

component present is methyl palmitate. If S, x, y and z represent palmitic, oleic, linoleic and C_{20} monoethenoid acids, the equations are:

$S + x + y + z = 9.86$ . . . . . (1)

$S/270 + x/296.3 + y/294.3 + z/324.3 = 9.86/298.9$ . . (2)

$85.6x + 172.4y + 78.2z = 104.0 \times 9.86$ . . . . . .(3)

$85.6x + 86.2y + 78.2z = 80.4 \times 9.86$ . . . . . .(4)

From (3) and (4), we have

$y = 2.70$ (linoleic acid ester)

Substituting the value of y in equations (1) and (2) and then eliminating S, we have

$331x + 618z = 2698$ . . . . . (5)

From equations (3) and (5), we have

$z = 1.69$ (C_{20} monoethenoid ester)

Substituting the values of y and z in equation (3), we have

$x = 5.00$ (oleic acid ester)

and $S = 0.47$ (palmitic acid ester)

4. Unsaponifiable Matter.

A. Isolation of sitosterol - Two hundred grams of filbert oil were saponified with alcoholic potassium hydroxide solution for eight hours. The resulting soap was dissolved in water and the aqueous solution was then extracted continuously with ether for 24 hours. The

ethereal solution was washed with water until free from soap and then dried over anhydrous sodium sulfate. The ether was distilled off. The sitosterol was recrystallized three times from absolute ethyl alcohol (25).

The acetyl derivative was prepared by acetylating the sitosterol with acetic anhydride. It was recrystallized from hot absolute ethyl alcohol after distilling off the excess of acetic anhydride under reduced pressure.

B. Analytical results - The elementary carbon and hydrogen of the sitosterols and their derivatives were determined by semimicrocombustion method (27). The specific rotation was determined in chloroform solution at 25°C. The data are summerized in Table 7.

5. The Influence of Storage Condition with Respect to the Chemical and Physical Changes in Filbert Oils.

A. Methods of Investigation - The oil, kernels and whole nuts of Barcelona and Du Chilly varieties were stored at four different temperatures. Five hundreds grams of kernels and 1000 grams of whole nuts were stored in paper sacks separately for each phase of the study; the expressed oil was kept in tightly sealed small glass jars. The samples which were held at -19°C. were analyzed after 12 months, at 0°C. after 6 months, at room temperature after 4 months and at 35°C. every two months. Therefore during the course

Table 7

Elementary Composition of Sitosterol and Its Derivative from Barcelona and Du Chilly Filbert Oils

	(Chloroform) α_D^{25}	M.P. C.	C		H	
			Theory %	Found %	Theory %	Found %
Barcelona Sitosterol[1]	-25.6°	136-8°	83.87	83.86	11.99	12.25
Du Chilly Sitosterol[1]	-35.2°	135-7°	83.87	83.95	11.99	12.09
Barcelona acetyl Sitosterol[2]	-	118°	81.25	81.27	11.29	11.32
Du Chilly acetyl Sitosterol[2]	-	116-8°	81.25	81.45	11.29	11.35

1 Calculated as $C_{27}H_{46}O$

2 Calculated as $C_{29}H_{48}O_2$

In all cases, a positive Liebermann-Burchard reaction was observed (42).

of one year twelve series were analyzed. In each case the expressed oil, oil from kernels which had been stored as shelled nuts, and oil from kernels which had been stored in the shell were included. Both Du Chilly and Barcelona varieties were examined. In all, 74 individual samples were analyzed.

The samples of kernels and whole nuts were weighed before expression of the oil to determine the loss of weight during storage.

The following physical and chemical constants were determined on the oil from each sample to determine if any significant changes had taken place under different storage conditions.

a. Peroxide oxygen value.

b. Iodine number.

c. Saponification number.

d. Free fatty acids.

e. Specific gravity.

f. Refractive index.

g. Color.

B. Procedures - The methods outlined previously for the determinations of iodine absorption number, free fatty acids, specific gravity, refractive index and color were followed.

A modified Lea's method (22) developed in this

laboratory was used for the determination of peroxide oxygen value. Approximately one gram of oil (depending on the degree of rancidity of the oil) was weighed into a 250 ml. Erlenmyer flask. Carbon dioxide was passed into the flask for about one minute to displace the air. About one gram of potassium iodide was added, followed by 20 ml. of a mixture of two volumes of glacial acetic acid to one volume of chloroform. Carbon dioxide was then passed in again and the mixture was heated gently under a reflux condenser for two minutes. The flask was cooled in cold water and 30 ml. of water was poured through the top of the condenser. The free iodine was then titrated with 0.002N thiosulfate. Since the coefficient of reabsorption of free iodine varies with size of sample used, all the peroxide oxygen values recorded in this study are corrected to eliminate the error due to reabsorption. In order to make this correction, a known amount of benzoyl peroxide equivalent to 16 ml. of 0.002N iodine solution was added to a peroxide free filbert oil sample. The free iodine liberated was then determined and the percent recovery was then calculated and plotted against the size of the sample as shown in Figure 1. The peroxide oxygen value was calculated as number of ml. of 0.002N NaS_2O_3 used for the titration of free iodine liberated by one gram of oil.

Fig. 1 PER CENT RECOVERY OF FREE IODINE AGAINST THE SIZE OF FILBERT OIL SAMPLE IN THE DETERMINATION OF PEROXIDE OXYGEN VALUE.

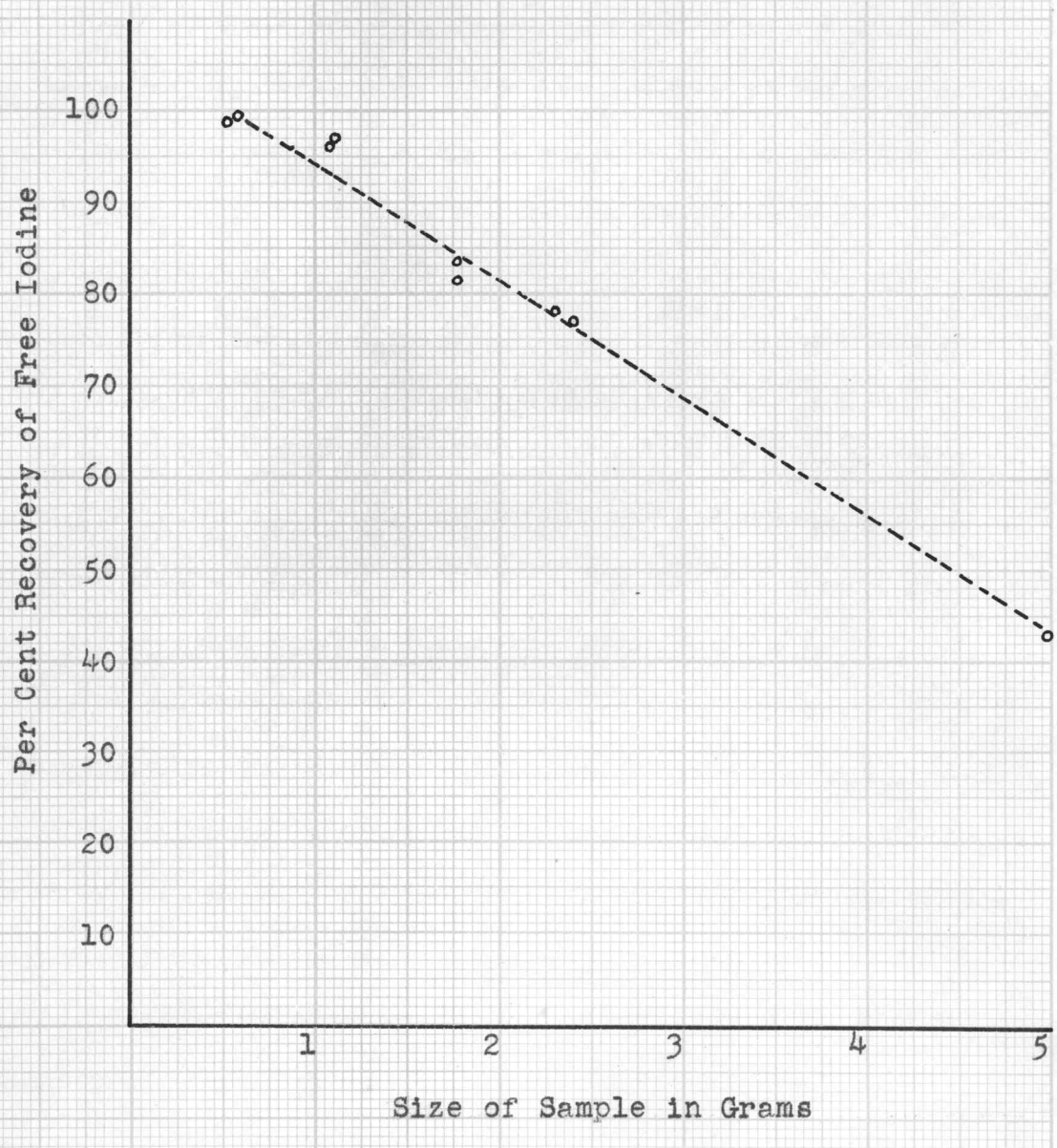

Table 8

Chemical and Physical Constants of Barcelona Filbert Oil Stored at 35°C

Period of Storage Months	0	2	4	6	8	10	12
Peroxide Value	4.03	13.4	29.0*	10.1	9.02	18.0*	9.24
Iodine Number	93.2	93.2	92.2	92.9	93.0	91.7	93.9
Saponification Number	188.1	188.7	187.8	192.4	190.6	192.1	190.4
Free Fatty Acids % as Oleic Acid	0.12	0.10	0.13	0.12	0.12	0.13	0.10
Specific gravity 25/4°C	0.9106	0.9104	0.9118	0.9114	0.9114	0.9120	0.9095
Refractive Index 20°C.	1.4694	1.4695	1.4698	1.4699	1.4699	1.4698	1.4696
Color Klett-units	304	295	290	301	303	276	279

* These oils did not show any change of taste and odor.

Table 9

Chemical and Physical Constants of Barcelona Filbert Oil Expressed from the Kernels Stored at 35°C

Period of Storage Months	0	2	4	6	8	10	12
Peroxide value	4.03	0.26	0.97	21.4*	0.79	2.03	2.45
Iodine number	93.2	96.3	95.9	93.7	94.0	93.0	96.0
Saponification number	188.1	188.8	188.3	193.3	193.5	192.3	191.4
Free fatty acids % as Oleic Acid	0.12	0.17	0.38	0.42	0.34	0.45	0.46
Specific gravity 25/4° C	0.9106	0.9113	0.9102	0.9132	0.9118	0.9121	0.9103
Refractive Index 20°C	1.4694	1.4699	1.4698	1.4699	1.4699	1.4698	1.4698
Color Klett-Units	304	337	344	318	332	299	300

* The high peroxide value of this sample was probably due to incorrect handling during the process of expression.

Table 10

Chemical and Physical Constants of Barcelona Filbert Oil Expressed from the Whole Nuts Stored at 35°C.

Period of Storage Months	0	2	4	6	8	10	12
Peroxide value	4.03	1.68	3.35	0.89	0.68	1.47	7.08
Iodine number	93.2	94.3	95.0	94.6	94.4	93.1	95.2
Saponification number	188.1	188.6	188.2	192.9	189.2	193.1	191.1
Free fatty acids % as Oleic acid	0.12	0.32	0.25	0.29	0.47	0.43	0.52
Specific gravity 25/4°C	0.9106	0.9120	0.9112	0.9116	0.9116	0.9115	0.9110
Refractive Index 20°C	1.4694	1.4697	1.4697	1.4698	1.4699	1.4697	1.4698
Color Klett Unit	304	670*	318	327	315	288	286

* The darker color of this sample was probably due to the contamination of the oil during expression.

Table 11

Chemical and Physical Constants of Barcelona Filbert Oils Stored at Room Temperature

Period of Storage Months	0	As Oil			Expressed from the Kernels			Expressed from the Whole Nuts		
		4	8	12	4	8	12	4	8	12
Peroxide Value	4.03	10.5	12.0	48.1	0.00	0.39	3.73	0.00	0.37	1.93
Iodine Number	93.2	92.3	92.6	94.7	94.0	94.3	96.7	94.1	94.7	95.2
Saponification Number	188.1	188.2	189.5	192.2	187.4	187.1	191.7	188.1	191.6	191.3
Free Fatty Acids % as Oleic Acid	0.12	0.12	0.10	0.12	0.26	0.25	0.24	0.17	0.35	0.23
Specific Gravity 25°/4° C.	0.9106	0.9104	0.9111	0.9102	0.9105	0.9114	0.9103	0.9109	0.9109	0.9105
Refractive Index 20°C	1.4694	1.4697	1.4699	1.4695	1.4698	1.4700	1.4697	1.4697	1.4698	1.4696
Color Klett Unit	304	300	302	308	321	287	315	318	296	284

Table 12

Chemical and Physical Constants of Barcelona Filbert Oil Stored at 0°C and -19°C.

		As Oil			Expressed from the Kernels			Expressed from the Whole Nuts		
Period of Storage Months	0	6	12	12	6	12	12	6	12	12
Storage Temperature °C		0	0	-19	0	0	-19	0	0	-19
Peroxide value	4.03	3.84	7.50	4.60	0.00	1.57	1.40	0.59	4.32	1.53
Iodine number	93.2	93.7	93.9	93.1	95.7	96.0	95.4	94.8	95.2	94.3
Saponification number	188.1	193.1	191.4	190.7	192.9	191.5	191.0	193.2	191.1	191.0
Free fatty acids % as Oleic acid	0.12	0.10	0.14	0.13	0.24	0.26	0.18	0.17	0.19	0.15
Specific gravity 25°/4° C	0.9106	0.9112	0.9103	0.9104	0.9112	0.9104	0.9112	0.9112	0.9103	0.9109
Refractive Index 20° C	1.4694	1.4697	1.4695	1.4695	1.4696	1.4696	1.4697	1.4700	1.4697	1.4697
Color Klett-Unit	304	323	299	301	318	335	338	308	318	306

Table 13

Chemical and Physical Constants of Du Chilly Filbert Oil Stored at 35°C.

Period of Storage Months	0	2	4	6	8	10	12
Peroxide Value	1.80	15.8	32.6	6.55	48.0	72.4	118.5
Iodine number	97.2	97.0	96.2	96.2	94.5	93.7	94.7
Saponification number	188.2	188.2	188.2	192.6	189.0	191.6	192.3
Free Fatty Acids % as Oleic acid	0.10	0.09	0.11	0.11	0,12	0.16	0.21
Specific gravity 25°/4° C	0.9116	0.9115	0.9115	0.9113	0.9118	0.9140	0.9136
Refractive Index 20°C	1.4698	1.4698	1.4697	1.4697	1.4699	1.4700	1.4710
Color Klett-Unit	475	454	430	400	335	118	80

Table 14

Chemical and Physical Constants of Du Chilly Filbert Oil Expressed from the Kernels Stored at 35°C.

Period of Storage Months	0	2	4	6	8	10	12
Peroxide value	1.80	0.52	0.61	1.34	0.67	3.54	1.69
Iodine number	97.2	98.2	96.5	96.7	96.8	95.4	96.0
Saponification number	188.2	188.6	188.3	193.2	190.5	192.3	191.5
Free fatty acids % as Oleic acid	0.10	0.15	0.21	0.30	0.31	0.34	0.32
Specific gravity 25°/4°C	0.9116	0.9114	0.9114	0.9123	0.9122	0.9123	0.9107
Refractive Index 20°C	1.4698	1.4698	1.4698	1.4700	1.4700	1.4700	1.4700
Color Klett unit	475	470	467	485	455	476	415

Table 15

Chemical and Physical Constants of Du Chilly Filbert Oil Expressed from the Whole Nuts Stored at 35° C.

Period of Storage Months	0	2	4	6	8	10	12
Peroxide value	1.80	0.48	0.54	0.92	0.95	1.21	2.41
Iodine number	97.2	97.3	95.2	95.2	95.6	95.4	97.2
Saponification number	188.2	188.3	188.5	192.8	192.0	192.9	191.2
Free fatty Acids % as Oleic Acid	0.10	0.13	0.25	0.25	0.31	0.31	0.28
Specific gravity 25°/40C	0.9116	0.9110	0.9118	0.9117	0.9116	0.9117	0.9110
Refractive Index 20°	1.4698	1.4698	1.4700	1.4699	1.4700	1.4699	1.4700
Color Klett Unit	475	478	450	431	447	452	398

Table 16

Chemical and Physical Constants of Du Chilly Filbert Oil Stored at Room Temperature

	As Oil			Expressed from the Kernels			Expressed from the Whole Nuts		
Period of Storage Months	0	4	8	12	4	8	12	4	8	12
Peroxide value	1.80	13.1	29.5	8.91	0.00	0.14	1.86	0.00	0.39	3.17
Iodine Number	97.2	96.1	96.0	97.4	97.3	97.3	98.5	96.3	95.8	97.4
Saponification number	188.2	188.5	186.7	192.1	188.1	194.2	192.1	187.5	191.4	192.0
Free Fatty acids % as Oleic acid	0.10	0.14	0.10	0.10	0.17	0.33	0.20	0.17	0.31	0.20
Specific gravity 25°/4°C	0.9116	0.9117	0.9127	0.9110	0.9115	0.9118	0.9111	0.9106	0.9116	0.9102
Refractive Index 20°C	1.4698	1.4698	1.4702	1.4700	1.4698	1.4700	1.4700	1.4698	1.4700	1.4700
Color Klett unit	475	455	433	370	459	466	425	465	468	420

Table 17

Chemical and Physical Constants of Du Chilly Filbert Oil Stored at 0°C and -19°C.

		As Oil			Expressed from the Kernels			Expressed from the Whole Nuts		
Period of Storage Months	0	6	12	12	6	12	12	6	12	12
Storage Temperature °C		0	0	-19	0	0	-19	0	0	-19
Peroxide Value	1.80	3.88	8.25	2.57	0.00	1.32	1.54	0.00	1.29	4.50
Iodine Number	97.2	96.7	97.2	97.5	97.6	97.8	98.0	96.8	97.7	96.3
Saponification number	188.2	193.4	191.0	191.3	193.8	191.1	191.9	192.8	191.7	191.1
Free Fatty Acids % as Oleic Acid	0.10	0.11	0.12	0.16	0.16	0.18	0.16	0.27	0.17	0.18
Specific gravity 25°/4°C	0.9116	0.9116	0.9108	0.9105	0.9113	0.9105	0.9108	0.9113	0.9106	0.9104
Refractive Index 20°C	1.4698	1.4700	1.4700	1.4699	1.4700	1.4699	1.4700	1.4700	1.4699	1.4700
Color Klett-unit	475	475	462	463	463	430	435	470	472	435

C. Results - The results are summerized in Table 8 to 17.

The peroxide oxygen value of Du Chilly filbert oil stored at 35°C increased considerably with the time of storage. A rancid taste was apparent after 8 months storage at this temperature, and after 12 months the oil had a peroxide number of 119 and a very strong rancid taste. However, Barcelona oil was more stable when stored under the same conditions, showing only slight rancidity after 12 months of storage. The oils stored at room temperature had a peroxide number of 48 and 8.9 for Barcelona and Du Chilly respectively after 12 months of storage. In this case, Barcelona oil seemed to be less stable than Du Chilly. Both oils stored at 0° C. and -19° C. showed practically no change of taste and had a low peroxide value. Nevertheless, all oils obtained from the kernels or the whole nuts which were kept under the same storage conditions had very low peroxide oxygen values and no change of taste or flavor was observed.

The free fatty acids remained constant in Barcelona filbert oil and were very slightly increased in Du Chilly oil after 12 months of storage at 35° C. Negligible change was observed when the temperature was low. In the case of oils obtained from the kernels or whole nuts which were kept under the same conditions, the fatty acids were found

to increase gradually and noticably with the time of storage. A greater increase was found in the oil stored at higher temperature. This indicated enzymatic splitting of glycerides in the kernels which does not take place in the oil after being expressed.

The density of yellow color decreased more rapidly at higher temperatures. The color of Du Chilly oil faded much faster than that of Barcelona oil when held under the same conditions.

In all cases, the specific gravity and refractive indices remained quite constant throughout the storage tests.

The slight and irregular variations in iodine absorption and saponification values which occured during storage do not appear to be significant.

IV. Investigation of the Nutritive Value of Filbert Nut Protein

1. Methods of Investigation.

It was recognized early in the nineteenth century that, in man, the body protein was synthesized only from ingested protein. Magendie soon demonstrated that all proteins are not of equal value (24). In 1841 he showed that gelatin would not take place of meat protein in the diet. About seventy years later, Osborne and Mendel (28) demonstrated that certain proteins which produced nutritive failure in rats when used alone were rendered satisfactory by the addition of certain missing amino acids. In 1930, W. C. Rose (33) of the University of Illinois began a brilliant series of studies using pure amino acids as the sole source of nitrogen. Of the 22 amino acids he studied, he concluded that 10 amino acids are essential and 12 nonessential for the growth of rats.

Protein of either animal or plant origin which are deficient in one or more essential amino acids will not provide adequate protein nutrition no matter how much is fed. Therefore different rations of same nitrogen content may have different value in nutrition and result in a different growth response.

To investigate the quality of the filbert nut

protein, the paired-feeding technique was chosen.

The animals used through out this study were young rats, 28 to 30 days of age, of the Evan-Long strain from the stock colony.

The experimental animals were placed in individual wire cages and were allowed water and the experimental diets. In paired-feeding, two animals are selected to be as nearly alike as possible in weight, sex and age. One animal is placed on the filbert meal ration and the other is placed on a ration exactly the same except casein is substituted for the filbert meal. Both animals are allowed approximately the same amount of food. Animal weights and individual food intake are measured daily.

2. Growth Response of Rats on Rations using Filbert Meals as Sole Source of Protein with or without Supplementation.

Rations containing 9% protein level and 8% butter fat suggested by Dr. P. H. Weswig* was first tried with unsatisfactory results. The animals showed no gain of weight in both casein and filbert meals rations for two weeks. Later the protein level was increased to 12.5% and 16.5% which was used for all rations throughout this study.

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* Associate chemist, Agricultural Experiment Station.

Rations prepared from oil free filbert nut meal were compared with the casein ration. The protein level in both cases was the same. Cellu flour was omitted in rations containing the filbert nut meal. The composition of these rations are shown in Table 20. The vitamins supplement (Table 18) was mixed directly into the ration.

Table 18

Vitamin Supplements Used in All Rations

Vitamins	mg. per kilo ration
Thiamin hydrochloride	5
Riboflavin	10
Pyridoxine hydrochloride	5
Nicotinic acid	5
Calcium pantothenate	25
p-Amino benzoic acid	300

The growth response of 28 day old rats to the casein ration was established by feeding six animals ad lib. The excellent growth proved the adequacy of this ration. The growth curves for male and female were shown in Figure 2.

The comparative study of growth responses on casein ration and oil free filbert nut meal rations was carried out by using paired-feeding technique. Later, thesame rations supplement with tryptophan, cystine, lysine, and tryptophan and cystine respectively were used in this study to determine whether or not the proteins from the filbert meals possess sufficient amounts of these amino acids.

The results were summerized in Tables 21 to 26.

It is obvious that the protein from both filbert nut meals is inferior in quality to that of casein. Du Chilly filbert nut protein promoted growth somewhat better than the Barelona variety. In both cases, supplementing with 0.2% of DL-tryptophan, 0.5% L-cystine or 0.2% DL-tryptphan and 0.3% L-cystine showed a slight improvement. When supplemented with 0.5% DL-lysine a distinct improvement resulted, and was almost equal to the casein ration, which was supplemented with the like amount of DL-lysine, in promoting growth. Nevertheless, the filbert meal rations even without supplementation still appeared to promote better growth than the stock diet prepared by a local feed company. This ration is given in Table 19. It has a protein level of approximately 19.5% (9).

Table 19

Composition of Stock Diet used in Oregon State Colony

Materials	Per cent
Whole yellow corn meal	38.0
Whole wheat flour	31.0
Ground alfalfa leaves	7.6
Skim milk powder	20.0
Irradiated yeast	1.0
Sodium Chloride	0.5
Calcium carbonate	0.5
Cod liver oil	0.5

Fig.2 GROWTH RESPONSE OF 28 DAY OLD MALE AND FEMALE RATS RECEIVING CASEIN BASAL RATION Ad Libitum.

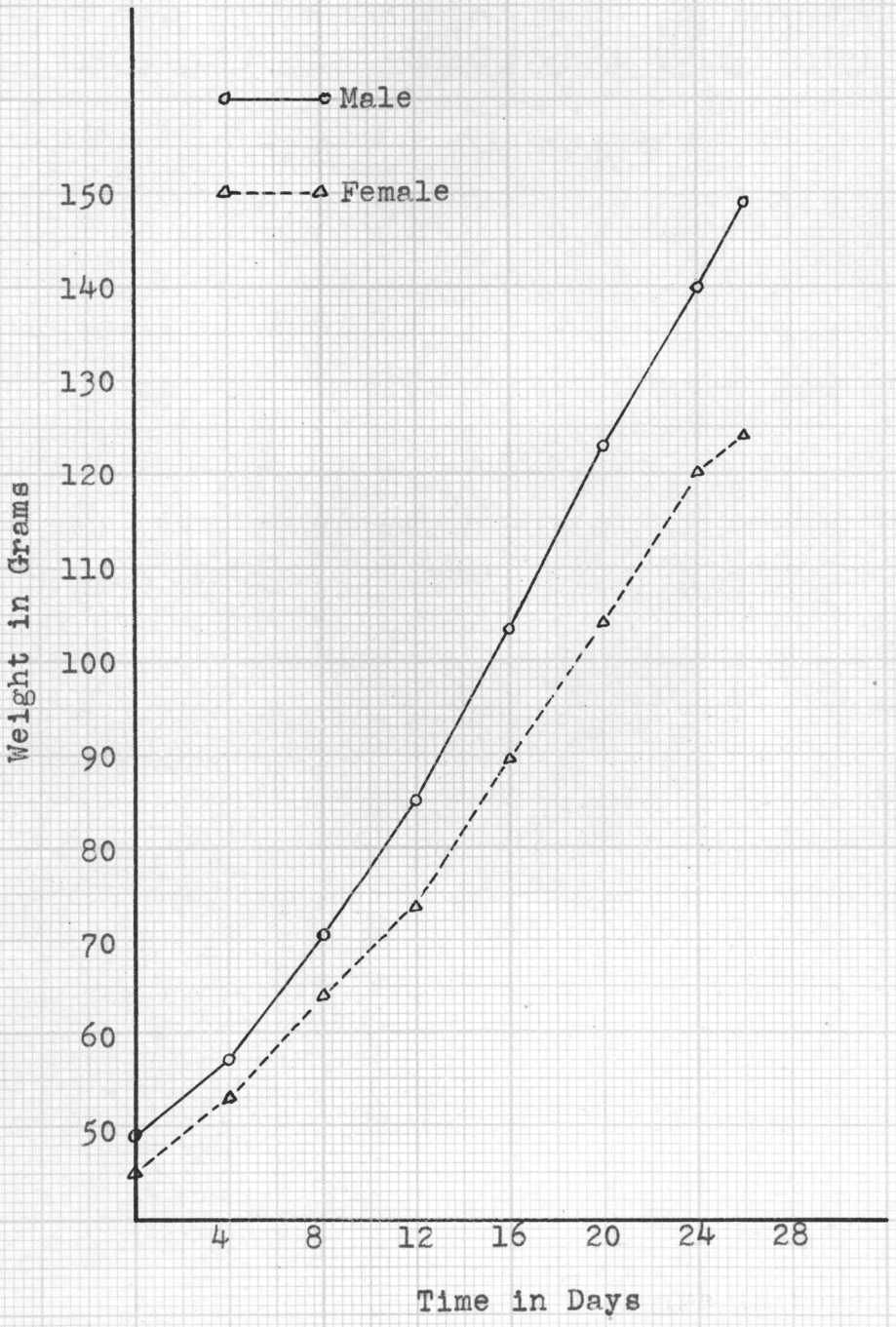


Table 20

Composition of the Oil Free Filbert Nut Meals and Casein Rations

Ration No.	C1 %	C2 %	C3 %	C4 %	C5 %	B1 %	B2 %	B3 %	B4 %	B5 %	D1 %	D2 %	D3 %	D4 %	D5 %
Corn oil	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Cod liver oil	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Jones salts mix***	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Inositol	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Choline HCl	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Liver extract	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Sucrose	74.3	74.3	74.1	74.1	74.3	57.2	57.4	57.1	57.1	57.8	50.4	50.7	50.4	50.4	51.1
Cellu flour	2.0	2.0	2.0	2.0	2.0	-	-	-	-	-	-	-	-	-	-
Casein	16.5	16.3	16.3	16.3	16.0	-	-	-	-	-	-	-	-	-	-
Barcelona meal*	-	-	-	-	-	35.6	35.2	35.2	35.2	34.5	-	-	-	-	-
Du Chilly meal**	-	-	-	-	-	-	-	-	-	-	42.4	41.9	41.9	41.9	41.1
DL-tryptophane	-	0.2	-	0.2	-	-	0.2	-	0.2	-	-	0.2	-	0.2	-
L-cystine	-	-	0.5	0.3	-	-	-	0.5	0.3	-	-	-	0.5	0.3	-
DL-lysine	-	-	-	-	0.5	-	-	-	-	0.5	-	-	-	-	0.5
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

* Barcelona filbert meal contains 46.3% crude protein
** Du Chilly filbert meal contains 38.9% crude protein
*** Jones, J. H. and Foster, C., J. Nutr., 24, 245 (1942).

Table 21

Paired-feeding Experiment with oil free Filbert Nut Meals and Casein Basal Rations
(Each experiment covers 28 days)

	Pair 1		Pair 2		Pair 1		Pair 2	
Ration	B_1	C_1	B_1	C_1	D_1	C_1	D_1	C_1
Animal #	10	9	12	11	14	13	16	15
Sex	♀	♀	♀	♂	♀	♀	♂	♂
Litter	3	3	4	4	5	5	5	5
Initial weight	59	57	67	50	42	41	42	39
Final weight	130	160	131	164	108	132	118	152
Total gain	71	103	64	114	66	91	76	113
Food Intake	348	342	329	324	288	284	317	309
$\frac{\text{Food Intake}}{\text{gram gain}}$	4.90	3.32	5.14	2.84	4.36	3.12	4.17	2.73

Table 22

Paired-feeding Experiment with Oil Free Filbert Nut Meals and Casein Basal Rations Supplemented with 0.2% DL-tryptophan.
(Each experiment covers 28 days)

	Pair 1		Pair 2		Pair 1		Pair 2	
Ration	B_2	C_2	B_2	C_2	D_2	C_2	D_2	C_2
Animal #	26	25	28	27	30	29	32	31
Sex	♂	♀	♀	♂	♂	♂	♂	♀
Litter	7	7	7	7	8	8	8	8
Initial weight	46	44	52	49	58	54	54	52
Final weight	94	102	118	132	139	160	124	137
Total gain	48	58	66	83	81	106	70	85
Food intake	212	205	282	281	293	291	282	281
Food intake / gram gain	4.42	3.53	4.27	3.39	3.62	2.75	4.03	3.31

Table 23

Paired-feeding Experiment with Oil Free Filbert Nut Meals and Casein Basal Rations Supplemented with 0.5% L-cystine.
(Each experiment covers 28 days)

	Pair 1		Pair 2		Pair 1		Pair 2	
Ration	B_3	C_3	B_3	C_3	D_3	C_3	D_3	C_3
Animal #	18	17	20	19	22	21	24	23
Sex	♂	♂	♂	♂	♂	♂	♀	♀
Litter	6	5	6	6	6	6	7	7
Initial weight	40	36	32	30	36	34	50	46
Final weight	102	138	78	114	92	123	133	144
Total gain	62	102	46	84	56	89	83	98
Food intake	264	267	201	201	225	225	294	290
$\frac{\text{Food intake}}{\text{gram gain}}$	4.25	2.62	4.37	2.39	4.01	2.53	3.54	2.96

Table 24

Paired-feeding Experiment with Oil Free Filbert Nut Mealsand Casein Basal Rations Supplemented with 0.3% L-cystine and 0.2% DL-tryptophan.
(Each experiment covers 28 days)

	Pair 1		Pair 2		Pair 1		Pair 2	
Ration	B_4	C_4	B_4	C_4	D_4	C_4	D_4	C_4
Animal #	40	39	42	41	44	43	46	45
Sex	♂	♂	♀	♀	♀	♀	♂	♂
Litter	10	10	10	10	11	11	11	11
Initial weight	42	40	44	40	43	40	44	38
Final weight	109	147	115	136	135	140	100	93
Total gain	67	107	71	96	92	100	56	55
Food intake	281	277	293	288	293	286	207	183
$\frac{\text{Food intake}}{\text{gram gain}}$	4.20	2.59	4.13	3.00	3.18	2.86	3.70	3.32

Table 25

Paired-feeding Experiment with Oil Free Filbert Nut Meals and Casein Basal Rations Supplemented with 0.5% DL-lysine.
(Each experiment covers 28 days)

	Pair 1		Pair 2		Pair 1		Pair 2	
Ration	B_5	C_5	B_5	C_5	D_5	C_5	D_5	C_5
Animal #	50	49	52	51	54	53	56	55
Sex	♀	♀	♀	♂	♂	♂	♀	♀
Litter	12	12	13	12	13	13	14	12
Initial weight	35	32	34	33	39	37	29	25
Final weight	105	105	105	118	146	145	94	102
Total gain	70	73	71	85	107	108	65	77
Food Intake	204	201	230	235	303	303	202	205
$\frac{\text{Food intake}}{\text{gram gain}}$	2.92	2.84	3.24	2.76	2.83	2.80	3.11	2.66

Table 26

Comparative Results of Growth Response of Young Rats on Rations containing Casein or Filbert Nut Meals as the Source of Protein with or without Supplements.

Source of protein	Supplement	Type of feeding	Sex	Number of animals used	Average Wt.gained	Average Food intake	Average food Intake per Gain
					gm	gm	
casein	none	ad lib	M	4	101	287	2.84
casein	none	ad lib	F	2	79	235	2.98
casein	none	p-f*	M	2	114	317	2.78
casein	none	p-f	F	2	97	313	3.23
casein	tryptophan	p-f	M	2	95	286	3.01
casein	tryptophan	p-f	F	2	72	243	3.37
casein	cystine	p-f	M	3	92	231	2.51
casein	cystine	p-f	F	1	98	290	2.96
casein	lysine	p-f	M	2	97	269	2.78
casein	lysine	p-f	F	2	75	203	2.75
casein	tryp.& cyst.	p-f	M	2	81	230	2.84
casein	tryp.& cyst.	p-f	F	2	98	287	2.93
B. meal	none	p-f	F	2	68	339	4.98
B. meal	tryptophan	p-f	F	2	57	247	4.34
B. meal	cystine	p-f	M	2	54	233	4.31
B. meal	lysine	p-f	F	2	71	217	3.08
B. meal	tryp.& cyst.	p-f	M & F	2	69	287	4.16
D. meal	none	p-f	M & F	2	71	303	4.26
D. meal	tryptophan	p-f	M	2	76	288	3.79
D. meal	cystine	p-f	M & F	2	70	260	3.72
D. meal	lysine	p-f	M & F	2	86	253	2.97
D. meal	tryp.& cyst.	p-f	M & F	2	74	250	3.38

* paired-feeding

3. Isolation of Globulins from the Filbert Meals

The second step in this study was isolation of pure protein from the oil free filbert meal. Two hundred and fifty grams of oil free Barcelona filbert meal were soaked with one liter of 10% sodium chloride solution at room temperature overnight. The liquid was then removed by squeezing the mass by hand in a several fold of cheese cloth. The liquid was then centrifuged to remove the residue which passed through the cheese cloth. The supernatant liquid was dialyzed for 48 hours in a cellophane bag until free from chloride ions. The precipitate was removed by centrifuge and designated as Barcelona globulin 1. To the liquid a stream of carbon dioxide gas was passed through slowly for five minutes. It was allowed to stand overnight in a refrigerator. The second portion of precipitate designated as Barcelona globulin 2 was removed again in the same manner. Both portions of Barcelona globulin were washed several times with methyl alcohol and finally with ether and allowed to dry at room temperature. 45.2 grams of globulin 1 and 12.3 grams of globulin 2 were obtained, which approximates 50% of the crude protein present in the meal.

The globulin from the Du Chilly filbert nut meal was isolated in the same way. Seventy per cent of the

crude protein present in the meal was obtained in the combined globulin fractions.

The total ash, nitrogen, carbon and hydrogen were determined and summerized in Table 27.

Table 27

Elementary Composition of Globulins from Barcelona and Du Chilly Filbert Nuts

Globulin prepn.	C %	H %	N %	Ash %	% Protein*
B. globulin 1	49.71	6.87	16.50	0.73	18.1
B. globulin 2	50.14	6.83	16.65	1.08	4.9
D. globulin 1	49.76	6.90	16.36	0.47	22.8
D. globulin 2	50.16	6.69	16.34	0.58	2.8

* Isolated from the Meal

4. Growth Response of Rats on Rations using Filbert Globulin as the Sole Source of Protein with or without Supplementation.

Two rations having the same protein level as the casein ration were prepared by using B. globulin 1 and D. globulin 1 as the sole source of protein. The paired-feeding technique was employed. One rat on Barcelona globulin 1 ration died after the 15th day. Another on the Du Chilly globulin 1 ration died after the 16th day. The others showed no gain in weight. On the 28th day, 0.2% of DL-tryptophan was added to these rations. The rats then

gained a total of 4 grams in ten days. In addition to the 0.2% DL-tryptophan, 0.2% of DL-methionine was added. After an additional period of seven days, no improvement occured. The DL-methionine supplement was increased to 0.6% to see if a better response was given. After a further sixteen days of feeding, the rat on Barcelona globulin 1 ration showed a gain of eight grams with a food intake of 72 grams, and the one on Du Chilly globulin 1 ration showed a gain of ten grams with a food intake of 73 grams. At this point all four rats were changed to the stock ration for seven days, a gain was observed in all animals. The growth response curves were shown in Figure 3 and 4 and the results were summerized in Table 28.

Other two rations having the same protein level, using B. globulin 1 and D. globulin 1 respectively as the sole source of protein, supplemented with 2.0% DL-lysine and 1.2% DL-methionine, were prepared. The growth responses of these rations on rats were found as good as the casein rations after a period of three weeks of paired-feeding. In the case of Barcelona globulin 1, the rat gained 65 grams in 22 days with a food intake of 173 grams as compared to the rat on the casein ration which gained 68 grams in the same period with a food intake of 174 grams. With Du Chilly globulin 1 ration, the rat gained 62 grams in weight with a food intake of 179 grams as

Table 28

Paired-feeding Experiment with Filbert Globulin and Casein Basal Rations
(Each experiment covers 28 days)

	Pair 1		Pair 2		Pair 1		Pair 2	
Ration	Barcelona Globulin	Casein	Barcelona Globulin	Casein	Du Chilly Globulin	Casein	Du Chilly Globulin	Casein
Animal #	6	5	8	7	2	1	4	3
Sex	♂	♂	♀	♀	♂	♂	♀	♀
Litter	1	1	2	2	1	1	1	1
Initial weight	47	44	50	45	44	40	46	44
Final weight	died 38	65	48	65	42	57	died 35	79
Total gain	-9	21	-2	20	-2	17	-6	35
Food Intake	59	146	145	145	121	135	62.3	164
Food Intake / gram gain		6.95		7.25		7.93		4.69

Fig.3 COMPARATIVE GROWTH RESPONSE OF 28 DAYS OLD RATS TO BARCELONA FILBERT NUT GLOBULIN AND CASEIN RATIONS USING PAIRED-FEEDING TECHNIQUE.

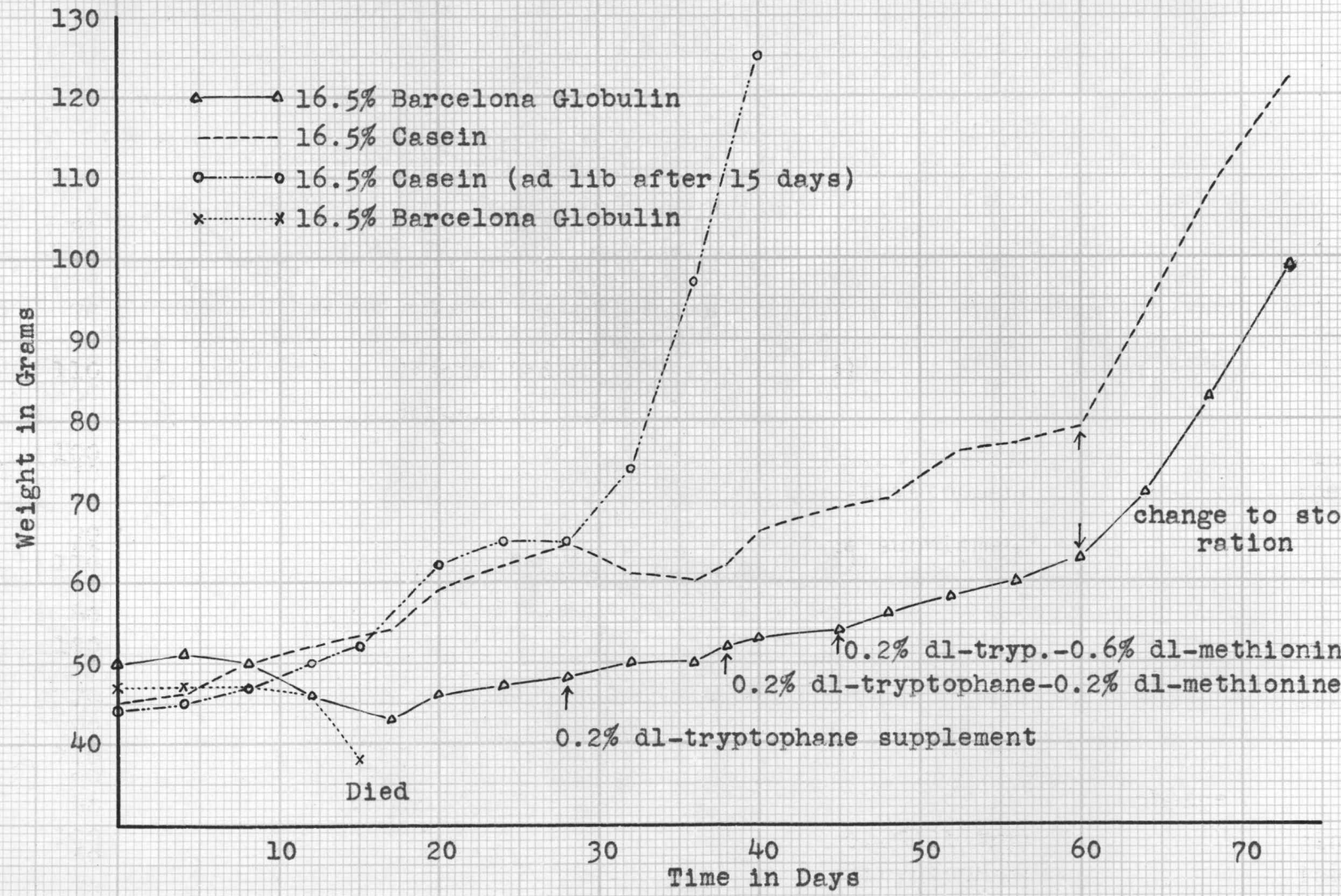


Fig.4 COMPARATIVE GROWTH RESPONSE OF 28 DAYS OLD RATS TO DU CHILLY FILBERT NUT GLOBULIN AND CASEIN RATIONS USING PAIRED-FEEDING TECHNIQUE.

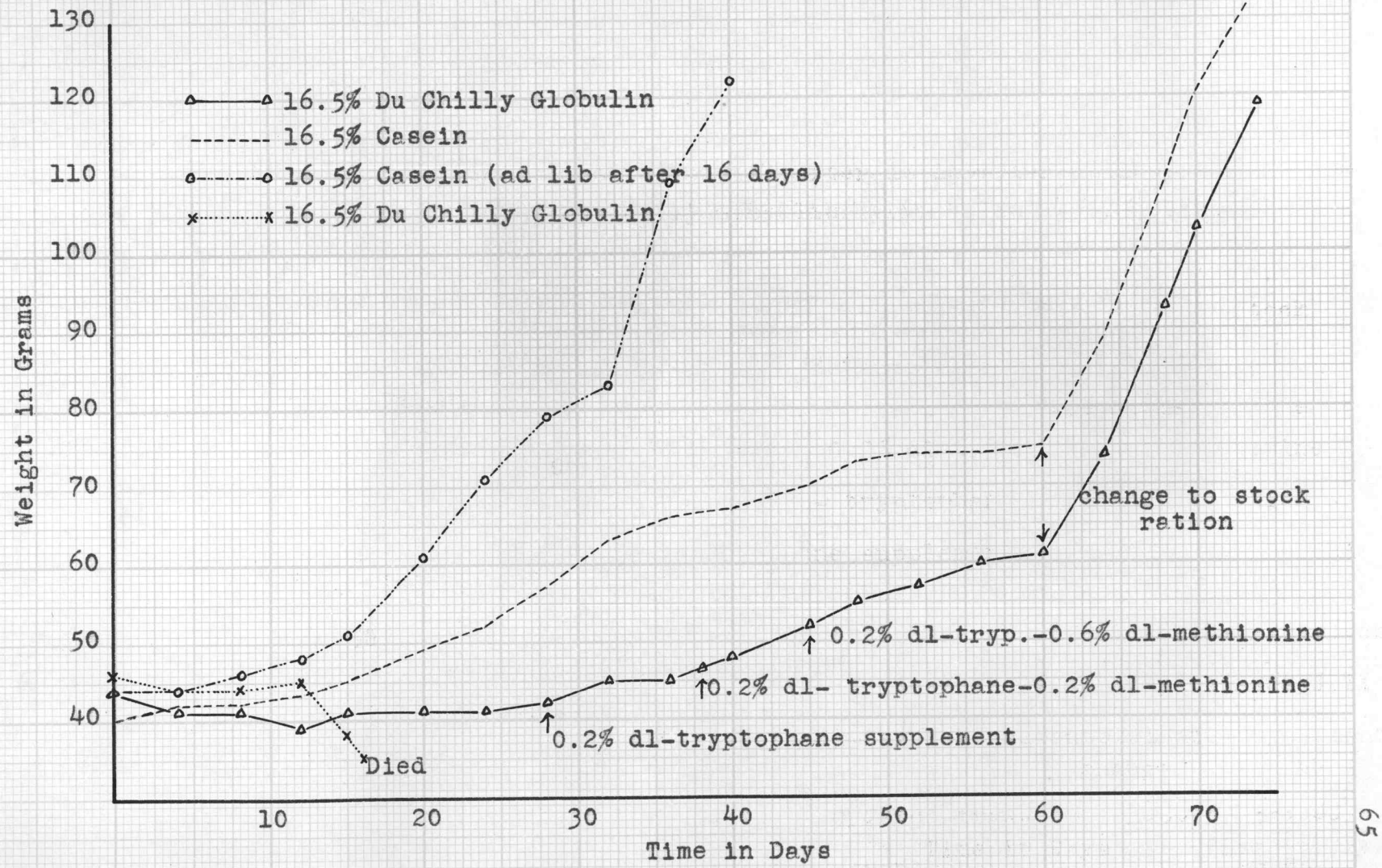


Table 29

Paired-feeding Experiment with Filbert Nut Globulins and Casein Basal Rations Supplemented with 2.0% DL-Lysine and 1.2% DL-Methionine. (Each experiment covers 22 days)

	Pair 1		Pair 1	
Ration	B. Globulin - lysine - methionine	Casein - lysine - methionine	D. Globulin - lysine - methionine	Casein - lysine - methionine
Animal #	58	57	60	59
Sex	Female	Female	Female	Male
Litter	14	14	14	14
Initial weight, gm.	37	35	39	39
Final weight, gm.	106	101	115	117
Total gain, gm.	69	66	76	78
Food intake, gm.	184	183	192	192
$\frac{\text{Food intake}}{\text{gram gain}}$	2.67	2.77	2.53	2.46

Fig.5 COMPARATIVE GROWTH RESPONSE OF 28 DAY OLD RATS TO BARCELONA GLOBULIN 1 RATION SUPPLEMENTED WITH DL-METHIONINE AND DL-LYSINE AND CASEIN RATION.

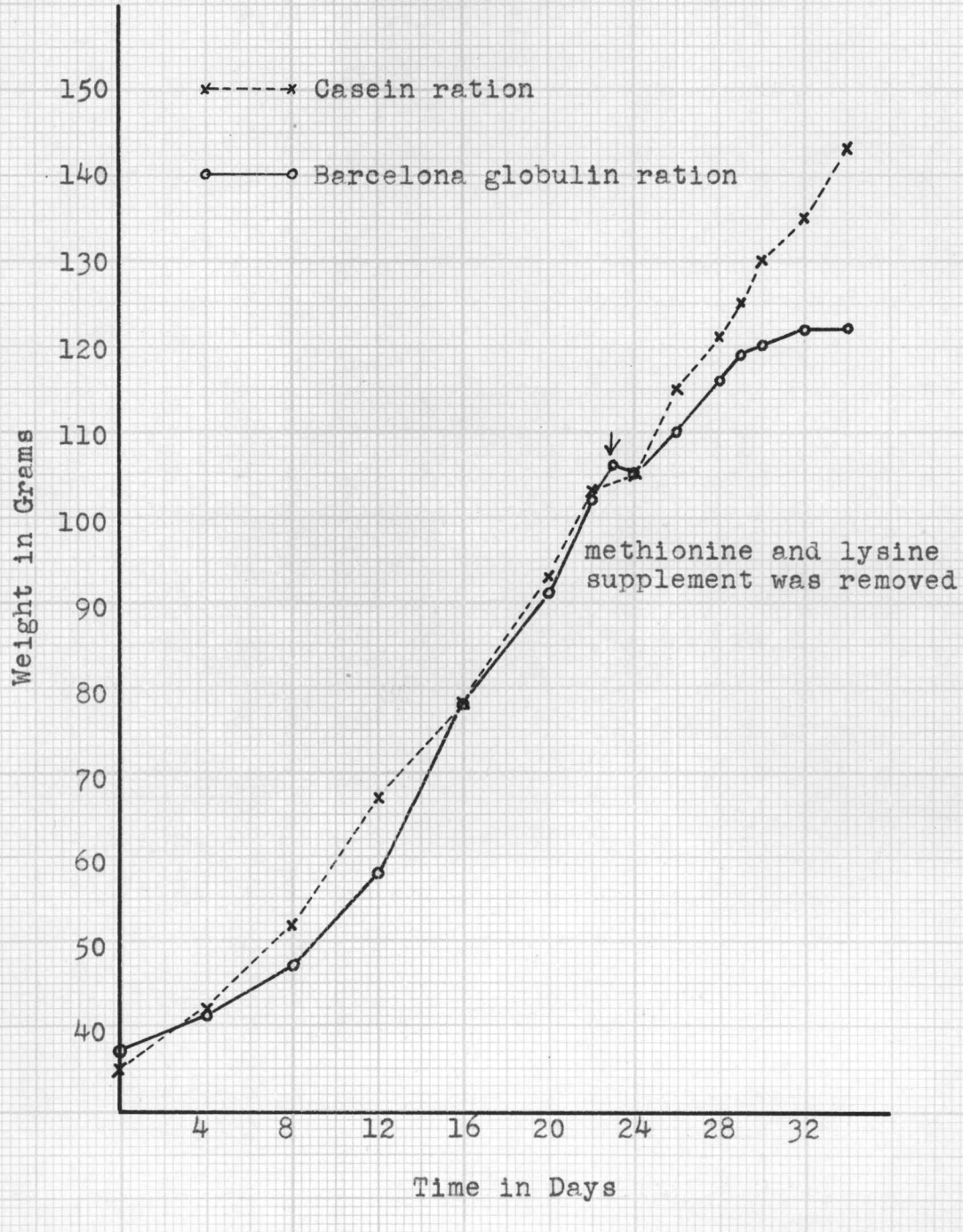
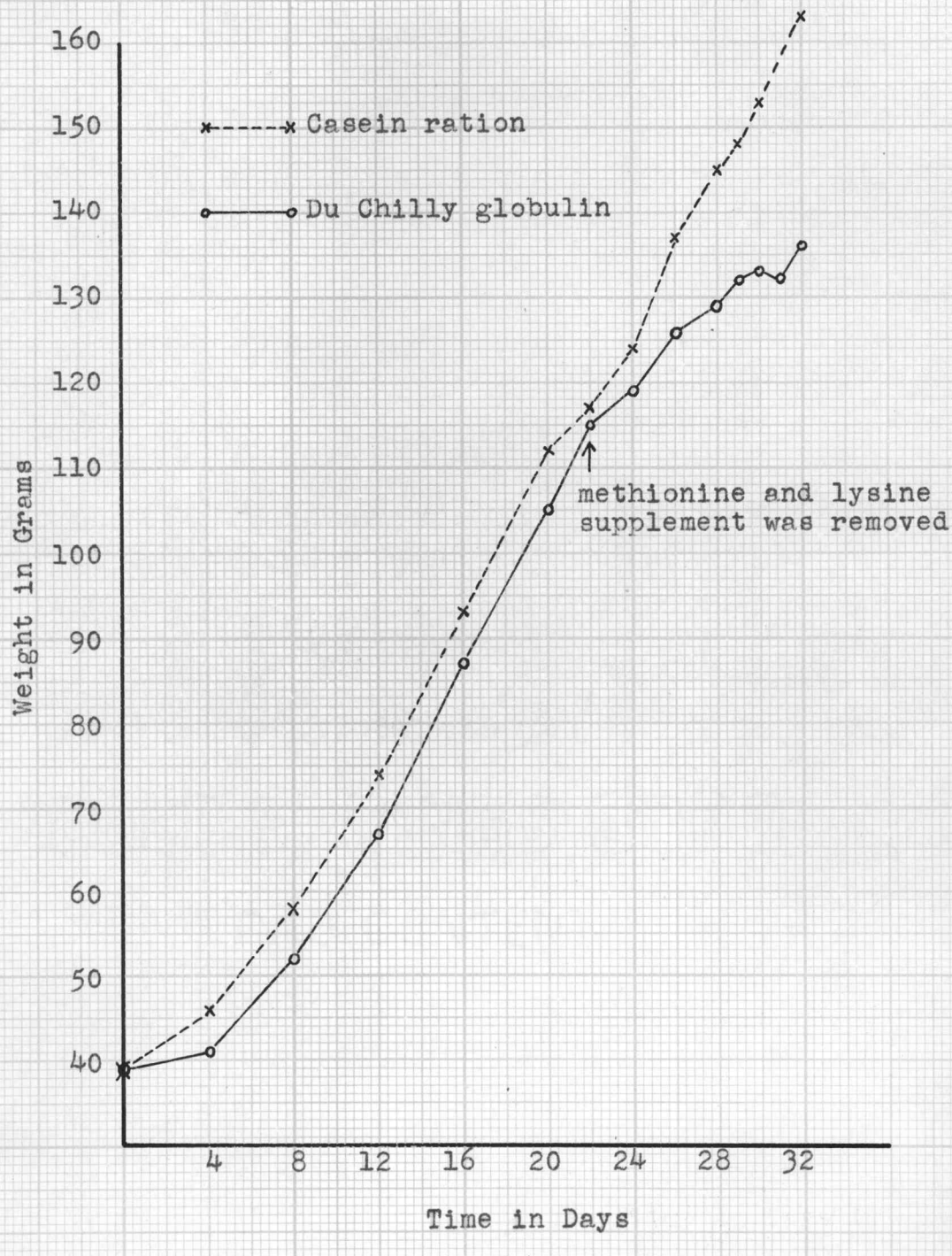


Fig.6 COMPARATIVE GROWTH RESPONSE OF 28 DAY OLD RATS TO DU CHILLY GLOBULIN 1 RATION SUPPLEMENTED WITH DL-METHIONINE AND DL-LYSINE AND CASEIN RATION.



compared to the rat on the casein ration which gained 65 grams with a food intake of 180 grams in 21 days. The animals were then changed to the filbert globulins rations containing no lysine and methionine supplement. After an period of seven days, the animals on the globulin rations showed a slight gain while as the animals on the casein ration showed much better growth. The growth response curves were shown in Figures 5 and 6.

The results indicated that both globulins from Barcelona and Du Chilly filbert nuts do not promote growth of young rats. When supplemented with 0.2% DL-tryptophan the rations showed no improvement. This indicated that the amount of tryptophan in this ration was sufficient. The slight improvement which occured when the rations were supplemented with 0.6% DL-methionine indicated that this amino acid is one of the several missing factors in these rations. A distinct improvement of the globulin rations in promoting growth of young rats when supplemented with an optimum amount of lysine and methionine indicates the deficiency of these amino acids in filbert globulins.

To confirm the biological results, the amino acid distribution of these two globulins were investigated by microbiological assay.

V. Investigation of Amino Acid Composition of Filbert Nut Globulin by Microbiological Assay

1. Introduction.

The amino acid composition of a protein food should parallel its nutritive or biological value as determined by animal test. The poor response to gelatin, observed early in the nineteenth century by Magendic and Prout (24) can be directly corrected to its deficiencies in essential amino acids. The minor deficiency in the sulfur amino acids, cystine plus methionine, in casein, which was first pointed out by Osborne and Mendel (28), is also evident from the analytical values. The high biological value of egg proteins found by Murlin (26) is also indicated in the well balanced distribution of the essential amino acids.

The filbert globulins from either Barcelona or Du Chilly did not give normal growth with rats. Supplements of dl-tryptophan to these rations did not show any improvement in the growth of the rats. Tryptophan plus methionine gave only slight growth improvement, whereas methionine and lysine supplementation showed a great improvement. Therefore investigation of amino acid distribution by microbiological method was carried out in order to confirm these results and at the same time to find if any other amino acid deficiencies occur in these two

filbert globulins.

After the discovery of 'bios' by Wildiers (49), microorganisms have been used to determine the presence of substances which increase the rate or extent of their growth. Because of this similarity in nutritive requirements it is now possible to use microorganisms to determine quantitatively many of those substances which are now known to be essential constituents of all living tissues.

The use of lactic acid bacteria for the determination of amino acids, which they require, is now widespread. The basic techniques used and the various individual methods proposed are very similar, and have been reviewed elsewhere (37,40,41). The uniform medium of Henderson and Snell (18) with slight modification, with the appropriate amino acid omitted, has been employed.

2. Methods

A. Cultures and Inocula - Organisms used were Lactobacillus arabinosus 17-5, Streptococcus faecalis R, Leuconostoc mesenteroides P-60 and Lactobacillus casei. These were carried by biweekly transfer as stab cultures in the medium containing 1% glucose, 1% Difco yeast extract and 2% agar. All cultures were incubated at 37° until good growth occured (24-48 hrs.) and were then refrigerated until the next transfer. The inoculum for

assay was prepared from a stab culture by growing at 37° for 16-24 hours in liquid inoculum medium of Snell (40). The cells were centrifuged and resuspended in an equal volume of sterile 0.9 per cent sodium chloride solution. A dilute inoculum was prepared by adding 0.1 ml. of the resuspended inoculum to 20 ml. of sterile saline. One drop of dilute inoculum was used for each assay tube.

B. Procedure - Assays were carried out in 6-inch lipless culture tubes and in a total volume of 10cc. The samples or standard were added in volumes from 0.5 to 5 ml., water being added to make to 5 ml., 5 ml. of the basal medium, prepared at twice the concentration shown in Table 29, was then added. Sterilization was accomplished by autoclaving the tubes for 10 minutes at 10 to 12 pounds pressure. A metal cover approximately 1 cm. deep and lined with a heavy padding of cotton was placed over each rack of 20 tubes. This cover was removed for approximately 1 minute while 1 drop of inoculum was added to each tube from a sterile pipette.

After incubation at 37° for 48 to 72 hours, lactic acid produced was titrated directly in the assay tubes with 0.1N NaOH using bromthymol blue as indicator.

C. Basal Medium - The compositions of assay medium suggested for use in assaying for amino acids with lactic acid bacteria are very similar to each other and to those

used earlier for assay of vitamins. The chief differences are in the number and amount of amino acids used to replace casein hydrolysate of earlier medium, and in the concentrations of vitamins included. The universal medium of Henderson and Snell (18) shown in Table 30 is used in this laboratory with slight modification.

D. Hydrolysis of Proteins - Hydrolysis was accomplished by autoclaving 0.5 gm. of the protein in sealed Pyrex tubes with 20 ml. of 3N HCl for 5 hours at 15 pounds pressure. The alkaline hydrolysates were prepared by autoclaving 0.5 gm. of protein with 10 ml. 5N NaOH in a sealed pyrex tube for 15 hours at 15 pounds pressure. The digest was neutralized to pH 4.0, then was extracted twice with an equal volume of ether, adjusted to pH 6.6.

3. Results

The results of these analyses are shown in Table 31 and are compared with the range of values from the literature found by microbiological assay of these same proteins.

The data represent the results of ten analyses of 3 to 5 different concentrations of protein hydrolysate.

The values of 16 amino acids found in casein are in very good agreement with the literature values except slightly high values were found in the case of proline, tyrosine and threonine, and low in the case of aspartic acid.

Table 30

Composition of Uniform Medium for Amino Acid Assay

Component	Quant./100 ml.	Component	Quant./100 ml.
Glucose	2 gm.	Thiamine	100 ug.
Na citrate	2 gm.	Riboflavin	100 ug.
Na acetate	0.1 gm.	Pyridoxal	20 ug.
NH_4Cl	0.3 gm.	Ca Pantothenate	100 ug.
K_2HPO_4	0.5 gm.	Niacin	100 ug.
Salts C	2 ml.	p-Aminobenzoic acid	20 ug.
$MgSO_4 \cdot 7H_2O$	100 mg.	Biotin	1 ug.
$FeSO_4 \cdot 7H_2O$	5 mg.	Folic acid	1 ug.
$MnSO_4 \cdot 7H_2O$	16 mg.	DL-Alanine	100 mg.
NaCl	5 mg.	DL-Aspartic acid	100 mg.
Adenine sulfate	1 mg.	L(+) Glutamic acid	100 mg.
Guanine-HCl	1 mg.	L(+) Arginine-HCL	20 mg.
Uracil	1 mg.	L(+) Lysine-$HCl \cdot H_2O$	20 mg.
Xanthine	1 mg.	L(+) Proline	20 mg.
		Other Amino Acids Natural form	10 mg.
		DL-form	20 mg.

The quantities indicated are for 100 ml. final volume or ten 10 ml. tubes. The first five components are added in dry form each time a determination is made. Adenine, guanine and uracil are kept in solution at concentrations of 1 mg./ml. with sufficient HCl to keep them in solution. The xanthine is kept in a solution of dilute ammonia at the same concentration. The vitamins are kept in a single solution at such concentrations that 1 ml. is used for the quantities indicated in the table. The amino acids exclusive of the one being determined are kept in a solution with such concentrations that 25 ml. of solution contains the amount indicated in the table. Cystine and tyrosine are dissolved first in the minimum quantity of 3N HCl, then other amino acids and water to make the required volume. To prevent seeding with small amounts of undissolved amino acids, with resulting crystallization at refrigeration temperatures, the solution should be heated until complete solution is effected. All solutions are kept under toluene, in the cold room and made fresh at intervals of one month. The medium is adjusted to pH 6.8-7.0.

Table 31

Amino Acid Composition of Filbert Nut Globulins and Casein

	Casein		Barcelona Filbert	Du Chilly Filbert
	Found	Literature	Globulin 1	Globulin 1
Arginine	3.39±0.26%	3.78±0.65%	14.44±0.60%	14.50±0.52%
Histidine	3.00±0.19%	3.03±0.24%	2.41±0.09%	1.96±0.06%
Lysine	7.96±0.16%	8.07±0.3 %	2.07±0.14%	1.99±0.10%
Tryptophan	1.36±0.04%	1.28 %	1.45±0.03%	1.52±0.07%
Phenylalanine	5.27±0.17%	5.45±0.34%	3.84±0.21%	3.54±0.17%
Cystine	0.70±0.01%	0.36±0.04%	2.22±0.08%	2.47±0.09%
Methionine	2.61±0.10%	2.69±0.26%	0.81±0.05%	0.84±0.09%
Serine	6.47±0.48%	7.5 %	5.39±0.30%	5.59±0.34%
Threonine	6.23±0.36%	4.28±0.28%	5.31±0.36%	5.59±0.18%
Leucine	10.68±0.45%	10.25±0.3 %	10.0 ±0.48%	6.14±0.46%
Isoleucine	8.30±0.26%	7.6 ±0.35%	5.13±0.25%	4.97±0.18%
Valine	7.06±0.28%	7.15±0.4 %	3.74±0.23%	3.38±0.17%
Glutamic acid	21.3 ±1.1 %	21.7 ±1.7 %	19.0 ±1.24%	19.4 ±1.44%
Aspartic acid	5.70±0.46%	7.4 ±0.03%	8.08±0.23%	8.09±0.33%
Proline	15.30±0.21%	11.6 ±0.7 %	4.40±0.16%	4.22±0.27%
Tyrosine	7.22±0.19%	5.8-±0.7%	3.26±0.10%	3.23±0.11%

The amino acids used as standard were dried in vacuo at 45° C. and kept in a desiccator containing anhydrous calcium chloride. DL forms of tryptophan threonine, aspartic acid, phenylalanine, serine, alanine, methionine, leucine, isoleucine, and valine were employed; the natural isomers of the other acids were used.
Lactobacillus arabinosus 17-5 was used for leucine, isoleucine, threonine, glutamic acid, cystine and tryptophan; Streptococcus faecalis for arginine, histidine, tyrosine and methionine; Leuconostoc mesenteroides P-60 for lysine, aspartic acid, proline and serine; Lactobacillus casei for valine and phenylalanine.

The results indicated that both filbert globulins were very deficient in lysine and methionine, thus confirmed the observation in the previous biological investigation.

VI. Results and Discussion

In this investigation an attempt has been made to study Barcelona and Du Chilly filberts which are grown commercially in the Northwest. This study has been focused mainly upon the chemical constituents of filbert nut shells and kernels, the physical and the chemical constants of filbert oils and their fatty acids components and the nutritive value of its meals and the globulins.

In comparing the results reported in parts II and III in this thesis with the studies of Wainio and Forles (47), and Bertram (6), Barcelona and Du Chilly filberts are shown to contain slightly more oil and less protein than that of European varieties. The amount of pentosans in the shells is approximately 27 percent in Barcelona and 25 per cent in Du Chilly as compared to from 24 to 28 percent in Corylus avellena (17, 29). A close agreement of those values which can be determined with a high degree of accuracy, such as specific gravity and refractive index, was found between the European hazelnuts and the domestic filberts (16, 35, 36). However, the iodine number of European oils reported by the same workers appeared to be somewhat lower than that of Barcelona and Du Chilly varieties which has an iodine value from 92 to 97 for the former and from 95 to 98 for the latter. The saponification

number has a range from 188 to 194 for both Barcelona and Du Chilly which showed no variation with the European varieties. There were some variations in percentage of fatty acids in the oil reported by Schuette and Chang (36), by Bertram (6) on European oils as compared to those of Barcelona and Du Chilly investigated in this laboratory. These variations were due to several factors. They were calculated from other constants such as iodine number, thiocyanogen number and saponification number, and any variation in these values would be reflected in the calculations.

Bertram, in the separation of solid acids by the Twitchell method, reported that the solid acids having high iodine value and had given no explanation for this abnormal behavior. Similar results were observed in this study on both Barcelona and Du Chilly filberts oils. Fractional distillation of methyl esters of both fatty acids fractions revealed that this abnormal behavior probably was due to the high content of C_{20} monoethenoid acid. Its lead salt is only slightly soluble in ethyl alcohol. No attempt had been made for the isolation and identification of this acid.

The oils obtained by extraction gave a darker color and more free fatty acids than those obtained by expression. This is true of other vegetable oils. The coloring matter

which is not removed with the oil during the expression process would be extracted out by the solvent. Therefore, it gave a darker color of the oil. However, the oleic and linoleic glycerides calculated from the iodine and thiocyanogen values seemed to have no significant difference.

Both Barcelona and Du Chilly filbert oil obtained by expression contained a very small amount of unsaponifiable matter. An examination of the data revealed that the sitosterol of both oils possessed properties similar to those obtained from other vegetable oils (2, 3, 4).

In the storage study, the results revealed that the rancidity developed much faster in Du Chilly oil than of Barcelona oil at high temperature. All oils obtained from the kernels or whole nuts in both varieties which were kept under the same conditions for one year showed no rancidity at all. It is believed that the kernels or the whole nuts must contain a natural antioxidant which prevents the oxidation of the oil. The free fatty acids were found to increase gradually and noticably with the time of storage in the oils obtained from kernels or whole nuts but not in the expressed oils. This result indicated that a fat splitting enzyme must be present in the kernels as the splitting of the ester into free fatty acids took place only in the kernels but not in the expressed oils. Again, the increase of free fatty acids was greater at higher temperatures

indicating the characteristics of an enzymic action.

The nutritive value of oil free filbert meal has been demonstrated by means of the rat growth method. The rations which were prepared from oil free filbert meals without supplement as the sole source of protein promoted the growth of young rats and were equal to or slightly better than the stock ration used in Oregon State colony. Such rations, if supplemented with lysine, showed a definite improvement. They promoted the growth of young rats as well as the ration which used casein as a source of dietary protein. When supplemented with other free amino acids, such as tryptophan, cystine or both to these filbert meal rations, a slight improvement was observed. It is safe to say that both Barcelona and Du Chilly filbert meal are adequate as source of protein even if they are slightly inferior when compared to the casein in promoting growth.

The elementary analysis indicated that globulin 1 and globulin 2 from both Barcelona and Du Chilly filberts are probably not the same. A diet containing globulin 1 from either Barcelona or Du Chilly filberts as the source of protein did not support growth of young rats or even permit the maintainance of body weight. Addition of tryptophan to these diets did not show any improvement. When supplemented with methionine, animals showed a slight gain

in weight. These observations revealed that tryptophan was not the deficiency factor and methionine was one of the missing essential amino acids.

With addition of minimum amounts of lysine and methionine to those globulin rations, an excellent growth response on young rats was observed in paired-feeding experiment. They promoted growth almost as well as the casein diet which was used as a reference. From the results, we may conclude that globulin 1 from either Barcelona and Du Chilly filbert nuts is difficient in lysine and methionine. However, the adequacy of using oil free filbert nut meals in this study indicated that there must have other protein in the meals which partially supply the requirement of methionine and lysine. No attempt had been made to isolate other proteins remaining in the meal or to conduct a similar type of experiments by using globulin 2 fraction.

In the microbiological assay of amino acids of Barcelona and Du Chilly globulin 1, a vitamin free casein was used as a reference. The values of proline, tyrosine and threonine in casein were found slightly high as compared to the values in the literature and low in the case of aspartic acid. All values of the other 12 amino acids determined were in very good agreement. The data revealed that both filbert globulins were very deficient in methionine and lysine, which is in agreement with the

animal experiments.

In Table 33, the percentages of 10 essential amino acids of 16.5% protein level rations were calculated from the data and compared to the minimum values of such amino acids which are required to promote the growth of young rats as recommended by Rose (34). It revealed that both globulins rations were very deficient in lysine and methionine and were on the boarderline with histidine, tryptophan, phenylalanine and valine. The well balanced distribution of these amino acids in 16.5% casein ration as shown in Table 33 revealed the superior quality of casein over that of filbert globulin as a dietary protein.

However, if one compared the amino acid distribution of Barcelona and Du Chilly filbert globulin 1 with some other plant proteins as shown in the Table 32 (8), it is surprising to notice that they are more evenly balanced than the other plant proteins, except the cottonseed globulin.

Table 32

Approximate Amino Acid Content of Some Plant Proteins (8)
(calculated to 16.0 g. of nitrogen)

Amino acids	Barcelona Globulin 1 %	Du Chilly Globulin 1 %	Corn Gluten %	Gliadin %	Zein %	Edestin %	Cotton-seed globulin %	Arachin %
arginine	14.4	14.5	3.1	2.7	1.6	14.3	12.2	12.2
histidine	2.4	2.0	1.6	1.9	0.8	2.1	3.0	1.9
lysine	2.1	2.0	0.8	1.1	0.0	2.2	5.2	1.5
tyrosine	3.3	3.2	6.7	2.8	5.9	3.9	3.4	4.1
tryptophane	1.5	1.5	0.7	0.8	0.2	1.3	1.3	0.6
phenylalanine	3.8	3.5	6.4		6.6	4.2	7.8	4.4
cystine	2.2	2.5	1.1	2.3	1.0	1.2	1.1	1.3
methionine	0.8	0.8	4	2.7	2.5	2.0	2.3	0.6
threonine	5.3	5.6	4.1	2.7	2.5		2.7	2.3
leucine	10.0	6.1	24		25	6.6	7.5	
isoleucine	5.1	5.0	5		5		2.3	
valine	3.7	3.4	5		3	5.1	6.7	
glycine			4.3		0	1.6		1.8
glutamic acid	19.0	19.4	24.5	42.2	35.6	17.8	21.2	22.1
aspartic acid	8.1	8.1		1.3	3.4	10.3		4.9
proline	4.4	4.2		12	9	5.4		
alanine					9.9	4.8		4.0
serine	5.4	5.6					2.7	4.6

Table 33

A Comparative Value of the Essential Amino Acids in 16.5% Casein and Filbert Globulin Rations calculated from the Microbiological Results.

Amino Acid	Minimum Requirement for Rats (34) %	Casein Ration %	Barcelona Globulin Ration %	Du Chilly Globulin Ration %
Lysine	1.0	1.31	0.34	0.33
Histidine	0.4	0.50	0.40	0.32
Tryptophane	0.2	0.22	0.24	0.25
Phenylalanine	0.7	0.87	0.63	0.58
Leucine	0.8	1.76	1.65	1.01
Isoleucine	0.5	1.37	0.85	0.82
Methionine	0.6	0.43	0.13	0.14
Valine	0.7	1.16	0.62	0.56
Arginine	0.2	0.56	2.38	2.39
Threonine	0.5	1.03	0.88	0.92

VII. Summary

1. The history, botany, varieties, acreage and production of filbert nut in the Northwest have been reviewed.

2. A number of quantitative analyses of the shell and the kernels of the Barcelona and the Du Chilly varieties have been made to ascertain what rating they should have on the basis of a plant material.

3. The chemical and physical constants of Barcelona and Du Chilly filbert oils were made to determine the type of oil present so that a comparison could be made with other vegetable oils.

4. The fatty acid components of these oils were established by fractional distillation of the methyl esters after a primary separation into solid and liquid fatty acids fractions by Twitchell's method. The high iodine value of solid acid fraction obtained by Twitchell's procedure was probably due to the high content of C_{20} monoethenoid acid.

5. In the work related here, the sitosterols from both oils were isolated from the unsaponifiable fraction and identified as similar to those obtained from other vegetable oils.

6. The influence of storage condition with respect to the chemical and physical changes in Barcelona and Du

Chilly oils have been studied. In general, Barcelona filbert oil is more stable than Du Chilly. The oils obtained from the kernels or the whole nuts after one year of storage at 35° C showed no change of taste or flavor whatsoever whereas expressed oils which were stored under the same conditions developed rancidity. It is not recommended to store the oil at 35°C for more than eight months. However, if stored at room temperature, the oil still would be good for human consumption even after one year.

7. The nutritive values of oil free Barcelona and Du Chilly filbert meals were evaluated by paired-feeding experiments on rats, using vitamin free casein as a reference. Evidence indicated that filbert meal rations promoted growth of young rats but were not equal to the casein ration. They may be greatly improved by supplementation with lysine and slightly improved by supplementing with tryptophan, cystine or both. The rations prepared by using filbert globulin as a sole source of protein did not promote growth of young rats. Feeding experiments showed that the rations were deficient in lysine and methionine.

8. Sixteen amino acids of these globulins were determined by microbiological assay. A sample of vitamin free casein was analyzed and compared with literature values in order to judge the accuracy of these determinations. The biological results were confirmed by microbiological evaluation.

VIII. Bibliography

1. Allen's Commercial Organic Analysis, vol. 2, 5th ed. (1924).

2. Anderson, R. J. and Moore, M. G., J. Am. Chem. Soc., 45, 1944 (1923).

3. Anderson, R. J. and Nabenhauer, F. B., J. Am. Chem. Soc., 46, 2113 (1924).

4. Anderson, R. J. and Shriner, R. L., J. Am. Chem. Soc., 48, 2976 (1926).

5. A. O. A. C. Methods of Analysis, 6th ed. (1945).

6. Bertram, S. H., Ole, Fette, Wachse, Seife, Kosmetik, 14, 2 (1936). Vide Chem. Abstracts, 31, 907 (1937).

7. Bevilotti, V., Quaderni Nutriz., 8, 378 (1942). Vide Chem. Abstracts, 38, 4979 (1944).

8. Block, R. J., in Anson, M. L. and Edsall, J. T., Advances in Protein Chemistry, New York, Academic Press, 2, 119 (1945).

9. Bubl, E. C., Thesis, Oregon State College, (1948).

10. Burrier, A. S. and Schuster, C. E., Oregon Station Bulletin 351 (1937).

11. Cajori, F. A., J. Biol. Chem., 43 #2, 583 (1920).

12. Dalmatov, V., Trudui Lab. Isucheniyu Belka Belkovogo Obmena Organizme, No. 4, 50 (1933). Vide Chem. Abstracts, 29, 6613 (1935).

13. De Caro, L. and Franceschini, J., Quaderni nutriz., 6, No. 1, 82 (1939). Vide Expt. Sta. Record 83, 707 (1940).

14. Engels, O., Seifensieder-Ztg., 70, 36 (1943). Vide Chem. Abstracts, 38, 3380 (1944).

15. Gray, A., Manual of Botany, 7th ed. (1908).

16. Gusserow, C. A., Arch. Pharm., 27, 153 (1928).

17. Helpert, R. S. and Kruger, W., Ber. 72B, 400 (1939)

18. Henderson, L. M. and Snell, E. E., J. Biol. Chem., 172 #1, 15 (1948).

19. Hilditch, T. P., The Chemical Constitution of Natural Fats. London, (1940).

20. Krusser, O. V., Biochem. Kul'tur. Rastenu, 7, 440 (1940) Vide Chem. Abstracts, 35, 3287 (1940).

21. Laughlin, K. C., Nash, C. W. and Whitmore, F. C., J. Am. Chem. Soc., 56, 1396 (1934).

22. Lea, C. H., Rancidity in Edible Fats, New York, 107 (1939).

23. Lewkowitsch, J., Chemical Technology and Analysis of Oils, Fats, and Waxes, vol. 1, 6th ed. (1938).

24. Magendie, F., Ann. chim. phys., 1st ser. 3, 66 (1816). Vide Maynard, L. A., Animal Nutrition, 2nd ed. New York, McGraw-Hill (1947) 92p.

25. Morrow, C. A. and Sandstrom, W. M., Biochemical Laboratory Methods for Students of the Biological Sciences, 2nd ed., New York, John Wiley & Sons, Inc. (1935).

26. Murlin, J. R., Nasset, E. S. and Marsh, M. E., J. Nutrition, 16, 249 (1938).

27. Niederl, J. B. and Niederl, V., Micromethods of Quantitative Organic Analysis, 2nd ed., New York, John Wiley & Sons, Inc. (1942).

28. Osborne, T. B. and Mendel, L. B., J. Biol. Chem., 17, 325 (1914).

29. Phillips, M. and Goss, M. J., J. Assoc. Off. Agr. Chem. 23, 662 (1940).

30. Reed, C. A., Better Fruit, 17, #1, 9 (1922).

31. Reed, C. A., Better Fruit, 17, #2, 20 (1922).

32. Rehder, A., Manual of Cultivated Trees and Shrubs. MacMillan, 152 (1927).

33. Rose, W. C., Physiol. Revs., 18, 109 (1938).

34. Sahyun, M., Outline of the Amino Acids and Proteins, Reinhold, 229 (1944).

35. Salvatore, S., Staz. sper. agrar. ital., 55, 34 (1922). Vide Chem. Abstracts, 17, 3424 (1922).

36. Schuette, H. A. and Chang, C. Y., J. Am. Chem. Soc., 55, 3333 (1933).

37. Schweigert, B. S. and Snell, E. E., Nutr. Abstr. and Rev., 16, 497 (1947).

38. Slate, G. L., New York State Sta. Bul. 588 (1930).

39. Slate, G. L., New York State Station Cir. 192 (1941).

40. Snell, E. E. in Anson, M. L. and Edsall, J. T., Advances in Protein Chemistry, New York, Academic Press, 2, 85 (1945).

41. Snell, E. E., Ann. New York Acad. Sci., 47, 161 (1946).

42. Sumner, J. B. and Somers, G. F., Laboratory Experiments in Biological Chemistry, New York, Academic Press, Inc. 22 (1944).

43. Thomas, M. D., Breithaupt, L. R. and Nielsen, N. I., Oregon State Extension Bul. 631 (1944).

44. Twitchell, E., J. Ind. Eng. Chem., 13, 806 (1921).

45. U. S. D. A. Miscellaneous publication #571.

46. U. S. D. A. Tree Nuts: Acreage, Production, Farm Disposition, Value, and Utilization of Sales 1909-45 (1947).

47. Wainio, W. W. and Forles, E. B., J. Agr. Research, 62, 627 (1941).

48. Wiegand, E. H., Oregon State Hort. Soc. Ann. Rept., 35, 107 (1943).

49. Wildiers, E., La Cellula, 18, 313 (1901). Vide Snell, E. E. Advances in Protein Chemistry, New York, Academic Press, 2, 85 (1945).