# Protein Nutritional Value of Extrusion-Cooked Wheat Flours

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(Received: 1 May, 1984)

#### ABSTRACT

The effect of extrusion cooking on the protein nutritional value of wheat flour and whole-grain wheat flour was studied. Amino acid analysis showed lysine retention between 63 and 100%. The retention was positively affected by an increase in feed rate, and negatively by an increase in screw speed. The prominent lysine damage under severe conditions was probably due to formation of reducing carbohydrates through hydrolysis of starch. The loss of other amino acids was small. The BV of extruded wheat flours was significantly reduced in the products showing the most prominent lysine loss. However, no effect was seen in TD. In contrast, a decrease in BV of extruded whole-grain flour was accompanied by a significant reduction in TD. Extrusion cooking of wheat starch did not affect the nitrogen balance in rats given casein/starch diets containing raw or extruded starch.

#### INTRODUCTION

Most studies on the protein nutritional value of extruded products have been performed on blended foods processed in single-screw extruders

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Food Chemistry 0308-8146/84/\$03.00 © Elsevier Applied Science Publishers Ltd, England, 1984. Printed in Great Britain

(Jansen *et al.*, 1978; Del Valle *et al.*, 1981; Harper & Jansen, 1981). Less biological data are available on twin-screw extruders, which are able to operate over a wider range of moisture contents.

The main mechanism responsible for protein deterioration during processing is the Maillard reaction or non enzymic browning. In a previous study on the protein quality of an extruded cereal-based biscuit containing sucrose, a significant decrease in the availability of lysine, as well as in protein digestibility, was observed under severe conditions (Björck *et al.*, 1983). It has been suggested that the rate of the Maillard reaction, during extrusion cooking of such a mixture, is limited by the formation of reducing sugars through hydrolysis of sucrose (Noguchi *et al.*, 1982). In addition, hydrolysis of starch (Kim, 1983; Owusu-Ansah *et al.*, 1983; Theander & Westerlund, 1983) may provide additional reducing carbohydrates.

Unabsorbed carbohydrates have been shown to affect the metabolic and endogenous losses of nitrogen in the rat (Asp & Dahlqvist, 1976; Nyman & Asp, 1982), possibly due to an increased activity of colonic bacteria. Formation of enzyme-resistant starch has been reported during food processing (Theander & Westerlund, 1983; Johansson *et al.*, 1984). Even if only a small fraction of starch were passed undigested into the colon, it would provide the colonic bacteria with a substantial amount of fermentable carbohydrates. This could have methodological implications when evaluating the protein nutritional value of processed products in nitrogen balance experiments.

The present investigation was performed to study the protein nutritional value of extruded wheat flours and whole-grain wheat flours. The products were processed in a Creusot-Loire BC 45 twin-screw extruder under different conditions, without additional carbohydrates present. The amino acid composition was determined by gas-liquid chromatography, and the degree of browning by reflectance measurements. Protein digestibility and biological value were evaluated through nitrogen balance studies in rats.

## MATERIALS AND METHODS

#### Materials

Commercially available wheat flour (80% extraction rate), whole-grain wheat flour (Vaasa Mills Ltd, Finland) and wheat starch (A-starch, Raisio

Factories Ltd, Finland) were used in the experiments. The materials were extruded in a Creusot-Loire BC 45 twin-screw extruder under different conditions as described in Table 1. The extruded products and the whole-grain wheat flour were ground to pass a 0.5 mm sieve.

#### **Chemical analysis**

Nitrogen content was determined by the Kjeldahl procedure. Amino acids were analyzed by gas-liquid chromatography as *N*-trifluoroacetyl*n*-butyl derivatives after acid hydrolysis of the protein and subsequent derivatization (Nair, 1977). Colour was measured with a Elrepho 21000 reflectance photometer. The sample cup was filled with approximately  $30 \text{ cm}^3$ , and the reflectance was measured at 577 nm with an opal glass standard.

#### Animal experiments

The nitrogen balance experiments were performed in young rats (Sprague Dawley, 75g) according to Eggum (1973). Each diet was tested on five animals. Food intake was restricted to 10g dry matter per day, containing 150 mg N.

In the case of wheat flour and whole-grain wheat flour, the flour itself was used as the protein source. In the groups fed raw or extruded wheat starch 69.3% (dry basis), the protein source was casein (ANRC reference protein, ICN Nutritional Biochemicals) supplemented with 0.2% Lmethionine (BDH). All diets contained 5% corn oil, 4.8% minerals and 1% vitamins, including choline chloride. The mineral and vitamin mixtures were as described previously (Björck et al., 1984). The sucrose content in the diets was varied to adjust the dry matter. Faecal metabolic and urinary endogenous excretion were determined with a diet containing 4.5% protein from diethyl ether extracted hen's egg. After a four-day adaptation period, a nitrogen balance was performed for five days. Urine and faeces were collected separately in metabolic cages, and the content of nitrogen was analyzed. Faeces was collected dry, removed every day and frozen at -20 °C. The faeces was then lyophilized, weighed and milled to pass a 0.4 mm screen, and stored at -20 °C until analysis. Calculation of true digestibility (TD), Biological Value (BV) and Net Protein Utilization (NPU) was performed with the Thomas-Mitchells' equations as described by Eggum (1973).

									1
		7	Vheat flou	r		Mhe	ole-grain fi	our	Starch
	WFI	WF2	WF3	WF4	WF5	WGFI	WGF2	WGF3	
Mass feed rate including moisture									
$(g \min^{-1})$	350	200	200	200	200	200	200	000	000
Feed moisture content $(\%)$	15	15	15	15	51	20	<b>7</b> 0		31
Screw speed (rev. min <sup>-1</sup> )	150	100	150	200	150	100	150	07 00	
Mass temperature <sup>b</sup> (°C)	161	161	171	171	156	164	166	166	180
<sup>a</sup> Creusot-Loire BC 45 twin-screw ex die/end plate 2 mm. Screw: combinat intermeshing screws. <sup>b</sup> Mass temperature was measured v	tion Die-F with a the	et tempera RCCCC-F	ature of b eed; R, re	arrel 150 verse fligt	°C. Die: d it screw ele	iameter 5 mm; ment; C, comp	capillary l ression scr	ength 27 mm ew element;	; clearance co-rotating
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Process Conditions<sup>a</sup> During Extrusion Cooking of Wheat Flour, Whole-Grain Wheat Flour and Wheat Starch **TABLE 1** 

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## **RESULTS AND DISCUSSION**

The amino acid composition before and after extrusion cooking of wheat flour is shown in Table 2. The lysine loss ranged from 0 to 37 %depending on process conditions. No significant losses occurred in other amino acids. The most prominent lysine loss (37%) occurred at the highest mass temperature, WF4. An increase in screw speed from  $150 \text{ rev min}^{-1}$  (WF3) to 200 rev min<sup>-1</sup> (WF4), under otherwise similar conditions, increased the lysine loss from 25 to 37% (P < 0.05). In

Amino acid	in raw material	in extrusion-cooked materials (%) <sup>a</sup>					
	(g per 16 g N)	WF1	WF2	WF3	WF4	WF5	
iso-Leucine	4.4	98	100	102	100	98	
Leucine	8.5	99	99	101	95	98	
Lysine	2.4	100	96	75***	63***	75***	
Phenylalanine	5.2	96	94	88	104	96	
Threonine	3.2	94	100	97	88	91	
Valine	5.3	96	102	109	94	98	
Alanine	4.1	90	100	102	107	93	
Glycine	4.0	100	105	105	103	105	
Proline	14.5	97	97	94	91	94	
Serine	5.4	106	91	85	92	98	
Aspartic acid	4.6	91	107	93	91	96	
Glutamic acid	36.7	100	_			101	

 TABLE 2

 Amino Acid Composition in Raw and Extrusion-Cooked Wheat Flour

<sup>a</sup> Per cent of the corresponding value in the raw material.

Significantly different from the corresponding raw material: \*\*\* P < 0.001 (Student's *t*-test).

contrast, an increase in feed rate, from  $200 \,\mathrm{g\,min^{-1}}$  (WF5) to  $350 \,\mathrm{g\,min^{-1}}$ , significantly improved the lysine retention (P < 2.01). No lysine loss was observed in product WF1, whereas the loss was considerable (25%) in the product processed at a lower feed rate.

The amino acid composition in the whole-grain wheat flour products was almost unaffected by processing (Table 3), except at the most severe conditions used (WGF3), where 9% of the lysine was lost.

As judged from the amino acid composition in the raw materials,

Amino acid	in raw material (a per 16 a N)	in extrus	materials	
	(870 10 811)	WGF1	WGF2	WGF3
iso-Leucine	3.8	111	105	105
Leucine	7.5	103	101	104
Lysine	3.2	106	94	91*
Phenylalanine	4.8	92	102	110
Threonine	3.1	113	107	107
Valine	4.7	102	104	113
Alanine	4.3	105	102	105
Glycine	4.4	107	102	107
Proline	11-1	110	103	106
Serine	4.9	104	99	101
Aspartic acid	5.8	107	100	96
Glutamic acid	30.3	108	100	104

 TABLE 3

 Amino Acid Composition in Raw and Extrusion-Cooked Whole-Grain Wheat Flour

<sup>*a*</sup> Per cent of the corresponding value in the raw material.

Significantly different from the corresponding raw material: \* P < 0.05 (Student's *t*-test).

milling of wheat flour caused a decrease in the content of especially lysine and aspartic acid, whereas the opposite was found for glutamic acid and proline (Tables 2 and 3). This is in agreement with other reports on the effect of milling on the amino acid pattern (Pedersen & Eggum, 1983).

In Fig. 1, reflectance has been plotted versus lysine content in the different extruded wheat flour products. Evidently, there is a correlation between the degree of browning and the lysine content in processed samples (r = 0.95). However, the reflectance obtained with raw flour (88.3) deviated considerably from all processed samples, including that with no lysine loss. This was probably due to other factors than Maillard products affecting the reflectance, e.g. starch gelatinization.

Results from nitrogen balance experiments with the wheat flour products and the most severely processed whole-grain product are shown in Table 4. With wheat flour, true protein digestibility (TD) was unaffected by processing even under severe conditions. In contrast, with whole-grain wheat flour (WGF3), there was a significant decrease in TD (6%) during processing. Under mild conditions, the biological value (BV)



Fig. 1. Product colour as a function of the total lysine content.

of the wheat flour products was similar to that of the raw wheat flour, about 50, whereas the BV and the net protein utilization (NPU) of product WF3–WF5 were significantly reduced. In the whole-grain product, there was also a significant decrease in BV and NPU. As lysine is the limiting amino acid in wheat, a diminished availability in processed products will be reflected by a decrease in BV. However, with lysine limiting at or below maintenance, the BV levels off at about 40 due to, for instance, endogenous synthesis or reduced catabolism (Bender, 1961).

As judged from the weight gains (close to zero) of the rats given the most severely processed products (WF3–WF5), the lysine content in these products was at the maintenance level. This could explain why the decrease in BV was less pronounced than the decrease in total lysine in these products. Consequently, although the lysine content in product WF4 was significantly different from product WF5 (P < 0.01) and WF3 (P < 0.05), no significant difference was observed in BV. However, an increase in feed rate from 200 g min<sup>-1</sup> (WF5) to 350 g min<sup>-1</sup> (WF1) significantly improved the BV (P < 0.01). Due to a higher lysine content in whole-grain wheat, the decreases in BV and lysine in product WGF3 were similar, about 10%.

	$TD \pm SD$	$BV \pm SD$	
Wheat flour	na falsa na mangana na		vor de son a provinción de la constante de la c
Raw	$94.4 \pm 2.6$	50.7 + 3.8	47.8 + 3.6
WFI	$96.3 \pm 0.9$	<b>7 +</b> 50.6 + 1.7	-+48.7+1.7
WF2	$95'5 \pm 0.7$	<b>7 * 7 * 2 0</b>	<b>7 1 1 6 5 1 1 8</b>
WF3	$93.9 \pm 2.1$	*** 40.2 + 2.3 (***) <sup>a</sup>	*** 37.8 + 2.6 (***)
WF4	$93.5 \pm 3.0$	*** 37.6 + 5.1 (***) <sup>a</sup>	35.3 + 5.8 (***) <sup>a</sup>
WF5	$95.5 \pm 1.3$	<b>** 40</b> ·8 + 3·0 (***) <sup>a</sup>	** ** 39.0 + 3.2 (***)
Whole-grain wheat flour	-	· ·	
Raw	$89.0 \pm 1.9$	55.8 + 2.4	49.6 + 2.5
WGF3	84·4 ± 1·7 (**) <sup>4</sup>	$49.7 \pm 2.9 \; (**)^a$	41.9 + 1.7 (***) <sup>a</sup>

**TABLE 4** True Digestibility (TD), Biological Value (BV) and Net Protein Utilization (NPU) of Raw and Extrusion-Cooked Wheat Samples

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Results from balance experiments with casein/starch diets, containing raw or extruded wheat starch, are shown in Table 5. No effect was seen in any of the parameters TD, BV or NPU due to extrusion cooking of the starch. Thus, the losses of nitrogen in the urine and faeces were not affected, indicating that insignificant amounts of undigestible starch were formed during processing of pure starch.

Casein with Raw or Extrusion-Cooked	Wheat Starch	as Source of Ca	arbohydrate
	$TD \pm SD$	$BV \pm SD$	$NPU \pm SD$
Casein <sup>a</sup> + raw wheat starch	$100.5 \pm 1.6$	$92.1 \pm 1.3$	$92.5 \pm 2.4$
Casein <sup>a</sup> + extrusion-cooked wheat starch	$99.8 \pm 1.5$	$93.4 \pm 2.1$	$93 \cdot 2 \pm 3 \cdot 1$

**TABLE 5**  $\mathbf{T}_{i}$  ,  $\mathbf{D}_{i}^{i}$  ,  $\mathbf{C}_{i}^{i}$  ,  $\mathbf{D}_{i}^{i}$  ,  $\mathbf{D}_{i}$ 

Casein<sup>a</sup> + extrusion-cooked wheat starch 99·8 <u>+</u> 1·5  $93.4 \pm 2.1$ 

<sup>a</sup> The diet was supplemented with 0.2% L-methionine (dry basis).

Rats given the most severely processed wheat flour (WF4) developed diarrhoea, making it difficult to separate urine and faeces in the metabolic cages. This may have affected the TD and BV, but not the NPU of this product. In this case, faeces was collected in 5 % H<sub>2</sub>SO<sub>4</sub> as described by Eggum (1973). The stools of rats given product WF3 and WF5 were slightly watery but did not present any problems during collection. These effects of severely processed wheat flour on faeces cannot be explained at present.

The loss of lysine in the extruded whole-grain wheat flours was much less pronounced than in the wheat flour products. This was probably due to a more narrow temperature interval and a higher moisture content of feed during processing of whole-grain wheat. The beneficial effect of a higher moisture content on lysine retention has been demonstrated previously (Noguchi et al., 1982; Björck et al., 1983). The lysine loss in whole-grain wheat was accompanied by a decrease in TD, whereas no effect was seen on TD in the wheat flour products. This observation is difficult to interpret. However, it has been suggested that extrusion cooking, processing of food, e.g. may reduce the protein digestibility due to interactions between protein and undigestible polysaccharides present in the cell walls (Linko et al., 1981; Knipfel et al., 1983). It is notable that the BV of the most severely processed whole-grain flour was still similar to that of unprocessed wheat flour.

Very little attention has been paid to the impact of feed rate on product characteristics (Linko *et al.*, 1981). In the present study, it was shown that an increase in feed rate significantly improved the protein nutritional value of the product. This is probably a result of a shorter residence time.

Conflicting results exist on the impact of screw speed on the protein nutritional value (Tsao, 1976; Noguchi *et al.*, 1982). An increase in screw speed, under otherwise similar conditions in our study, increased the lysine loss. This could not be verified in the biological assay, as the lysine loss was too prominent in these products. Although the lysine destruction was much more pronounced when fructose, a reducing carbohydrate, was added during extrusion cooking of a cereal-based mixture, the loss was considerable (30 %) also with only starch present (Beaufrand *et al.*, 1978). An increase in shear rate has been reported to promote dextrinization of starch (Sahagun & Harper, 1980), thus providing reducing carbohydrates that can participate in the Maillard reaction (Racicot *et al.*, 1981). According to Andersson *et al.* (1981), an increase in screw speed during extrusion cooking of a starch/gluten/bran mixture led to an increase in burnt taste, which may correspond to a higher level of Maillard compounds.

Colour measurements are easy to perform, and can provide information about the extent of lysine damage in processed products. In a study by Lorenz *et al.* (1974), an increase in extrusion temperature during processing of triticale increased product colour, whereas an increase in moisture content had the opposite effect. In this study, a correlation was found between colour and lysine content in extruded wheat products. However, the colour measurements could not be used to predict the lysine loss relative to the raw material.

In conclusion, under carefully selected extrusion conditions, wheat products with high protein quality can be obtained. However, although only minute amounts of reducing sugars were present originally in the raw flours, the lysine loss was considerable (37%) under severe process conditions, possibly due to the formation of reducing carbohydrates through hydrolysis of starch.

#### ACKNOWLEDGEMENTS

The extrusion processing and colour measurements were performed by J. Olkku and A. Hagqvist, VTT Technical Research Centre of Finland,

Espoo, Finland. This investigation was supported by grants from the National Swedish Board for Technical Development, project nos 79-5253, 80-3799, 81-3749 and 81-5717.

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