Effect of Water Activity on Autoxidation of Raw Peanut Oil

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ABSTRACT

The effect of water activity (a_w) on rate of autoxidation of raw peanut oil at 37 C was studied. Up to 3 weeks of storage, no marked effect of a_w on the rate of peroxide formation was noticeable. However, with prolonged storage, the rate of peroxide formation decreased rapidly at a_w of 0.67 and higher as compared to a_w of 0.50 and lower. At the end of 9 weeks' storage, the peroxide value at a_w=0.02-0.50 was in the range of 31-35, while it was only ca. 10 at 0.79 and 0.92 a_w. There was no noticeable influence of a_w on the rate of free fatty acid formation. The oil stored for 7 weeks at 0.02 and 0.11 a_w had fresh flavor, although the peroxide values were high; whereas the oils at 0.79 and 0.92 a_w were stale, although the peroxide values were less than 10. The anisidine value at 0.02 a_w (348) was higher than at 0.92 a_w (62). The carbonyl values at 0.02 and 0.11 a_w were also higher (1090, 1021) than at 0.79 and 0.92 a_w (573, 780). The results indicate that the protective effect on the oil against peroxide formation at high a_w may be due to certain components present in raw peanut oil.

INTRODUCTION

Vegetable oils are usually stored and transported in the raw unrefined form for the overseas trade. In countries like India, more than 75% of the total edible vegetable oils is sold and consumed in the raw form. During processing and marketing the oils are exposed to the ambient humidity. The raw oils invariably contain gums which entrap moisture. Water plays an important role in the autoxidation of lipids (1). Recently it has been demonstrated, with freshly expressed raw walnut oil, that water activity (a_w), besides affecting the primary reactions, also influences the secondary reactions of autoxidation (2), which are more important from the angle of consumer acceptability. The Codex Alimentarius commission has specified a maximum peroxide value of 10 meq peroxide/kg oil as the limit of acceptability for all virgin and nonvirgin edible oils (3). Although much information is available on the influence of a_w on the autoxidation of lipids in model systems, the paucity of such information on the commercial virgin oils necessitated the present work wherein raw peanut oil has been used.

EXPERIMENTAL PROCEDURES

Materials

Fresh raw peanut oil was purchased from the local market. Its chemical characteristics are given in Table I.

Analyses

For carrying out the autoxidation studies at different a_w, 250-mL flasks with 100-mL side arm flasks (similar to Warburg flask) were used. Peanut oil (150 mL) was placed in the main flask and 15 mL of saturated salt solution were taken in the side arm of the flask to obtain the desired a_w (Table II). The flasks were then placed in a 37 C incubator and after 5 hr equilibration, the stoppers of each flask were closed. Periodically the samples were withdrawn for analysis.

Peroxide, free fatty acid and iodine values were determined by AOCS methods (4). Total carbonyl value was determined by passing a solution of the sample (1 g oil) in carbonyl free hexane (25 mL) through a column of 2,4-DNPH/phosphoric acid supported on celite. The effluent from the column was made up to a known volume and the optical density (OD) was read at 340 nm in a Beckman Du Spectrophotometer. The carbonyl concentration was calculated using an E=22,500 (5). Anisidine value was determined using 2-10 mg of the oil samples using the procedure of Jirousova (6). Total phospholipid content was estimated by digesting raw peanut oil (50 mg) with 70% perchloric acid (0.9 mL) on a sand bath until clear. Phosphorus content was estimated in the digested sample using the micromethod as outlined by Marinetti (7). The phosphorus content was multiplied by a factor of 25 to obtain the phospholipid content.

For determining the fatty acid composition, the oil was saponified and the liberated fatty acids were converted into methyl esters (FAME) using diazomethane (8). The FAME were analyzed using a CIC gas chromatograph equipped with a flame ionization detector (FID) under the following conditions: column 8 ft × 1/8 in. tubing packed with 15% diethylene glycol succinate supported on chromosorb w, 60-80 mesh; column temperature 185 C; carrier gas (nitrogen), 15 mL/min; and hydrogen 20 mL/min. The fatty acids were identified by comparing the retention time with

TABLE I

Chemical Characteristics of Raw Peanut Oil

Iodine value (wijs)	101.5
Fatty acid composition (%)	
16:0	15.6
18:0	1.1
18:1	48.5
18:2	29.2
20:0	3.7
24:0	1.9
Phospholipids (%)	0.52
Free fatty acids (as % oleic acid)	1.5
Peroxide value (meq peroxide/kg oil)	3.8
Total carbonyl value (μmol/kg oil)	721
Anisidine value (100 × OD of 1% fat solution)	20

TABLE II

Water Activity of Saturated Salt Solutions at 37 C

Saturated salt solution	a_w
Calcium chloride (fused) dry lumps only	0.02
Lithium chloride	0.11
Potassium acetate	0.22
Magnesium chloride	0.32
Potassium carbonate	0.44
Magnesium nitrate	0.50
Sodium acetate	0.67
Ammonium sulfate	0.79
Sodium phosphate dibasic	0.92

TABLE IV

Flavor Notes of Raw Peanut Oil Stored at Different a_w for Six Weeks at 37 C

a_w	Odor and taste evaluation by a panel of three members
Fresh oil	Fresh, slightly rancid, bitter after-taste
0.02	Fresh, slightly rancid, bitter after-taste
0.11	Fresh, no rancid smell, strong fresh flavor, no bitter after-taste
0.22	Rancid, lacks fresh flavor, no bitter after-taste
0.32	Rancid, bitter after-taste (less rancid compared to sample autoxidized at 0.79 a_w)
0.44	Rancid, highly bitter after-taste
0.50	Quite rancid, slightly bitter after-taste
0.67	Rancid, bitter after-taste
0.79	Rancid, lacks fresh flavor (less rancid compared to sample autoxidized at 0.22 a_w)
0.92	Stale, highly rancid, bitter after-taste, a different (other than rancid) off-flavor

those of authentic FAME. The area of the peak was measured by triangulation, and the results expressed as proportional weight percent of the components in the sample.

Sensory Evaluation

After 6 weeks' storage, the samples at each a_w were evaluated for odor and flavor by a panel consisting of the staff members of the laboratory. Samples were taken in duplicate for each evaluation and the odor and flavor as perceived by the panel were recorded (Table IV).

RESULTS AND DISCUSSION

Water activity (a_w) had no significant influence on the formation of free fatty acids (Table III). The peroxide values at a_w 0.02, 0.11, 0.22, 0.32, 0.44 and 0.50 increased uniformly with storage, there being no marked difference between them at each storage period (Table III). However, the peroxide values at these a_w were markedly higher than at a_w 0.67, 0.79 and 0.92 at each storage period, except at one week storage. The differences in the peroxide values between the lower range of a_w (0.02-0.50) and the higher range of a_w (0.67-0.92) became more distinct as the period of storage increased. The peroxide values particularly at a_w 0.79 and 0.92 were much lower (ca. 10 after 9 weeks of storage) compared to values at other a_w. The rate of oxygen uptake is known to be high at very low and very high a_w compared to medium a_w in oxidizing lipid model systems (1). The low peroxide values at high a_w (Table III) in the oxidizing raw peanut oil could be due either to some component(s) of the oil acting as antioxidant(s) only at high a_w or to decomposition of the peroxides formed faster than at lower a_w. However, the second possibility was less likely, since the panel evaluation on the 6 weeks' storage samples showed no intense rancid flavors (although a peculiar after-taste was noticed) (Table IV). Also, the carbonyl values at lower a_w (Table V) were noticeably higher than at a_w 0.79 and 0.92. The anisidine values were also considerably lower for 0.92 a_w compared to values for 0.02 a_w. If the rate of peroxide decomposition at high a_w were to be more than at low a_w, then this should be reflected in the secondary products content, i.e., the carbonyl and the anisidine values at 0.92 a_w should have been higher than at 0.02 a_w. As this was not so, faster rate of decomposition of the peroxides could not be the reason for low peroxide values recorded at high a_w. Incidentally, at lower a_w the oil had a fresh flavor although the peroxides were high; and at a_w 0.67 and above, the oil was stale and had a peculiar after-taste although the peroxide values were low. This could be due to the differences in the composition of

TABLE III

Effect of Water Activity on Peroxide and Free Fatty Acid Values in Autoxidizing Raw Peanut Oil Stored at Different a_w at 37 C

Storage period (weeks)	Water activity[c] 0.02		0.11		0.50		0.67		0.79		0.91	
	PV[a]	FFA[b]	PV	FFA	PV	FFA	PV	FFA	PV	FFA	PV	FFA
1	5.7	1.7	5.8	1.6	5.1	1.6	5.0	1.6	5.2	1.6	5.5	1.7
3	10.2	1.6	10.8	1.6	10.0	1.6	7.6	1.7	7.8	1.7	6.2	1.9
5	21.0	1.6	21.6	1.6	19.2	1.5	13.4	1.7	10.4	1.8	7.5	2.0
7	35.1	1.7	26.1	1.6	22.4	1.6	15.2	1.7	8.8	2.0	7.3	2.3
8	29.0	–	29.5	–	27.0	–	18.5	–	9.2	–	9.1	–
9	34.0	–	35.9	–	31.4	–	21.5	–	10.4	–	9.6	–

[a]PV: Peroxide value expressed as meq peroxide/kg oil; initial value 3.8 meq/kg.

[b]FFA: Free fatty acid value expressed as % oleic acid; initial value 1.5%.

[c]PV and FFA at 0.22-0.44 a_w are not given as they were almost similar to those at 0.02 and 0.11 a_w.

TABLE V

Effect of Water Activity on Total Carbonyl and Anisidine Values in Autoxidizing Raw Peanut Oil at 37 C Stored for Seven Weeks

Chemical parameter	Water activity 0.02	0.11	0.22	0.32	0.44	0.50	0.67	0.79	0.92
Total carbonyl value[a]	1090	1021	804	714	837	790	870	573	780
Anisidine value[b]	348	–	–	–	–	235	–	–	62

[a]Total carbonyl value expressed as μmol/kg oil. Initial total carbonyl value was 721 μmol/kg oil.

[b]Anisidine value expressed as OD × 100 of a 1% fat solution. Initial anisidine value was 20.

the secondary products of oxidation (2).

Verma and Prabhakar (9) have shown that the peroxide values in oxidizing refined safflower oil are similar at low and high a_W at each storage period. The unusual low peroxide values at high a_W, therefore, can be attributed to some components like phospholipids present in the raw peanut oil (degummed peanut oil has been found to oxidize rapidly at high water activities). Reports on antioxidant and synergistic effects of phospholipids are conflicting (10,11) and the information on the role of phospholipids in oils at different a_W is scanty. These studies indicate that it would be better to trade the raw oils at high a_W in the international markets as the rate of peroxide formation is minimum at high a_W.

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Protein Conformations and Their Stability

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ABSTRACT

Our understanding of the conformations of proteins and their stability has increased substantially in recent years. A reaction of considerable interest is native (N) $\rightleftharpoons$ denatured (D) where N is the globular, native state of the protein which is now well defined as a result of numerous structural determinations by X-ray diffraction studies, and D represents unfolded, denatured states of the protein whose structure depends on the denaturant used to promote unfolding. Through experimental studies much is known about the kinetics, thermodynamics, and mechanism of this reaction. For example, it is known that the free energy change for this reaction under physiological conditions, ΔG_D, is between 3 and 15 kcal/mol for a fairly wide range of globular proteins. Thus, the globular conformation which is absolutely essential for the biological function is only marginally stable. In addition, these ΔG_D values are remarkably sensitive to small changes in the structure of the protein. It has been shown that single amino acid substitutions can dramatically increase or decrease ΔG_D values and some substitutions surely lead to unfolding of the polypeptide chain. Most chemical alterations in the structure of a protein, e.g., cleavage of a peptide bond, or modification of an amino acid side chain, lead to decreases, often sizable, in the conformational stability. The remarkably low conformational stability of globular proteins is important, in part, because many properties of the protein, e.g., solubility and proteolytic digestibility, change substantially when the protein unfolds. Recent developments in these areas of interest to protein chemists and food scientists are illustrated and discussed.

INTRODUCTION

The important tasks in living systems are accomplished through specific interactions which depend on the globular, native conformations of proteins. (I suggest to my students that this is "the secret of life," at least at the molecular level.) The complexity and sophistication of some of these systems is awe inspiring. Thus, the interest and admiration which biochemists have for the globular conformation of proteins is understandable and is reflected in an eloquent statement by Richardson (1):

> "... high-resolution electron-density maps ... are like intricate, branched coral, intertwined but never touching. β-sheets do not show a stiff repetitious regularity but flow in graceful, twisting curves, and even the α-helix is regular more in the manner of a flower stem, whose branching nodes show the influences of environment, developmental history, and the evolution of each separate part to match its own idiosyncratic function."

The aim of the article is to review recent research on the conformational stability of globular proteins and related topics. Three recent reviews (2-4) and a book (5) have discussed conformational stability in more depth. Other reviews (6-8) and symposia (9,10) have considered protein folding from a more general point of view.

DISCUSSION

Protein Conformations

The best characterized conformation of proteins is clearly the unique, globular conformation that most proteins form spontaneously under physiological conditions in order to carry out their appointed task. This is exemplified by the title of Richardson's recent review, "The Anatomy and Taxonomy of Protein Structure" (1). Many interesting generalizations about details of the globular conformation are emerging as more and more crystal structures become available (1,6,7,11). Here we summarize some of the key features relative to conformational stability.

Figure 1 shows a globular protein model which emphasizes the difference in polarity between the interior and exterior of the molecule (see reference 11 for a more detailed