EFFECT OF HEAT ON THE DIGESTIBILITY OF LEAF PROTEINS

Part 1.—Toxicity of the Lipids and their Oxidation Products

F.H. Shah, Riaz-ud-Din and A. Salam

West Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Lahore

(Received April 25, 1966)

The digestibility of the leaf protein concentrates (LPC) by enzymes, decreased when they were heated at 100°C. This seems to be due to the oxidation of the highly unsaturated lipids present in the proteins. The oxidation products of the lipids and their polymers with proteins were toxic to trypsin, pepsin and the enzymes of pancreatic extract. However, the digestibility of the heated as well as the fresh protein samples increased when the protein was defatted with chloroform-methanol.

Introduction

Leaf-protein concentrates (LPC) are of increasing interest in the supply of high-quality protein. The LPC contains higher proportion of essential amino acids, with the exception of lysine, isoleucine and methionine, than many animal food-stuffs.[1] The biological value of LPC has been reported to be higher than the values for beef, casein, soyabean and yeast.[2] The quality of the product could be further improved by supplementing it with methionine.[1] However, the high moisture contents (60-75%) and the presence of enzymes in the concentrates affect its nutritional as well as keeping qualities.

Heating of the fresh cake increases its shelf-life but affects the quality of the product.[3] This has been attributed to the denaturation of the proteins. The presence of highly unsaturated fatty acids in the lipids of LPC[4] also affects its quality. The oxidation products of unsaturated fatty-acids have been reported to be toxic to many enzymes.[5] The present work was carried out in order to study the effect of heat on the digestibility of LPC and the toxic effect of oxidation products of the lipids on the proteolytic enzymes of human digestive system.

Experimental

Protein Extraction

Leaf-proteins were extracted from a mixed crop of *Trifolium resupinatum* (shatalla), *Medicago sativa* (lucerne) and *Brassica compestris* (sarson) by the method of Morrison and Pirie.[6]

Drying of the LPC

500 g lots of the homogenized LPC were heated at 60°, 80° and 100°C under identical conditions. The dried product (D.M. 90%), was ground and the protein which passed through a 60-mesh sieve was used in the experiments. The samples were stored at -10°C.

Removal of the Lipids

50 g lots of the LPC were defatted by the method of Folch *et al.* and the lipids present in the extract were estimated by evaporating it at 55°C under reduced pressure. The defatted protein was heated at 40°C under vacuum to remove the solvent present in it.

Preparation of Pancreatic Extract

Fresh pancreas were purchased from the local market and freed from the adherent fat and the external membranes. 600 g of the defatted pancreas were deep frozen, cut and minced in ice-cold water. The blend was quickly filtered. The process was repeated twice and 800 ml of the extract was obtained.

Nitrogen Determination

The nitrogen determinations were made by a micro-Kjeldahl's method using the copper-selenium catalyst. Proteinous nitrogen (PN) and non-proteinous nitrogen (NPN) in the samples was estimated as trichloroacetic acid (TCA) insoluble and TCA soluble N respectively. The protein contents of the samples were calculated as protein $N \times 6.0$.[8]

Determination of Digestibility

Trypsin and pepsin (E. Merck) and the enzymes present in the ox pancreas were used for determination of the digestibility of the protein samples.

1.0 g of the protein samples was suspended in 20 ml of the buffer and incubated at an optimum temperature after the addition of the enzyme.[9]

The samples were taken after 3, 6, 9 and 24 hours. The undigested protein present in 2 ml of the suspension was precipitated by the addition of 2 ml of 20% TCA. The samples were centrifuged at 2500×g for 20 minutes and the nitrogen contents of the supernatant (NPN) was determined. The NPN×6.0 is referred in the text as digestible protein. Blanks were run to find the extent of autolysis of the enzyme and the hydrolysis of the protein during incubation.

Results and Discussion

LPC (D.M. 25.0%) contained 18.6% chloroform-methanol extractable lipids, whereas the samples dried at 60°, 80° and 100°C yielded 18.8, 19.0 and 18.0% lipids respectively. Amount of lipids extracted with petroleum ether (40-60°) was 10-11%.

10.1% of the protein present in the fresh cake was digested after 9 hours of incubation in the presence of trypsin (Table 1 A). Drying at 60° and 80°C affected the digestibility of the protein only slightly, indicating that the excessive production of simple peptides during heating counterbalanced the loss in the digestibility of LPC due to the denaturation of the protein and formation of oxidation products of the lipids. However, the sample heated at 100°C showed a marked decrease in digestibility. This seems to be due to denaturation of the protein as well as the formation of protein complexes with lipids [10] and carbohydrates.[11]

Extraction with $CHCl_3$-CH_3OH raised the tryptic digestibility of all the samples except that dried at 80°C, which was slightly lowered. The higher digestibility of the defatted samples proved that the lipids and their oxides, produced during heating, were toxic to trypsin and hence responsible for the decrease in the digestibility. The decrease in the digestibility of the sample dried at 80°C (defatted) appears to be due to the removal of simple peptides by $CHCl_3$-CH_3OH which extracts non-lipids as well. This was confirmed when petroleum ether (40-60°) was employed for the extraction of the lipid (Table 1 A.)

Pancreatic extract digested 39.0% of the proteins present in LPC after 33 hours (Table 1 B). Drying at 60°C did not affect the digestibility, whereas heating at 80° and 100°C appreciably lowered it. The decrease in the digestibility could be due to toxicity of the oxidation products of the lipids as well as denaturation of LPC. The extraction of the samples with $CHCl_3$-CH_3OH established the fact that the loss in the digestibility was mainly due to the toxic effect of lipids and their oxides.

TABLE 1.—DIGESTIBILITY OF LPC.

Enzyme	Time in hours	% digestibility of LPC		% digestibility of LPC dried at 60°C			% digestibility of LPC dried at 80°C			% digestibility of LPC dried at 100°C		
		Whole	De-fatted*	Whole	De-fatted*	De-fatted**	Whole	De-fatted*	De-fatted**	Whole	De-fatted*	De-fatted**
A. Trypsin 250 mg	3-	4.10	5.11	4.98	5.76	6.15	4.47	4.73	7.61	3.63	4.69	7.24
	6-	8.67	8.34	7.31	8.48	7.96	6.29	5.26	9.62	5.07	4.83	8.43
	9-	10.14	11.68	8.63	9.75	10.30	7.42	8.21	12.66	5.94	6.86	10.82
	24-	8.13	18.34	5.06	10.94	13.52	9.89	7.16	13.15	3.46	8.30	13.32
B. Pancreatic extract 1 ml	3-	10.46	8.09	5.62	6.02		4.42	5.39		1.38	3.12	
	6-	13.57	17.66	8.12	12.06		8.35	10.55		4.21	4.83	
	9-	17.88	22.63	10.82	16.13		7.00	13.22		7.41	10.18	
	24-	31.15	32.72	26.86	28.65		21.65	24.03		15.62	20.25	
	27-	30.18	38.48	29.61	31.87		23.49	22.54		18.15	21.15	
	30-	36.22	41.26	31.11	33.87		25.73	27.71		20.17	23.44	
	33-	38.98	41.56	39.95	35.49		25.02	29.92		22.89	23.63	
	48-	31.10	45.60	34.95	39.95		26.69	34.21		13.21	30.24	
C. Pepsin 600 mg	3-	15.00	17.13	7.04	15.69		9.39	9.23		11.68	9.28	
	6-	19.18	21.19	11.79	16.02		10.42	13.10		14.70	9.27	
	9-	21.55	25.10	16.13	22.02		12.58	16.34		16.32	14.86	
	24-	28.26	48.24	25.58	41.79		18.03	26.30		25.80	20.60	

* By chloroform: methanol (2:1)
** By petroleum ether.

All lipid-containing samples except those dried at 80°C showed a decrease in NPN after 9 and 33 hours of incubation with trypsin and pancreatic extract respectively. The decrease was maximum in the case of the sample dried at 100°C. This decrease in NPN can be attributed to adsorption of amino acids on the lipids and their oxides or the interaction of metabolic products of the enzymic reaction with the enzymes. Venolia and Tappel[12] while studying the formation of brown colour in the emulsions of the menhaden and herring oils with the aqueous dispersions of egg albumin observed that the rate of formation of brown colour was a function of the pH and that the adsorption mostly took place at a pH inclined towards alkalinity. As this decrease in NPN was only observed at pH 7.0 and not at pH 1.8, it is evident that it was due to the adsorption of amino acids on the oxides of lipids. This was further confirmed by the results of the experiments carried out with defatted samples. The exceptional behaviour of the sample dried at 80°C can be attributed to the excessive production of simple peptides which were easily attacked by trypsin.

28.3% of the protein present in the fresh cake was digested by pepsin after 24 hours of incubation (Table 1c.). The digestibility seems to have decreased only slightly in case of the samples dried at 60° and 100°C. However, it was seriously affected when the sample was dried at 80°C. Although drying at elevated temperatures severely affects the quality of the protein, the loss in the case of the sample dried at 100°C seems to have been compensated by the simultaneous production of large amounts of simple peptides which were easily attacked by pepsin. This was confirmed by the results of the experiments carried out with defatted ($CHCl_3$-CH_3OH) samples.

Trypsin digested 18.3% of the fresh defatted LPC after 24 hours of incubation, while the digestibility of the protein dried at 60°C (defatted) was 10.9% (Table 1A.). This decrease in the digestibility was caused by the denaturation of proteins during heating and the removal of simple peptides by $CHCl_3$-CH_3OH. LPC dried at 60°C and defatted with petroleum ether showed a digestibility of 13.5% instead of 10.9% in case of $CHCl_3$-CH_3OH, which confirmed that the decrease in the digestibility of the latter was partly due to the removal of the simple peptides by $CHCl_3$-CH_3OH.

In view of the above discussion, it can be concluded that lipids and their oxidation products produced during heating are toxic to trypsin, pepsin and the enzymes present in the pancreatic extract and the loss in the digestibility of the heated LPC samples was mainly due to these oxidation products.

Acknowledgment.—The authors' thanks are due to Mr. J.I. Javaid for help and assistance.

References

1. E.D. Gerloff, I.H. Lima, and M.A. Stahmann, J. Agr. Food. Chem., **13,** 139 (1965).
2. W.R. Akeson, and M.A. Stahman J. Agr. Food Chem., **13,** 145 (1965).
3. J. Duckworth, and A.A. Woodham, J. Sci. Food Agr., **12,** 5 (1961).
4. I.H. Lima, T. Richardson, and M.A. Stahmann, J. Agr. Food Chem., **13,** 143 (1965).
5. E.D. Wills, Biochem. Pharmacol., **7,** 7, (1961).
6. J.E. Morrison, and N.W. Pirie, J. Sci. Food Agr., **12,** 1 (1961).
7. J. Folch, M. Lees and G.H.S. Stanley, J. Biol. Chem., **226,** 497 (1957).
8. A.C. Chibnell, J. Biol. Chem., **55,** 333 (1923).
9. A. Salam, and F.H. Shah, Pakistan J. Sci. Ind. Res. (In press).
10. K.A. Narayan, and F.A. Nummerow, J. Am. Oil. Chem., Soc., **35,** 52 (1958).
11. K.J. Carpenter, C.B. Morgan, C.H. Lea, and L. Parr., J. Brit. Nutr., **16,** 451 (1962).
12. A.W. Venolia and A. L. Tappel, J. Am. Oil Chem. Soc., **35,** 135 (1958).