there is no evidence for these in the plants examined and this is supported by the fact that compound II is short of free organic groups that can promote conjugation reactions and compound III was not found among the nonconjugated metabolites. Compound V, other than from hydrolysis of its conjugate, can eventually derive from action, only on metalaxyl, of the strongly acid medium used to hydrolyze conjugated compounds. As nonconjugated metalaxyl was completely extracted in the ethyl acetate–methanol phase and as it is a compound short of free hydroxyl, carboxyl, or amino groups, it could not be present as a conjugate; there is evidence that the conjugated metabolite is compound V. This is supported by the presence of compund V among nonconjugated compounds.

No conjugated metabolites were found in the solid residue.

In conclusion, although metalaxyl is generally regarded as a stable compound, particularly in the cultural conditions used in these experiments, it was found to be subject to a certain decomposition that lead to hydrolysis of the molecule, giving compound V, which would seem to be the only responsible metabolite for the formation of conjugated derivative and, in a cyclization, for giving nonconjugated metabolite II.

Registry No. II, 88945-75-9; III, 52888-49-0; IV, 67617-64-5; V, 87764-37-2; metalaxyl, 57837-19-1.

LITERATURE CITED

Marucchini, C.; Patumi, M.; Zazzerini, A. *J. Agric. Food Chem.* **1983**, *31*, 1123–1125.

Matsumura, F.; Krishna Murti, C. R. "Biodegradation of Pesticides"; Plenum press: New York, 1982.

Tafuri, F.; Businelli, M.; Giusquiani, P. L. *IWOSC Proc.* **1973**, 113–118.

Tafuri, F.; Businelli, M.; Patumi, M.; Marucchini, C. *Pestic. Sci.* **1977**, *8*, 331–336.

Tafuri, F.; Businelli, M.; Scarponi, L. *J. Sci. Food Agric.* **1972**, *23*, 1417–1423.

Tafuri, F.; Marucchini, C.; Patumi, M.; Businelli, M. *J. Agric. Food Chem.* **1981**, *29*, 1296–1298.

Received for review August 8, 1983. Revised manuscript received December 7, 1983. Accepted January 9, 1984.

Cultivar Differences in Gelling Characteristics of Soybean Glycinin

Takashi Nakamura, Shigeru Utsumi, Keisuke Kitamura, Kyuya Harada, and Tomohiko Mori*

The gelation and gel properties of glycinins were examined for five soybean cultivars having different subunit compositions. The gelling characteristics of glycinin differed significantly among cultivars, arising from the differences in the nature of protein itself as well as protein concentration. Glycinins of three of the cultivars studied formed a gel within half the time required for the other two cultivars, where the former cultivars contained acidic subunit AS-IV, which is linked with a basic subunit to form an intermediary subunit through some bonds other than disulfide bonds, and the latter cultivars did not contain acidic subunit AS-IV. Hardness of the gels was different among cultivars, depending on the percentage of AS-III, which is the largest constituent acidic subunit of glycinin. Turbidity of the gels had a tendency to increase with increasing content of sulfhydryl groups of glycinins.

The seed storage proteins of legumes contain legumin that occurs in large amounts and appears to be made up of twelve subunits, of which six subunits are acidic and six subunits are basic in nature (Derbyshire et al., 1976). The diversity of the subunit composition of legumin among cultivars has been shown by Harada (1972) and Kitamura et al. (1980) for soybean, by Tombs (1965) for groundnut, by Blagrove and Gillespie (1978) for lupin, by Thomson and Schroeder (1978) and Casey (1979) for pea, and by Utsumi et al. (1980) for broad bean. We have recently demonstrated that the subunit compositions of glycinins isolated from the seeds of various cultivars of soybean vary among the cultivars and may be classified into five groups according to differing molecular charges of the subunits (Mori et al., 1981).

The Research Institute for Food Science, Kyoto University, Uji, Kyoto 611, Japan (T.N., S.U., and T.M.), Department of Agriculture, Faculty of Agriculture, Iwate University, Morioka 020, Japan (K.K.), and National Institute of Agricultural Sciences, Minsitry of Agriculture, Forestry and Fishery, Tsukuba, Ibaraki 305, Japan (K.H).

On the other hand, it is generally known that the major components of seed storage proteins are responsible for contributing to the quality, particularly the physical properties, of foods made from these seeds, their flour, and protein products. It has been reported that the quality of tofu gel (a traditional Japanese food made from soybeans) differs according to the cultivars used (Smith et al., 1960; Saio et al., 1969; Wang et al., 1983). The diversity of subunit composition of the major components of seed storage proteins among cultivars is most likely to be related to the physical properties of the foods made. 11S globulin (referred to as glycinin), one of the major components of soybean storage protein, has been shown to have intermediary subunits (AB), disulfide-bonded acidic (A) and basic (B) subunits, and the 6 (AB) structure (Badley et al., 1975; Kitamura et al., 1976; Mori et al., 1979). The acidic subunits have been separated by DEAE-Sephadex column chromatography into four fractions designated as AS-I (M_r 34 800), AS-II (M_r 34 800), AS-III (M_r 38 000), and AS-IV (M_r 34 800) in the order of elution from the column (Mori et al., 1982a). Acidic subunits I, II, and III are linked to their basic subunit counterpart by disulfide bridges. However, AS-IV is an exception; the linkage is noncovalent

0021-8561/84/1432-0647$01.50/0

(Mori et al., 1979, 1981, 1982a). In a previous paper (Mori et al., 1982a), we investigated the formation of pseudoglycinins, which have different subunit compositions from the native glycinin in terms of reconstitution from combinations of the native acidic and basic subunits, and their gel hardness was examined. The acidic subunits contribute differently to the hardness of gels, and the acidic subunit (AS-III), having a larger molecular weight than the other acidic subunits, plays an important role for increasing the hardness of the gels. The studies reported here were conducted to investigate the relation of structural characteristics of protein to the gelation and gel properties of glycinin by utilizing glycinins isolated from various cultivars that have different subunit compositions compared to each other.

MATERIALS AND METHODS

Materials. Five cultivars of soybean seeds (*Glycine max*), Shiro Tsuru-no-ko, York, Hill, Matsuura, and Raiden, which belong to groups I, II, III, IV, and V, respectively, were obtained as described previously (Mori et al., 1981). DEAE-Sephadex A-50 was purchased from Pharmacia Co., Ltd. Sodium dodecyl sulfate ($NaDodSO_4$), extrapure reagent, was obtained from Wako Pure Chemicals (Japan). Urea and 2-mercaptoethanol, extrapure reagent, were obtained from Nakarai Chemicals (Japan). Other chemicals were guaranteed reagent grade.

Preparation of Glycinin. A crude glycinin fraction was prepared from soybeans according to the method of Thanh et al. (1975). Chromatographic fractionation of the crude glycinin fraction was performed on a column of DEAE-Sephadex A-50 as described previously (Mori et al., 1979), where the column was eluted with 35 mM potassium phosphate buffer (pH 7.6) containing 10 mM 2-mercaptoethanol, 0.02% NaN_3, and NaCl in a linear gradient concentration of 0.25 to 0.5 M.

Method of Gelation and Determination of Hardness of the Gel. Twenty-microliter aliquots of the protein solutions in 35 mM potassium phosphate buffer (pH 7.6) containing 0.4 M NaCl (heating buffer) were taken in micropipets (Drummond Scientific Co., 200 μL) and heated at 100 °C for 20 min in a water bath, and the hardness of gels formed was then measured with a texturometer (General Foods Corp., GXT-2). The hardness of gels was expressed as a texturometer unit (kgw). The details of the procedure have been described in the previous papers (Mori et al., 1982a; Utsumi et al., 1982). On the other hand, in order to determine the minimum time for gelation, the glcyinin solutions of various protein concentration were heated and the formation of self-supporting gel was examined at time intervals of either 15 s or 1 min depending on the protein concentration.

Measurement of Turbidity of the Gel. The gels formed in the micropipets as described above were scanned as such on a Shimadzu dual-wavelength chromatoscanner, Model CS-910. Scanning was carried out at 600 and 750 nm for the "sample" and "reference", respectively.

Heating of Glycinin. Fifty-microliter aliquots of the glycinin solutions of 5% protein concentration in the heating buffer were heated at 100 °C in thin test tubes as described previously (Mori et al., 1982b).

Sucrose Density Gradient Centrifugation. The heat-treated glycinin solutions were centrifuged at 20 °C in 12 mL of 15–40% (w/v) linear sucrose gradient in the heating buffer at 152000*g* for 60 min in a Hitachi RPS 40T rotor. After centrifugation, the gradient was examined for absorbance at 280 nm with an ISCO density gradient fractionator. For sucrose gradient centrifugation, 2.5 mg of protein was used per experiment.

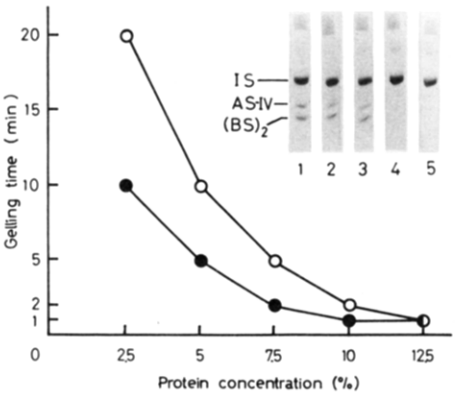

Figure 1. Minimum time for gelation of glycinin as a function of protein concentration. Soybean cultivars: (●) Shiro Tsuru-no-ko, York, and Hill; (○) Matsuura and Raiden. Inset shows the $NaDodSO_4$–polyacrylamide gel electrophoretic profile in the absence of 2-mercaptoethanol of glycinins: 1, Shiro Tsuru-no-ko; 2, York; 3, Hill; 4, Matsuura; 5, Raiden. IS, intermediary subunits in which the acidic and basic subunits are linked by disulfide bridges in a 1:1 ratio; $(BS)_2$, dimeric form of the basic subunits; AS-IV, acidic subunit of glycinin described in the text.

Electrophoresis. $NaDodSO_4$ gel electrophoresis was performed according to the method of Laemmli (1970) at room temperature with 10% polyacrylamide gels in the presence or absence of 2-mercaptoethanol, and then the gels were stained with amido black 10B as described previously (Mori and Utsumi, 1979). Fifty micrograms of each glycinin was used for electrophoresis.

Determination of Percentage of AS-III of Glycinin. The glycinins were electrophoresed on $NaDodSO_4$–polyacrylamide gels in the presence of 2-mercaptoethanol as described above. The percentage of AS-III, which is the high molecular weight acidic subunit of glycinin (Mori et al., 1982a), was calculated from densitometric scanning of the gel.

Determination of Sulfhydryl Groups. The sulfhydryl groups in glycinin were measured by the method of Ellman (1959). In the case of the measurement of surface sufhydryl groups, 0.05 mL of protein solution (150 mg/mL) was mixed with 0.7 mL of 35 mM potassium phosphate buffer (pH 7.6) containing 0.4 M NaCl. Subsequently 0.05 mL of DTNB reagent [39.6 mg of 5,5′-dithiobis(2-nitrobenzoic acid) in 10 mL of phosphate buffer (μ = 0.1, pH 7.0)] was added to the above solution, and after 7 min the color developed was measured at 412 nm. The total sulfhydryl groups were measured by a similar procedure as described above except that the phosphate buffer containing 8 M urea was used. A molar extinction coefficient of 13 600 was used for estimating the number of sulfhydryl groups.

Protein Determination. Protein was determined by the method of Lowry et al. (1951).

RESULTS AND DISCUSSION

Gelation. The glycinins isolated from soybean seeds of five cultivars were heated for appropriate times, and the minimum times required for the formation of self-supporting gel were measured. The degree of gelation of sol, gellike but not self-supporting, and self-supporting gels could be judged macroscopically. The higher the protein concentration, the shorter the time required for gel formation, in all the cultivars, as shown in Figure 1. However, gelation behavior could be classified into two groups: one group (Shiro Tsuru-no-ko, York, and Hill) gelled within half the time required for the other group (Matsuura and Raiden). Of all the cultivars studied those which contained acidic subunit AS-IV (Figure 1, inset) showed

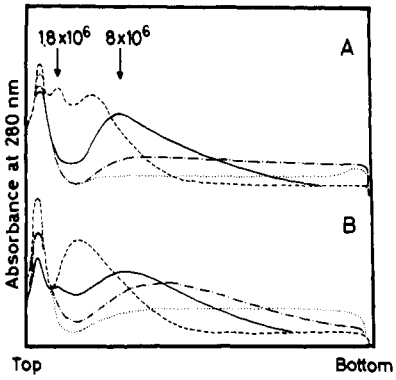

Figure 2. Sucrose density gradient centrifugation of heated glycinin. Soybean cultivars: (A) Shiro Tsuru-no-ko; (B) Matsuura. Heating time: (- - -) 30 s; (—) 1 min; (-·-), 2 min; (···) 4 min. Figures indicate molecular weights that were evaluated by using ribosome and tobacco mosaic virus as the molecular weight markers as described previously (Mori et al., 1982b).

distinctly shorter gelling time than those that did not contain AS-IV.

It has previously been demonstrated that a soluble aggregate with a molecular weight of 8×10^6 is formed at early stages of the heat-induced gelling process and subsequent heating causes formation of highly polymerized aggregates (Mori et al., 1982b). The rates of formation of soluble aggregate and subsequent polymerization of the glycinins were examined by sucrose density gradient centrifugation. As shown in Figure 2, the soluble aggregate was formed at 1 min of heating and subsequent polymerization proceeded, resulting in the formation of an aggregate that sedimented to the bottom of centrifugation tube at 2 min in the case of Shiro Tsuru-no-ko, while, in the case of Matsuura, the formation of soluble aggregate and subsequent polymerization were still under way at 1 and 2 min, respectively. York and Hill and Raiden exhibited similar behavior to Shiro Tsuru-no-ko and Matsuura, respectively (data not shown). Our recent investigation concerning the mechanism of the formation of soluble aggregate reveals that AS-IV is liberated during the formation of the soluble aggregate in the case of Shiro Tsuru-no-ko (unpublished experiments). As mentioned earlier, AS-IV is linked to its basic subunit counterpart only through noncovalent interactions, which could break due to the heat treatment. Liberation of AS-IV could result in conformational change of the glycinin molecule and stimulate the formation of soluble aggregate and subsequent polymerization resulting in rapid gel formation. The existence of AS-IV is similar in Hill and York (Figure 1, inset). Therefore, it may be assumed that in these cultivars too, the formation of soluble aggregates may be preceded by the release of AS-IV. Thus, AS-IV may be responsible for the differences in gelation time of these two groups.

Hardness of Gels. The glycinins of five soybean cultivars were heated to make gels, and the hardness of the gels was determined (Figure 3). The gel hardness increased with time of heating and then reaches a plateau at 20 min (Utsumi et al., 1982). The lowest gelation concentration for glycinin was 2.5% in all the cultivars, and gel hardness increased with increasing concentrations of protein. However, the gel hardness of the gylcinins studied differed at the higher protein concentrations: Raiden exhibited the highest gel hardness followed by Shiro Tsuru-no-ko, York, Hill, and finally Matsuura. Since we have previously demonstrated that the high molecular weight acidic subunit (AS-III) mainly contributes to the gel hardness (Mori et al., 1982a), the percentage of AS-III

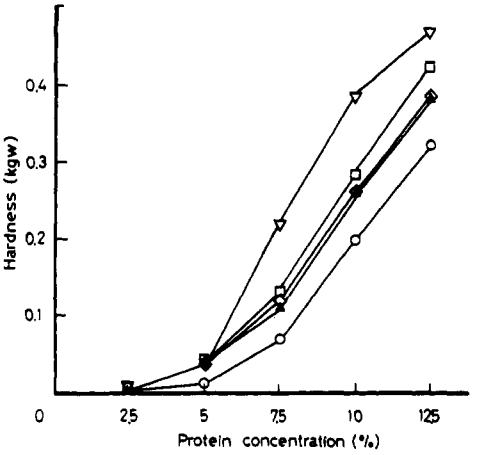

Figure 3. Hardness of heat-induced gels of glycinin as a function of protein concentration. Soyben cultivars: (□) Shiro Tsuru-no-ko; (▲) York; (◇) Hill; (○) Matsuura; (▽) Raiden.

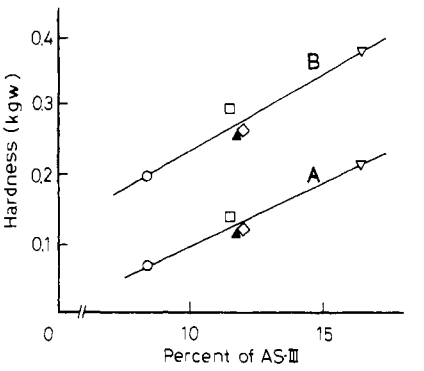

Figure 4. Relationship between gel hardness and percent of AS-III of glycinin. The glycinin solutions used were 7.5% (A) and 10% (B). The gel hardness was plotted against the percent of AS-III (expressed in percent of total acidic subunits) of the corresponding glycinin. The indicators of soybean cultivars were similar to those in Figure 3.

of the glycinins and their relation to the gel hardness were examined. As expected, the hardness of the gels was directly proportional to the percentage of AS-III as shown in Figure 4.

The acidic and basic subunits are linked together in specific combinations with the resulting formation of intermediary subunits in the glycinin molecules (Kitamura et al., 1976; Staswick et al., 1981). It has recently been reported that the acidic and basic subunits are synthesized as high molecular weight precursors of a single polypeptide chain (Tumer et al., 1982; Barton et al., 1982), which is analogous to the synthesis of legumin from *Pisum* and *Vicia*, as has been reported by Croy et al. (1980, 1982). These facts indicate that each acidic subunit is always paired with a specific basic subunit; that is, the difference in percent of AS-III among cultivars is equivalent to that of the paired basic subunit. Whether AS-III and/or its paired basic subunit contribute to gel hardness is not clear since we have not yet examined the paried basic subunit.

Turbidity of Gels. The glycinins of five soybean cultivars were heated to make gels, and the turbidity of the gels was determined (Figure 5). The gel turbidity decreased with increasing protein concentration in all the cultivars. The gel turbidity differed among cultivars: Matsuura exhibited the highest turbidity, followed by Shiro Tsuru-no-ko, York, Hill, and finally Raiden. The difference between the turbidity of Matsuura and the other cultivars was significant. It has been reported that the source of turbidity was the basic subunits of glycinin, which are dissociated from glycinin during heating (Hashizume

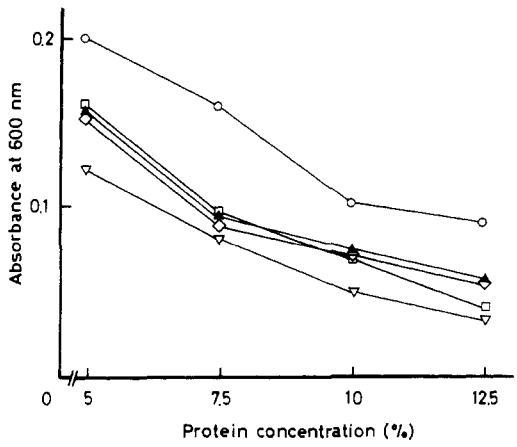

Figure 5. Turbidity of heat-induced gels of glycinin as a function of protein concentration. The indicators of soybean cultivars were similar to those in Figure 3.

Table I. Amount of Sulfhydryl Groups of Glycinin

cultivars	amount of sulfhydryl groups, mol/mol of glycinin		
	surface	internal[a]	total
Shiro Tsuru-no-ko	0.4	1.2	1.6
York	0.5	1.2	1.7
Hill	0.5	1.2	1.7
Matsuura	1.4	1.4	2.8
Raiden	0.5	1.4	1.9

[a] Internal was calculated by subtracting surface from total.

and Watanabe, 1979; Yamagishi et al., 1980; Mori et al., 1982b). The dissociation of basic subunits is accelerated by 2-mercaptoethanol and depressed by a sulfhydryl-blocking agent of *N*-ethylmaleimide (Wolf and Tamura, 1969; Mori et al., 1982b). Considering this and the fact that the basic and acidic subunits are linked by disulfide bridges in a 1:1 ratio (Kitamura et al., 1976; Mori et al., 1979), a sulfhydryl group may be correlated with the turbidity generated by the intermolecular disulfide exchange reactions between the acidic and basic subunits. Draper and Catsimpoolas (1978) have demonstrated that glycinin contains two sulfhydryl groups per mol of protein. When the sulfhydryl groups of each glycinin of five cultivars were determined, the number of sulfhydryl group differed among cultivars (Table I). The glycinin of Matsuura contained approximately three sulfhydryl groups per mol of protein, while that of the other cultivars contained two. The number of internal sulfhydryl groups did not differ significantly among cultivars. The additional sulfhydryl group in Matsuura was on the surface. The difference in turbidity of the gels between Matsuura and the other cultivars thus correlated well with the content of sulfhydryl groups. On the other hand, the addition of cysteine to the glycinin solution of Raiden, which exhibited the lowest turbidity, caused an increase in the turbidity of heat-induced gel linearly with cysteine concentration as shown in Figure 6. Due to the addition of cysteine to a level of more than 3 mol/mol of glycinin, where the total sulfhydryl groups in the heating system becomes more than 5 mol/mol of glycinin of Raiden, the turbidity of Raiden became the same as that of Matsuura (Figures 5 and 6). This may be because free cysteine molecules do not necessarily exhibit similar reactivity as the sulfhydryl groups in glycinin molecule. The cysteine residues in glycinins of different cultivars may have different degrees of reactivity, thus resulting in small differences in the

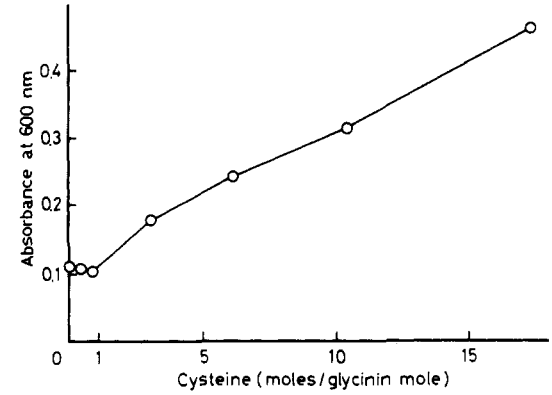

Figure 6. Effect of exogenous sulfhydryl groups addition on gel turbidity of glycinin. The glycinin of Raiden (5% solution) was heated to make gels in the presence of cysteine.

turbidity. Even though Raiden contained higher amount of sulfhydryl group (Table I) than Shiro Tsuru-no-ko, York, and Hill, the former showed lower turbidity (Figure 5) than the rest.

We are aware that the phenomenon of gelation is complex and still not completely elucidated. From the present study, we have evidence that the content of AS-III and the content and reactivity of sulfhydryl groups in glycinin molecule relate to the hardness and turbidity of gels, respectively. Besides this, there may be additional contributors to the above phenomenon; for example, the release of basic subunits may affect the nature of the soluble aggregate strands, which is a unit of the networks of gel (Nakamura et al., 1984), and thereby contribute to the hardness of gel. The inverse relationship of gel hardness and turbidity (Figures 3 and 5) points toward that direction.

LITERATURE CITED

Badley, R. A.; Atkinson, D.; Hauser, H.; Oldani, D.; Green, J. P.; Stubbs, J. M. *Biochim. Biophys. Acta* **1975**, *412*, 214.
Barton, K. A.; Thompson, J. F.; Madison, J. T.; Rosenthal, R; Javis, N. P.; Beachy, R. N. *J. Biol. Chem.* **1982**, *257*, 6089.
Blagrove, R. J.; Gillespie, J. M. *Aust. J. Plant Physiol.* **1978**, *5*, 651.
Casey, R. *Biochem. J.* **1979**, *177*, 509.
Croy, R. R. D.; Gatehouse, J. A.; Evans, I. M.; Boulter, D. *Planta* **1980**, *148*, 49.
Croy, R. R. D.; Lycett, G. W.; Gatehouse, J. A.; Yarwood, J. N.; Boulter, D. *Nature (London)* **1982**, *295*, 76.
Derbyshire, E.; Wright D. J.; Boulter, D. *Phytochemistry* **1976**, *15*, 3.
Draper, M.; Catsimpoolas, N. *Cereal Chem.* **1978**, *55*, 16.
Ellman, G. L. *Arch. Biochem. Biophys.* **1959**, *32*, 70.
Harada, K. *Ikushugaku Zasshi* **1972**, *22* (Suppl. 2), 108.
Hashizume, K.; Watanabe, T. *Agric. Biol. Chem.* **1979**, *43*, 683.
Kitamura, K.; Takagi, T.; Shibasaki, K. *Agric. Biol. Chem.* **1976**, *40*, 1837.
Kitamura, K.; Toyokawa, Y.; Harada, K. *Phytochemistry* **1980**, *19*, 1841.
Laemmli, U. K. *Nature (London)* **1970**, *227*, 680.
Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.
Mori, T.; Nakamura, T.; Utsumi, S. *J. Agric. Food Chem.* **1982a**, *30*, 828.
Mori, T.; Nakamura, T.; Utsumi, S. *J. Food Sci.* **1982b**, *47*, 26.
Mori, T.; Utsumi, S. *Agric. Biol. Chem.* **1979**, *43*, 577.
Mori, T.; Utsumi, S.; Inaba, H. *Agric. Biol. Chem.* **1979**, *43*, 2317.
Mori, T.; Utsumi, S.; Inaba, H.; Kitamura, K.; Harada, K. *J. Agric. Food Chem.* **1981**, *29*, 20.
Nakamura, T.; Utsumi, S.; Mori, T. *J. Agric. Food Chem.* **1984**, *32*, 349.
Saio, K.; Kamiya, M.; Watanabe, T. *Agric. Biol. Chem.* **1969**, *33*, 1301.

Smith, A. K.; Watanabe, T.; Nash, A. M. *Food Technol. (Chicago)* **1960**, *14*, 332.
Staswick, P. E.; Hermodson, M. A.; Nielsen, N. C. *J. Biol. Chem.* **1981**, *256*, 8752.
Thanh, V. H.; Okubo, K.; Shibasaki, K. *Plant Physiol.* **1975**, *56*, 19.
Thomson, J. A.; Schroeder, H. E. *Aust. J. Plant Physiol.* **1978**, *5*, 281.
Tombs, M. P. *Biochem. J.* **1965**, *96*, 119.
Tumer, N. E.; Richter, J. D.; Nielsen, N. C. *J. Biol. Chem.* **1982**, *257*, 4016.
Utsumi, S.; Nakamura, T.; Mori, T. *Agric. Biol. Chem.* **1982**, *46*, 1923.
Utsumi, S.; Yokoyama, Z.; Mori, T. *Agric. Biol. Chem.* **1980**, *44*, 595.
Wang, H. L.; Swain, E. W.; Kwolek, W. F. *Cereal Chem.* **1983**, *60*, 245.
Wolf, W. J.; Tamura, T. *Cereal Chem.* **1969**, *46*, 331.
Yamagishi, T.; Yamauchi, F.; Shibasaki, K. *Agric. Biol. Chem.* **1980**, *44*, 1575.

Received for review October 28, 1983. Accepted February 13, 1984. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (No. 57760101).

Pattern Recognition Analysis of Fatty Acids. Application to Beef Fat Tissue Classification

Emile M. Gaydou,* Marie-Christine Iatrides, Romaine Ramananarivo, Jacques Artaud, and Gilbert Peiffer

Thirty-six fat samples of hump, kidney, and depot fat of Malagasy zebu (*Bos indicus*), eight samples of kidney and tallow of *Bos taurus*, and two butter samples of *Bos taurus* were analyzed for 36 fatty acids by gas–liquid chromatography (GLC). Repeatability and GLC reproducibility analyses of the main fatty acids were checked. Intercorrelations among the fatty acids and their relations to fat tissue origins were investigated by analyzing the data with pattern recognition techniques. Nineteen fatty acids and four combinations of measurements were used for principal component analysis (PCA) and discriminant analysis (DA). By DA, 36 fat tissue samples of hump (15) and kidney (15) of *B. indicus* and tallow (6) of *B. taurus* were classed in three categories. Supplementary fat tissues were also successfully checked. Misclassifications were observed by DA, when only the main fatty acids were used.

Nutritionists need detailed data on the amounts of fatty acids in foods, in order to evaluate current dietary habits and to execute nutritional research. Such data would require quantitative analysis of a vast number of foods. The use of conventional gas chromatography has allowed the identification of many fatty acids in beef tallows. The latest advances in glass capillary gas chromatography enable even finer separation of fatty acids in complex mixtures such as depot fats and fats from organs of monogastric species, polygastric species, and fishes (Flanzy et al., 1976; Ramananarivo et al., 1981). The use of long (60 and 100 m) and highly efficient glass capillary columns can solve the problems of the separation between peaks, the positional isomers, and the separation of the geometric isomers (cis and trans) for the determination of the amount of trans fatty acids (Slover and Lanza, 1979). However, the analysis is relatively long (1–3 h) with animal fats. The use of short glass capillary columns reduces considerably analysis time, and a comparative study on 100-, 10-, and 2-m glass capillary columns coated with SP 2340 was made by Lanza et al. (1980). The identification of every fatty acid can be time-consuming, and generally the workers limit their studies to fatty acids having concentrations higher than 0.1%.

In this study, the fatty acid composition of various depot fats of French beef lipids (*Bos taurus*) and Malagasy zebu lipids (*Bos indicus*) was investigated. The repeatability and GLC reproducibility will be discussed. Multivariate statistical techniques, including discriminant and cluster analyses, which have been successfully applied in enological research (Kwan and Kowalski, 1980; Noble et al., 1980), were used to distinguish among various animal depot fats. Pattern recognition techniques were applied to 46 samples and proved successful in the distinction of various origin depot fats. The data presented in this investigation show the importance of minor fatty acids for the characterization of beef fat tissues.

EXPERIMENTAL SECTION

Fat Samples. Fifteen samples of hump tissue and fifteen samples of kidney fat, extracted from fifteen male zebu animals (*B. indicus*) were given by Sevima Co. (Antananarivo, Madagascar). Three kidney tissue samples and three external depot fat samples of zebu were collected from a local market in Antananarivo. Six beef tallow samples, two kidney fat samples, and two samples of butter (*B. taurus*) were collected from a local market in Marseilles.

Physicochemical Determinations. Determination of the iodine value (Wijs) and the saponification value were

Laboratoire de Phytochimie, Université de Droit, d'Economie et des Sciences, Centre de St. Jérôme, 13397 Marseille Cedex 13, France (E.M.G.), and Laboratoire des Organo-Phosphorés et Institut Universitaire de Technologie, 13337 Marseille Cedex 14, France (M.-C.I., R.R., J.A., and G.P.).

0021-8561/84/1432-0651$01.50/0