PROTEIN SUPPLEMENTATION

Influence of Processing on Supplementary Value of Vitamin B_{12} and Amino Acids to Proteins in Whole Wheat

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The influence of processing on the supplementary value of vitamin B_{12} to the proteins in soft whole wheat, in the presence of lysine, valine, and threonine, was studied. The processed wheat was a commercial shredded wheat product. The greatest influence of vitamin B_{12} in increasing the protein efficiency of the processed wheat was observed in the presence of lysine and threonine. Addition of 0.1 γ of vitamin B_{12} per animal per day, in the presence of 0.4% L-lysine and 0.2% DL-threonine, produced an increase of 14.1% in protein efficiency ratio in albino rats on the untreated wheat, but an increase of 42.1% in rats on the processed wheat, fed at the same 7% protein level. These results may have practical applications in the commercial production of cereal breakfast foods, when the amino acids used in this study become economical for enrichment of cereal grains.

Cereal products contribute about 30% of the protein intake of the American diet and account for about 10% of the total money spent for food (*9*). The proteins of cereal products are offered for human consumption in a variety of forms, some of which as breakfast foods require a degree of processing. In 1951 it became evident (*5*) that breakfast foods could be classified into three groups based on their protein nutritional values: poor, good, or excellent, depending largely on the methods of preparation.

Melnick and Oser (*3*) have shown that when cereals are properly cooked no undesirable changes in the protein component occur and their nutritive value appears to be equal to that of the unheated protein. Beaudoin, Mayer, and Stare (*1*) reported that the cooking of whole wheat in the laboratory, as in the preparation of shredded wheat, under optimum conditions, results in a significant improvement in nutritional value. The fact that the protein efficiency of a whole wheat cereal breakfast food could be improved considerably by the addition of small amounts of amino acids and vitamin B_{12} (*4*) stimulated a study of the influence of processing on the supplementary value of this vitamin to the proteins in soft wheat in the presence of the amino acids, lysine, valine, and threonine.

This investigation was carried out on the Wistar strain albino rat. The basal diets without amino acid additions had 24 animals in each group. In the rations with amino acid additions each group had 12 animals. The sexes were equally divided. The animals were about 30 days old when started on the experiments and weighed 50 to 56 grams each. The experimental period was 10 weeks. The only source of proteins was soft whole wheat and processed wheat, prepared from the soft wheat both fed at a 7% protein level. Both soft wheat and shredded wheat were furnished in liberal quantities by the Quaker Oats Co., Chicago, Ill., and had protein contents of 8.4 and 9.3%, respectively.

The soft wheat and shredded wheat were used to furnish 7% proteins in the rations, which contained 4% of Sure's salt mixture No. 1 (*7*), 7% hydrogenated vegetable shortening, 2% cod liver oil, 1% wheat germ oil, and the rest, percentagewise, glucose (Cerelose). The fat-soluble vitamins A, D, and E were furnished by the cod liver oil and wheat germ oil in the rations. All rations were supplemented with a liberal supply of crystalline B vitamins (*6*). The animals were weighed once weekly and accurate records were kept of food consumption. From these data the protein efficiency ratio was determined, and is expressed as gains in body weight per gram of protein intake. As the amino acid additions contributed some foreign nitrogen, data are also furnished on gains in body weight per gram of nitrogen intake. The results of this study are summarized in Tables I and II.

Preparation of Shredded Wheat

According to the manufacturers, the shredded wheat was prepared as follows.

Clean whole soft wheat is cooked in an atmospheric cooker in an excess of boiling water for 35 to 40 minutes. The grain from the cooker has a moisture content of 50 to 51%. Coming from the cooker the grains are first cooled and then put into storage tanks, where they are tempered for 6 to 8 hours. They are then passed through shredding rolls. The shreds are rolled into a round biscuit form and these biscuits are passed through a toasting oven for 17 minutes at 450°. Following the toasting, the biscuits are passed for 23 minutes through a dryer chamber, where hot air at a temperature of 235° F. is blown through the product.

It will be noted from Table I that the animals on basal ration 1, containing the soft whole wheat flour as the only source of proteins, gained an average of 49.3 grams each during the experimental period of 10 weeks, while the rats on ration 6, containing the processed soft shredded wheat, gained 39.9 grams each on the same protein level; in other words, the animals on the processed wheat gained 81% as much as the animals on the untreated wheat from which it was made. The findings of Beaudoin, Mayer, and Stare (*1*) that the shredding of whole wheat results in increase in biological value of its proteins are ascribed to different methods used in the laboratory for preparation as contrasted with the commercial methods employed in the manufacturing of this product.

The addition of 0.4% L-lysine and 0.2% DL-threonine was followed by 108.7% increase in growth and 70.9% increase in protein efficiency ratio, and

Table I. Influence of Processing on Supplementary Value of Vitamin B_{12} to Proteins in Soft Whole Wheat in Presence of Lysine, Valine, and Threonine

(7% protein in rations. Experimental period 10 weeks. Average results per animal. 12 males and 12 females in groups 1 and 6. 6 males and 6 females in each of other groups)

Ration	Type of Ration	Gains in Body Weight, G.	Gains in Body Weight, %	Total Food Intake, G.	Protein Intake, G.	Protein Efficiency Ratio[a]	Increase, %	Nitrogen Intake, G.	Gains in Body Weight per G. Nitrogen Intake, G.	Gains in Body Weight per G. Nitrogen Intake, %
1	SWF	49.3 ± 8.5[b]	...	554.1	38.8	1.27 ± 0.05[c]	...	6.80	7.24	..
2	SWF + 0.4% L-lysine +0.2% DL-threonine	102.9 ± 9.4	108.7	675.9	47.3	2.17 ± 0.05	70.9	8.79	11.71	61.7
3	SWF + 0.4% L-lysine +0.2% DL-threonine +0.1 γ B_{12}/animal/day	127.9 ± 8.9	159.4	777.9	54.5	2.35 ± 0.11	85.0	10.12	12.63	74.4
4	SWF + 0.4% L-lysine +0.2% DL-threonine +0.5% DL-valine	120.4 ± 8.3	144.2	701.1	49.1	2.45 ± 0.14	92.9	9.24	13.03	80.0
5	SWF + 0.4% L-lysine +0.2% DL-threonine +0.5% DL-valine +0.1 γ B_{12}/animal/day	138.0 ± 9.9	180.0	788.7	55.2	2.50 ± 0.06	96.8	10.49	13.15	81.6
6	PSW	39.9 ± 8.5	...	500.5	35.0	1.14 ± 0.09	...	6.14	6.50	..
7	PSW + 0.4% L-lysine +0.2% DL-threonine	76.4 ± 7.6	91.5	687.7	48.1	1.59 ± 0.06	39.5	8.93	8.54	31.4
8	PSW + 0.4% L-lysine +0.2% DL-threonine +0.1 γ B_{12}/animal/day	105.2 ± 7.5	163.6	726.7	50.9	2.07 ± 0.10	81.6	9.45	11.12	71.1
9	PSW + 0.4% L-lysine +0.2% DL-threonine +0.5% DL-valine	91.6 ± 8.2	129.6	640.6	44.8	2.04 ± 0.07	79.0	8.50	10.77	65.7
10	PSW + 0.4% L-lysine +0.2% DL-threonine +0.5% DL-valine +0.1 γ B_{12}/animal/day	122.9 ± 10.3	208.0	747.2	52.3	2.35 ± 0.08	106.1	9.92	12.38	90.5

[a] Expressed as gains in body weight per gram of protein intake.
[b] Standard deviation.
[c] Standard deviation of means.
SWF. Soft wheat flour.
PSW. Processed soft wheat.

the further addition to ration 2 of 0.1 γ of vitamin B_{12} per animal per day resulted in additional 50.7% increase in growth and 14.1% increase in protein efficiency ratio. In contrast, the supplementation of basal ration 6, containing the processed wheat, with the same amount of L-lysine and DL-threonine resulted in 91.5% increase in growth and 39.5% increase in protein efficiency ratio. However, the influence of the addition of 0.1 γ of vitamin B_{12} per animal per day, in the presence of lysine and threonine, was much more pronounced in the case of the processed than in the untreated soft whole wheat—i.e., 72.1% increase in growth and 42.1% increase in protein efficiency ratio.

The increases in body weight per gram of nitrogen intake, while somewhat less, followed the same pattern as the protein efficiency ratios. That there is a response to the addition of valine is apparent in the case of untreated wheat (rations 2 and 4), 35.5% increase in growth and 22.0% increase in protein efficiency ratio; and in the processed wheat (rations 7 and 9), 38.1% increase in growth and 39.5% increase in protein efficiency ratio. In the untreated wheat, 0.1 γ of vitamin B_{12} per animal per day, in the presence of lysine, valine, and threonine (rations 4 and 5) produced 35.8% increase in growth and only 3.9% increase in protein efficiency ratio, while in the processed wheat, this vitamin with same amino acid supplements produced 78.4% increase in growth and 27.1% increase in protein efficiency ratio (rations 9 and 10).

In order to determine the nature of the marked gains in body weight and increase in protein efficiency produced by the daily dose of 0.1 γ of vitamin B_{12} supplementing the basal ration containing the processed wheat fortified with lysine and threonine, the animals were sacrificed at the termination of the experiment and proteins, fat, and minerals (ash) were determined (*8*). There was no difference in the water content of the carcasses. However, vitamin B_{12} was influential in increasing the fat, proteins, and minerals: 52.2, 33.8, and 15.3%, respectively.

Liener (*2*) studied the effect of heat processing on the nutritive value of pro-

Table II. Influence of Vitamin B_{12} on Chemical Composition of Body Gains of Animals in Groups 7 and 8

(7% protein in rations. Experimental period 10 weeks. Average results per animal. 6 males and 6 females in each group)

Ration	Type of Ration	Protein Intake, G.	Increase in Body Weight, G.	Chemical Composition of Body Gains, G.: Fat	Protein	Ash
7	Processed soft wheat flour[a] +0.4% L-lysine +0.2% DL-threonine	48.1	76.4	+12.05	+13.03	+2.88
8	Processed soft wheat flour[a] +0.4% L-lysine +0.2% DL-threonine +0.1 γ B_{12}/animal/day	50.8	105.2	+18.34	+17.44	+3.32
% gains in fat, protein, and ash due to addition of 0.1 γ B_{12}/animal/day				52.2	33.8	15.3

[a] Shredded wheat breakfast food.

teins in cereal grains. After a thorough review of the literature he concluded that the following sequence of events ensues as a heat-vulnerable protein is subjected to a progressive increase in the severity of heat treatment:

The enzymic release of lysine during intestinal transit is delayed, so that a substantial portion of it is no longer available for the mutual supplementation of the other essential amino acids absorbed earlier in the digestive process.

The enzymic release of lysine may be delayed to such an extent that a substantial portion of the lysine is excreted unabsorbed. This is accompanied by a depression in the digestibility of the heated protein.

Lysine may be destroyed, as evidenced by a decrease in the amount of lysine recoverable from acid hydrolyzates of the heat-processed protein. The fate of lysine in heat-processed proteins may be true of other amino acids, such as threonine and valine used as amino acid supplements in this investigation.

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FLAVOR ORIGIN

Flavor and Odor Components in the Tomato

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In an investigation of the preservation of flavor during processing, suitable methods for the isolation of tomato flavoring constituents were shown to be vacuum distillation at 20 mm., or solvent extraction followed by molecular distillation of the solvent extract. Vacuum distillates contained: esters, 2 p.p.m. in both cooked and fresh juice; volatile acids, 1 p.p.m. in fresh and 0 in cooked; carbonyls, 33 p.p.m. in fresh and 43 p.p.m. in cooked. The main carbonyl constituent was acetaldehyde. Isovaleraldehyde was also present, and probably citral and vanillin. Three different types of tomato odor fractions were isolated chromatographically from the concentrates: a typical tomato odor fraction, relatively nonvolatile; a green tomato odor fraction, also relatively nonvolatile; and a raw tomato odor fraction, relatively volatile. The typical tomato odor fraction, largely retained by present processing methods, contained alcohols, carbonyl compounds, and unsaturated compounds, and these were modified by many other odor fractions, some terpene in nature. Changes in the unsaturated compounds may be involved when the flavor of stored tomato products deteriorates.

Determining the flavoring constituents of plant products is complicated by the number of components and their minute quantities. Yields are conditioned by arbitrary decisions: The investigation may be made on components distillable by steam at atmospheric pressure, at 20-mm. pressure or at less than 1-micron pressure. Lyophilization may be employed, or extraction with volatile solvents with or without distillation of the extract. Not only do yields vary, depending on methods used, but composition of the oil itself is affected.

Difficulties are enhanced by two problems after identification is made. How important is the component to the flavor? Is the component an artifact or a natural constituent of the flavoring matter? Changes in structure, particularly those of an oxidative character, constitute a hazard. In handling the necessarily large quantities of raw material, in spite of careful inspection for damaged and bruised fruit, delays in sorting and treating are inevitable and may give rise to off-flavors.

In this study, methods of isolating flavoring components from tomatoes were first compared. After the initial recovery steps, the oils obtained were separated by chromatographic methods, and the physical and chemical properties of the separated components were investigated.

Isolation of Flavor Concentrates

Solvent extraction, vacuum distillation at 20 mm., and steam distillation at

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