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BIOSYNTHESIS OF FAT IN RIPENING COCONUT: THE *IN VIVO* 'QUANTUM' SYNTHESIS OF TRIGLYCERIDES

By A. R. S. KARTHA

Fats from ripening coconut at oil development stages of 3–100% showed practically no acetyl value by a new sensitive gravimetric technique. It is suggested that biosynthesis of triglycerides in ripening coconut takes place by a 'quantum' mechanism, the unit of esterification consisting in the conversion of one glycerol to a triglyceride molecule.

Introduction

Until recently the idea current about fat synthesis in ripening seeds[1a] was that the free fatty acids were first synthesised and accumulated and were then gradually esterified to triglycerides as ripening progresses. This conception was based almost entirely on the fact that fats extracted from seeds in the earliest stages of ripening showed high acid values and that this progressively decreased during ripening to near zero figures in the fully ripe seeds.[1a] The delay in the synthesis of the triglycerides finally produced could be due to insufficient glycerol or lipase being present in the earlier stages[1b] or to the fact that concentration of water in the seed cells in the earlier stages is too great to permit of complete synthesis of neutral fat,[2] since it had been proved that under *in vitro* conditions the extent of esterification of glycerol and fatty acids by castor bean lipase[3] and also pancreas and intestinal lipases[4–6] was controlled by the proportions of water present. Although it has been known for a long time that fats from mature seeds show very low acetyl values and hence consisted almost exclusively of triglycerides,[7] no acetyl value determinations of fats from seeds in the earlier stages of ripening seem to have been recorded in the literature and this has left indeterminate the possible proportions of mono-, di- and tri-glycerides in the neutral fat fractions in unripe seeds.

Recent analyses of the free acidic fractions from seeds at different stages of ripening from mustard (*Brassica juncea*),[8] coconut (*Cocos nucifera*)[9] and the garden nasturtium (*Tropeolum minus*)[10] revealed that the free acids present in all these were entirely of a water-soluble type and did not contain more than traces of higher fatty acids, if any. This demonstrated that complete and immediate conversion of all higher fatty acids synthesised to neutral glycerides is taking place at all stages in the ripening seeds and that lipasic esterification of higher fatty acids present in ripening seeds is independent of the concentration of water present in the seeds. Further, there could also be no possible insufficiency of glycerol or lipase at any stage of the ripening.

There is therefore only one more aspect to be classified in the overall picture of esterification of fatty acids with glycerol in the fat depots, namely, whether the neutral fat consists entirely of triglycerides at all stages of ripening or whether appreciable proportions of mono- and di-glycerides are present during the earlier stages.

In vitro esterifications of glycerol and fatty acids by the agency of bacteriolipases,[11] castor bean lipase[12] and pancreas lipase[13] have invariably produced mixtures of mono-, di- and tri-glycerides. In the earlier stages of *in vitro* esterifications triglycerides are not detectable and the proportions of diglycerides themselves are small :[11] the relative proportions of di- and tri-glycerides increase progressively as esterification continues.[13] However, approximately complete conversion to triglycerides, as invariably happens in natural fat depots, has not so far been attained in *in vitro* esterifications. These results favour the view that the unit esterification involved in the *in vitro* esterifications is the conversion of an individual hydroxyl to an ester group, and it is generally considered that plant lipases (and also the animal lipases) esterify glycerol to triglycerides in three separate unit esterification stages. To visualise this one has to conceive of the lipase molecule adsorbing the glycerol at one of the three hydroxyls and desorbing the whole molecule as soon as the hydroxyl involved is converted to ester, the next hydroxyl being esterified only when it gets a chance to be adsorbed on another lipase molecule, which may perhaps arise only according to probability considerations. If the *in vivo* mechanism of esterification is the same as that *in vitro*, then measurable quantities of mono- and di-glycerides should be present in the fat from seeds in the earlier stages of ripening, although these would decrease progressively as ripening progresses.

Experimental

Coconut kernels at different stages of ripening were obtained from nuts at various stages of growth collected from the same tree at the same time. The nuts were dehusked immediately after being plucked from the tree, shells split at the middle and dried in the sun until the kernels dried partially and shrank somewhat, when the latter were removed from the shells with a knife. They were subsequently dried in the sun for a few days until they reached a hard consistency and then in an air oven at 60° to constant weight. The dried kernels were stored in glass bottles with ground stoppers until required for use. Two sets of kernels from two different trees were used for the studies : in one the total kernels including the brown testa at the bottom were dried as such[14] and in the second set the brown testa at the bottom of the kernels was carefully pared off with a sharp knife as soon as the kernel was removed from the shell and then subsequently dried to constant weight.[9] Both series had been earlier examined for oil content at different stages of growth of kernel.[9, 14] The results for the % development of kernel and oil at the different growth stages are reproduced in Table I.

For the present experiments 2–5 g. of the kernels were powdered with glass powder and anhydrous sodium sulphate (~1 g. for each g. of sample) and extracted by the cold percolation procedure[15, 16] with carbon tetrachloride as solvent (30–60 ml. depending on the weight of sample). The percolate passed directly to a weighed 100-ml. conical flask with ground-glass neck and the solvent was evaporated off on the water-bath to constant weight. The weight of oil was usually 1·5–3·0 g. and this was acetylated directly in the same flask without any further processing.

The *acetyl value* determination was conducted by a new gravimetric procedure recently developed in this laboratory.[17] This is conducted as follows : 1·5–3·0 g. of fat is acetylated by refluxing with 5 ml. of acetic anhydride for 2 h. in an all-glass apparatus with water-cooled condenser. After cooling, 15 ml. of 99–100% ethanol are introduced and the mixture refluxed for 1 h. to convert most of the excess acetic anhydride to ethyl acetate. The reaction mixture, after being cooled, is transferred to a weighed 150-ml. evaporating dish containing a cylindrical ring of filter paper, 10 cm. long and 1·2 cm. high, and the flask is washed out with a solvent mixture of 75% carbon tetrachloride and 25% ethanol (99–100%) in which the fat is freely soluble. The solvent is evaporated off on the water-bath, the filter-paper ring producing regular gentle boiling. The residue is evaporated to dryness repeatedly three times after addition of 5 ml. of the solvent mixture each time. It is then heated in an oven at 100–105° for 10 min., cooled and weighed. To ensure that all excess acetic anhydride has been removed,

Table I

Increase in weight on acetylation of coconut oils from unripe nuts at different stages of ripening

Stage No.	Time of collection (approx.), months before maturing	Kernel development %	Oil development, %	Wt. of oil acetylated	Increase in wt., mg. ion acetylation
		A. Total kernels including testa			
1	5	9·5	7·0	1·1642 1·1612	0·0 0·0
2	4	33·8	33·5	1·3134 1·3148	0·2 0·0
3	3	50·0	51·0	1·3130 1·3148	0·0 0·0
4	2	65·5	66·0	3·5312 3·5330	0·6 0·0
5	1	90·4	91·3	2·2018 2·2002	−0·2 −0·2
6	Mature nut	100·0	100·0	1·4298 1·4255	0·4 −0·4
	Control: castor oil			2·0442 2·1446	246·8 258·0
		B. White kernel alone (without testa)			
1	6	5·3	2·9	2·0966 2·0970	0·0 0·2
2	5	20·0	17·9	2·5318 2·5280	0·0 0·2
3	4	38·6	36·0	2·5936 2·5952	0·2 0·0
4	3	42·0	40·1	2·0092 2·0216	0·4 0·4
5	2	59·0	59·3	2·1904 2·1932	0·2 0·0
6	1	72·0	70·8	2·1696 2·1802	0·6 0·6
7	Mature nut	100·0	100·0	1·5928 1·6040	0·4 0·0
	Control: castor oil			2·0040 2·2932	241·0 275·6

the process of evaporating down with 5 ml. of the solvent mixture, heating for 10 min. at 100–105°, cooling and weighing is repeated until constant weight is reached, which usually occurs with the first re-weighing. The acetyl value is calculated from the increase in weight observed[17] which can easily be determined correct to 0·2 mg. by use of ordinary equipment. A blank determination is made on the acetic anhydride and blank value deducted from the experimental increase in weight.

Results

The increase in weight observed on acetylation of 13 samples of coconut oil from ripening nuts at different stages, of development, along with the % of kernel and % oil development at these stages are given in Table I. From these results it is seen that the carbon tetrachloride-extracted fats from the coconut kernels, whether unripe or ripe, show no detectable acetyl value at any stage whatever, since an increase in weight of 0·1% of the weight of sample corresponds to an acetyl value of 1·3[17] and in none of the samples was there any increase in weight of even 0·05%, corresponding to acetyl value of 0·6.

Discussion

The present results show that even at below 10% fat development, the fat present in the ripening coconut consists exclusively of triglycerides. This is not in agreement with the

lipasic esterification mechanism shown by *in vitro* studies wherein the unit of esterification consisted in the conversion of a single hydroxyl to ester group.

The absence of any detectable amount of mono- and di-glycerides at any stage of oil development in the coconut can be explained only on the assumption that during *in vivo* lipasic esterification, the unit of esterification is the conversion of a glycerol molecule to a triglyceride : in other words a ' quantum ' synthesis mechanism of production of triglycerides seems to prevail during the biosynthesis of fats in nature. This may be interpreted as being due to the fact that once a glycerol molecule is adsorbed on to a lipase molecule, it is not desorbed until all the three hydroxyl groups are esterified to give a triglyceride and the fully esterified molecule alone is desorbed.

The present results suggest another possibility. If the general assumption that all plant lipases have more or less the same characteristics and mechanism of action is correct, then the results show a complete difference in the mechanism of action of the same lipases under *in vitro* and *in vivo* conditions. This can be explained only on the basis that, during the process of isolation, changes in the enzyme complex take place which ultimately lead to differences in the mechanism of action. This possibility is supported by the fact that whereas *in vitro* esterifications are dependent on the concentration of water in the reaction mixture, the *in vivo* esterifications have been found to be entirely independent of water concentration in the seeds.[8–10] The alternative explanation that lipases in ripening seeds may be different from the lipases present in the mature seeds which have so far been used for the *in vitro* studies, and that this may be the reason for the difference in behaviour, does not appear to be feasible, since preliminary experiments have shown that the lipases in mature seeds display on germination a ' quantum ' mechanism of hydrolysis of triglycerides to fatty acids and glycerol with little or no accumulation of intermediate mono- and di-glycerides.[18]

Acknowledgments

The author is indebted to Dr. B. P. Pal, Director, and Dr. N. P. Datta, Head of the Division of Chemistry, for facilities, and to Sri J. M. S. Mathur, for technical assistance.

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Received 16 September, 1963

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