

TABLE II

Maillard Intermediates as Flavor Precursors

Formula	Compound
$\text{HO-CH}_2\text{-(CHOH)}_n\text{-CO-CH}_2\text{-NH-CRH-COOH}$	1-amino-1-deoxy-2-ketose (Amadori rearrangement product)
$\text{HO-CH}_2\text{-CHO}$	Glycolaldehyde
CHO-CHO	Glyoxal
$\text{CH}_3\text{-CO-CHO}$	Pyruvaldehyde
$\text{HO-CH}_2\text{-CO-CH}_2\text{-OH}$	Dihydroxyacetone
$\text{HO-CH}_2\text{-CHOH-CHO}$	Glyceraldehyde
$\text{CH}_3\text{-CHOH-CO-CH}_3$	Acetoin
$\text{CH}_3\text{-CO-CO-CH}_3$	Diacetyl
$\text{CH}_3\text{-CO-CO-CH}_2\text{-OH}$	Hydroxydiacetyl
$\text{H}_3\text{C-CHSH-S-CH}_3$	1-methyl thio ethanethiol
$\text{HO} \begin{array}{c} \diagdown \\ \diagup \end{array} \begin{array}{c} \diagup \\ \diagdown \end{array} \text{O}$	Hydroxy furanone
$\text{R}_1 \begin{array}{c} \diagdown \\ \diagup \end{array} \begin{array}{c} \diagup \\ \diagdown \end{array} \text{O} \text{R}_2$	
$\text{HS-CH}_2\text{-CHNH}_2\text{-COOH}$	Cysteine

quired to give the right impact.

Second, interaction frequently takes place between flavor compounds and the soy proteins, which may lead to a change in character of the flavor. Especially the interaction with some spices has a dramatic effect, sometimes leading to new off-flavors.

Third, when flavors are added before texturing, the heat treatment may change the character of the flavor. Hydrolyzates and reaction flavors are generally a failure as they turn into a burnt and bitter taint.

An elegant way to incorporate positive flavors in a bland soy protein material is to utilize the Maillard reactions,

which in many heat-treated foods are responsible for flavor formation. Suitable precursor mixtures can be added to the soy protein material before extrusion in such levels that the texturing process provides the right conditions for the reactions. The optimum precursors are well known intermediates in the Maillard reaction, like short chain aldehydes and ketones, sulfur compounds, or cysteine as a hydrogen sulfide releasing agent, and furanones. Some examples are listed in Table II.

The use of Maillard reaction intermediates provides a promising method to utilize the texturing process for incorporating positive flavors in refined soy protein products.

The Role of Processing in Changing Protein Characteristics

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ABSTRACT

In our efforts to design and produce proteins which deliver optimal benefits in products both reproducibly and cost effectively, we must keep in mind the following points. Protein "functionality" is only meaningful in a specific product and process context. Techniques are becoming available to investigate "molecular protein characteristics;" how we can best use them to understand how proteins contribute to "product attributes" remains to be elucidated. Different proteins respond differently to typical process regimes. Use of these proteins as functional ingredients may require formulation or process modifications.

The underlying theme of this presentation is embodied in the question: "Is it possible to relate protein molecular characteristics to the observed contribution of those proteins to product attributes, in a way which is both meaningful to the ingredient supplier, and to the product developer?" Therefore, I shall draw attention to some experimental observations which seem to be – intuitively at least – perhaps to relate to this rather fundamental question. What I shall not do is present hard data which could suggest that the answer is an emphatic yes!

In any food product, the consumer-perceived product quality reflects many interrelated factors. Two of the most important are: (a) the juxtaposition of the different com-

ponents – fat, meat fibres, water etc. – which have intrinsically different textural characteristics, (e.g., hardness, juiciness) as well as different economic parameters; and (b) the strength of the interactions, or bonds between the ingredients, which underlies how the components will hold together during processing and product manufacture, and will then be broken down during consumption by the consumer. In theory at least, the key contribution of protein to the consumer-perceived attributes of food products reflects: the intrinsic *molecular characteristics* of the protein, e.g. molecular weight, amino acid composition, sequence etc.; the *processing history* of the protein both to become a discrete ingredient, and/or during product manufacture; the physical and chemical *environment* of the protein ingredient, at all stages during isolation and utilization.

TABLE I

Protein Characteristic or Functionality Evaluated-Fat Stabilization (e.g., for Meat Products)

Approach adopted:

- Evaluate technical performance of a range of proteins, under simulated product and process conditions.
- Determine "molecular protein characteristics" by appropriate physical techniques, such as, N.M.R. – availability, flexibility of apolar residues; D.S.C. – "conformational state" of the proteins.
- Correlate observations from a.) and b.).

TABLE II

Evaluation of Emulsion Stability

Protein	Emulsion prepared		Conventional protein descriptions
	"Cold"	"Hot"	
"Caseinate"	a	b	Aggregated, random coil protein
Blood albumen	c	b	Globular protein
Soy ingredient "a"	c	b	Aggregated, multi-subunit protein
Soy ingredient "b"	b	b	Aggregated, multi-subunit protein

aVery unstable, considerable breakdown.
bVery stable, essentially no breakdown.
cModerately stable, some fat release.

TABLE III

Molecular Characteristics of Protein Ingredients

Protein		N.M.R.	D.S.C.	Comments
"Caseinate"	Cold ^a	Essentially No Apolar Signal		Apolar residues present, but not available under cold preparation conditions?
	Hot ^b	Strong Apolar Signal	No transitions	
Blood Albumen	Cold ^c	Some Apolar Signal		Heating increases the number/availability of Apolar residues?
	Hot ^b	Strong Apolar Signal	Unfolding transition indicated.	
Soy ingredient "a"	Cold ^c	Some Apolar Signal		Heating increases the number/availability of apolar residues?
	Hot ^b	Strong Apolar Signal	Unfolding transition indicated.	
Soy ingredient "b"	Cold ^b	Strong Apolar Signal		Processing has generated an ingredient with optimal apolar residue availability.
	Hot ^b	Strong Apolar Signal	No unfolding indicated	

^aVery unstable, considerable breakdown.

^bVery stable, essentially no breakdown.

^cModerately stable, some fat release.

To explore the question raised above, I would like to focus our attention on one key protein functionality — that is the ability to emulsify and stabilize fat in a typical meat product environment. Several proteins, blood albumen, caseinate, and various soy ingredients, are widely used for this purpose in current commercial practice in various countries.

The approach adopted is summarized in Table I. I have chosen four proteins — none of them as pure and well characterized as a rigorous scientific experimentation would require. I have chosen them deliberately not only because they are being used commercially, but also because their molecular structure is known to be significantly changed by typical process conditions such as, for example, heating.

If we are concerned with fat emulsification and stabilization, it seems reasonable to assume that the apolar amino acids of the proteins might be somehow involved. Furthermore, since the effects of processing conditions (e.g., heating), might change the molecular structure of the protein thereby either increasing or decreasing the number and availability of the apolar amino acids, techniques are needed which should inform us of these apolar amino acids and how they may be influenced by typical process regimes. The techniques of nuclear magnetic resonance (N.M.R.) and differential scanning calorimetry (D.S.C.) would seem, in theory at least, to be well suited to this purpose.

The first of these techniques is likely to be of interest, because in principle at least, N.M.R. analysis enables us both to detect the presence of different broad classes of functional amino acids, i.e., the apolar, hydrophobic amino acids leucine, valine etc., and the more hydrophilic residues such as lysine, glutamic acid, etc., and also to monitor their structural availability and flexibility. D.S.C. analysis is complementary since this technique enables us to determine something about "comparative stability" of proteins during thermal processing, and the protein under going a conventional, cooperative polypeptide chain unfolding "denaturation."

Unfortunately, for technical reasons I have had to *infer* the required protein characteristics from the available literature rather than measure them directly in the product situations under discussion — this, hopefully, is acceptable in the context of the round table discussion.

I have been able to show that NMR signals from apolar amino acid residues appear at characteristic regions (0.9 and 7.5 delta). A comparison of soy protein heated to 30 and 90 C shows a substantial increase in signals at 0.9 and 7.5.

Before discussing the experimental results in detail, I need to mention that the act of emulsifying fat in a predominantly continuous aqueous environment needs an emulsifier. Conventionally we think of the emulsifier — of which certain proteins can be considered highly efficient, biologically important examples — as being amphipathic, i.e., having (because of their intrinsic bimodal distribution of polar and apolar moieties) the ability to bridge the aqueous and nonaqueous phases. We need to bear in mind that other mechanisms of emulsification are now well established, and that the stabilization of fat in typical meat products certainly involves many other parameters — e.g., viscosity effects — than I have time to consider today. In the work to be schematically outlined, I believe that the initial act of fat emulsification at least may reflect the contribution of the apolar residues of the various protein ingredients.

It is appropriate to outline how the emulsions are prepared and evaluated. These emulsions were made in the conventional way in a small scale bowl chopper, under either hot or cold (both terms being relative) conditions. The stability of the emulsions was assessed by a simple centrifugation test after heating to 95 C.

The technologically related results are shown in Table II, and I have used a very simple coding to indicate the effectiveness of the various proteins in emulsifying and stabilizing the fat in this simple model system.

It is essential to draw attention to four points in Table II.

1. First, and most important, these results refer to only one set of conditions (e.g., protein concentrations, mixing regime, etc.). They are chosen for illustration only as it is well known that the technical performance of protein stabilizers does exhibit significant concentration effects.
2. Table II indicates the descriptions conventionally applied to these proteins and illustrates that they do have very different intrinsic molecular characteristics as previously mentioned.
3. Under the very schematic conditions outlined, we see an increase in technical performance depending upon whether the preparation regime was hot or cold.
4. Under cold processing conditions, there also appears to be a difference in technical performance among the various ingredients.

In Table III these technological observations have been combined with the molecular characteristics as they have been *inferred* from the best available N.M.R., or D.S.C. data. The author feels it appropriate to stress that the information in Table III is intended to be illustrative rather than definitive.

Comparing the relative performance of the four proteins under cold preparation conditions, caseinate would seem (under the specific conditions used) to be the least effective. All of the other proteins considered, however, appear to have significant emulsifying ability even under cold preparation conditions. When we look at the N.M.R. results shown in Table III, there does seem to be a possible correlation between the N.M.R. observable apolar residues and emulsifying ability. Thus the *actual* presence, and *availability* of appropriate apolar residues would seem to be a necessary protein characteristic in the initial fat emulsification being discussed. This is further substantiated by the effectiveness of caseinate under hot preparation conditions, and the observation of a well developed apolar residue N.M.R. spectrum at higher temperatures. Blood albumen has some emulsifying ability under cold preparation conditions, but this ability is clearly enhanced under hot preparation conditions. The combined N.M.R., and D.S.C. results for albumen show that the availability-mobility of the apolar residues increase with temperature (N.M.R.), and that a molecular unfolding (denaturation) also occurs upon heating (D.S.C.). It is tempting to infer that increased emulsifying ability of albumen with temperature rise corre-

lates directly with increased *availability* of apolar residues.

The two soy ingredients differ in technical performance, but only soy ingredient "a" appears to undergo a molecular unfolding giving rise to a D.S.C. transition. The technological results would seem to be consistent with the statement that ingredient "a" undergoes a structural change upon heating allowing more apolar residues to become available. On the other hand, ingredient "b" has — during manufacture — clearly undergone some processing step resulting in a loss of native structure — hence, no D.S.C. transition is evident. However, the relatively modest indication of mobile apolar amino acids as indicated by the N.M.R. results cautions us not to seek too simple a correlation in this highly complex product environment!

Though detailed analysis of the kind of information schematically presented in Table III could suggest that there is a correlation between the state of the protein and the availability of the apolar amino acids and its fat emulsification ability, it is essential to bear in mind that many other factors (e.g., viscosity, geleation) are very likely to be involved in this situation. Furthermore, as stressed previously, the results are intended to be indicative of the types of information that it should now be possible to generate, rather than purporting to be the actual observations in those technological situations. However, combining the indications presented in Tables II and III should enable us to infer that in fat emulsification two protein characteristics of major importance are likely to be: a.) availability-distribution of polar-apolar residues both for solubility and amphipathicity; and b.) conformational flexibility at appropriate temperatures.

It is not possible to give a meaningful answer to the question raised in the introduction. The purpose of this presentation was merely to indicate that techniques and approaches may now be becoming available to enable us to ask the question in a scientifically acceptable way, and to draw attention to the magnitude of our ignorance of the role of processing in changing protein characteristics and particularly our ignorance of how protein characteristics really relate to product situations. It is suggested that considerable systematic work, possibly using techniques such as those briefly outlined in this presentation, and selecting very carefully defined model systems, could go some way towards rectifying this situation.

Yield and Functional Properties of Air-Classified Protein and Starch Fractions from Eight Legume Flours

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ABSTRACT

Eight legumes were pin-milled and air-classified into protein (fine) and starch (coarse) fractions and their functional properties compared with those of soybean and lupine flours. The fine material which represented 22.5 to 29% of the original flours contained from 29 to 66% protein as well as a high proportion of the flour lipids and ash. The coarse material contained 51 to 68% starch and much of the crude fiber which was dense and concentrated in the starch fraction. Generally legumes which showed highly efficient starch fractionation gave lower recoveries of protein in the fine material. High values

for oil absorption, oil emulsification, whippability and foam stability were characteristic of the protein fractions, while starch fractions gave high water absorptions, peak and cold viscosities. Gelation occurred in both air-classified fractions. Pea and northern bean, chickpea and lima bean flours, and air-classified fractions gave generally higher values in the functional property tests, while fababeans, field pea, mung bean and lentil gave high protein fractionation in the air classification process.

INTRODUCTION

Grain legumes are normally consumed as whole or split