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PRODUCTS EXTRUDED FROM BUCKWHEAT FLOUR AND ITS MIXTURES WITH MILK PROTEINS. II. CHEMICAL CHARACTERISTICS AND PHYSICO-CHEMICAL PROPERTIES OF PROTEINS AND STARCH OF PRODUCTS EXTRUDED FROM BUCKWHEAT FLOUR AND ITS MIXTURE WITH MILK PROTEINS

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Key words: buckwheat, flour, milk proteins, starch-protein complexes, starch-lipid complexes.

The effect of extrusion on the chemical changes and physico-chemical properties of proteins and starch of buckwheat flour and its mixture with milk proteins was examined. It was found that the temperature of the process has an influence on the formation of starch-protein and starch-lipid complexes. The solubility and swelling power of starch was also changed.

INTRODUCTION

The published results of the latest studies give a more detailed characteristic of the extrusion process of cereal raw materials, pointing to its effect on changes in the physico-chemical properties of proteins and starch, having an important effect on the formation of the structure, its stability and the nutritive value of the products [3, 6].

The action of heat and water in this technological process may also cause certain chemical changes. In this field, particular attention is being paid to formation of starch-protein [4] and starch-lipid complexes [13] and to an increase of the assimilability of non-hydrolyzing polysaccarides [1].

Earlier results of our experiments pointed to the possibility of utilizing buckwheat flour and its mixture with milk proteins in obtaining extruded products [20]. The aim of the present work was to determine chemical changes and selected physico-chemical properties of proteins and starch in these products.

EXPERIMENTAL

MATERIALS

The material for the experiment was constituted by products extruded from buckwheat flour, buckwheat flour a 25% addition of milk proteins and disintegrated roasting buckwheat groats. The method of preparation of raw materials and the conditions of extrusion have been described in an earlier publication [20].

In order to demonstrate the influence of the temperature of extrusion on the chemical composition and changes in physico-chemical properties of proteins and starch, products extruded at 100, 120, and 130°C were examined. It was also interesting to show whether the process of extrusion intensified chemical changes caused by hydrothermal treatment of buckwheat grain. To this end, a product extruded at 130°C, obtained from disintegrated buckwheat groats was examined.

ANALYTICAL METHODS

Determination of moisture (water content)

Moisture was determined according to Polish standard PN-70/1-74011.

Determination of nitrogen compounds content

The content of total nitrogen and of nitrogen soluble in water, 5% KCl, 70% ethanol and 0.2% KOH was determined by the Kjeldahl method [15].

Charasteristics of nitrogen compounds

Proteins were characterized by the method of column chromatographic separation on Sephadex gel [17]. For the isolation of proteins, a sodium chloride solution with concentration 2% and 10% was applied and their separation was performed on Sephadex gel G-100 fine.

Determination of hysteresis field of titration of protein suspensions [10]

A suspension with concentration of 0.5% protein in 0.01 n NaCl after homogenization, was titrated with 0.05 n HCl, the pH value being measured every 0.2 units. Then, it was titrated with 0.05 n NaOH till reaching the initial value of pH. Titration curves were determined on the basis of the quantity of acid or base used in a pH range from 4 to the initial value. Titration curves served for a determination of the hysteresis loop, taking the total amount of the acid used as 10.

Determination of carbohydrates content

The reducing sugars were determined directly in water extracts and oligosaccharides, after hydrolysis — by the method of Luff-Bertrand [15].

Content of starch was determined by the polarimetric method after previous extraction of the sample with water and 80% methanol [15].

Isolation and characterisation of starch [11]

Buckwheat flour or the examined extruded products were soaked in water with a supplement of toluene. After 20 h, the residues were mixed in a mixer for 3 min and the suspension was rubbed through a 200 and 220 mesh sieve. After centrifugation, the sediment was extracted successively with water and 0.2% KOH. Then it was washed with water and methyl alcohol with a concentration increasing from 5 to 90%. The sediment was dried, disintegrated and sieved through a 80 mesh screen.
Determination of selected physico-chemical properties of starch

Swelling power and solubility were determined on the basis of the studies of Leach [11].

The degree of gelatinization [2] was determined by measuring the capacities of forming a colourful complex with iodine of starch chemically and thermally glued.

Determination of free and bound lipids content [8]

The content of free lipids were determined after extraction with petroleum ether in a Soxhlet apparatus for 16 h. After extraction of free lipids, the samples were hydrolyzed with 2 n HCl and the bound lipids were extracted with the use of a methanol-chloroform mixture (ratio 2 : 1 v/v) and its weight was determined.

DISCUSION OF THE RESULTS

EFFECT OF EXTRUSION ON NITROGEN COMPOUNDS

The obtained results of the experiments have shown that the total content of nitrogen compounds in buckwheat flour before and after extrusion was not changed (Table 1). A similar dependence was recorded when extruding the mixture of buckwheat flour and milk proteins and of disintegrated buckwheat groats. No distinct influence of the temperature of the process was found. Although quantitative changes were

Table 1. Level of soluble nitrogen compounds

Product	Total nitrogen % DM	Soluble N — % in relation to total N				
		H_2O	5% KCl	C_2H_5OH	0.2% KOH	insoluble
Buckwheat flour	2.65	12.06	21.87	1.13	15.81	48.03
extruded at 100°C	2.78	12.23	5.03	1.07	18.70	62.94
extruded at 120°C	2.63	12.92	4.63	1.14	15.20	64.25
extruded at 130°C	2.54	17.71	6.61	1.57	14.96	57.08
Buckwheat flour + 25% milk proteins	5.32	13.34	21.61	3.94	44.18	8.46
extruded at 100°C	5.27	11.00	19.16	3.79	41.36	24.66
extruded at 130°C	5.04	11.11	11.70	4.16	33.13	39.88
Disintegrated buckwheat groats	2.22	18.46	4.50	1.35	24.32	51.31
extruded at 130°C	2.16	13.42	4.62	1.38	10.18	70.37

not observed, the results of solubility of nitrogen compounds of products extruded at 100 and 120°C from buckwheat flour revealed a lowering of their solubility in water and solution of hydroxide and an increase of the insoluble nitrogen compounds content (Table 1). These changes are similar to those caused by the action of heat and water during torrefaction of buckwheat grain [18]. Temperature 130°C during the process caused an increase of solubility of nitrogen compounds in water in comparison to the raw material. Other relationships were indicated by the results obtained for products extruded from the mixture of buckwheat flour and milk proteins. A decrease of water-soluble nitrogen compounds content and an increase of insoluble nitrogen compounds content was observed; it was the greater, the higher the temperature of extrusion.

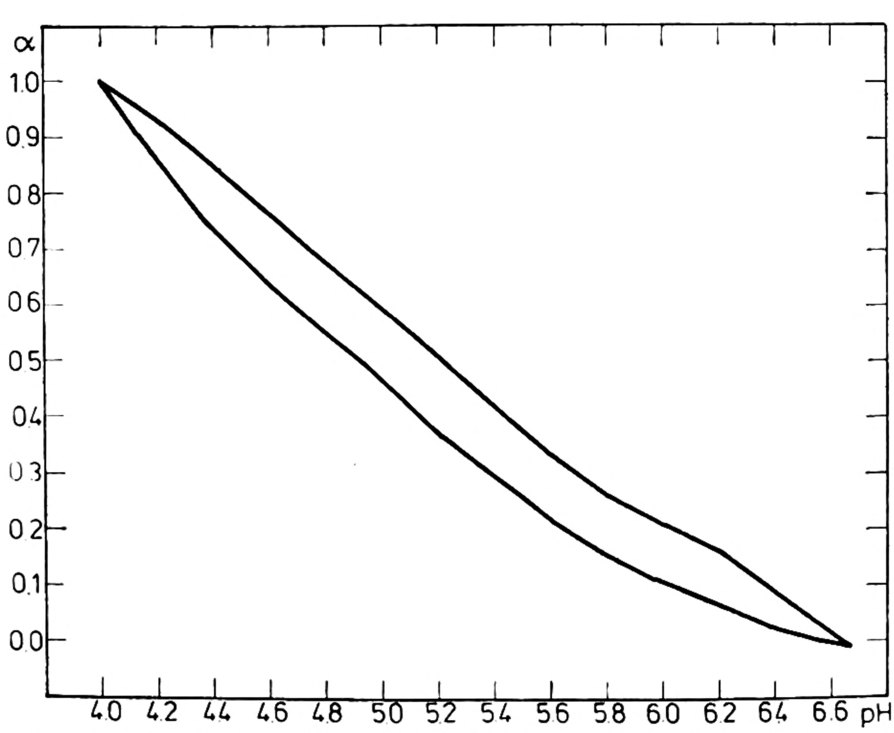


Fig. 1. Hysteresis of titration of 0.5% solution of proteins of buckwheat flour in 0.01 n NaCl with the use of acid and base

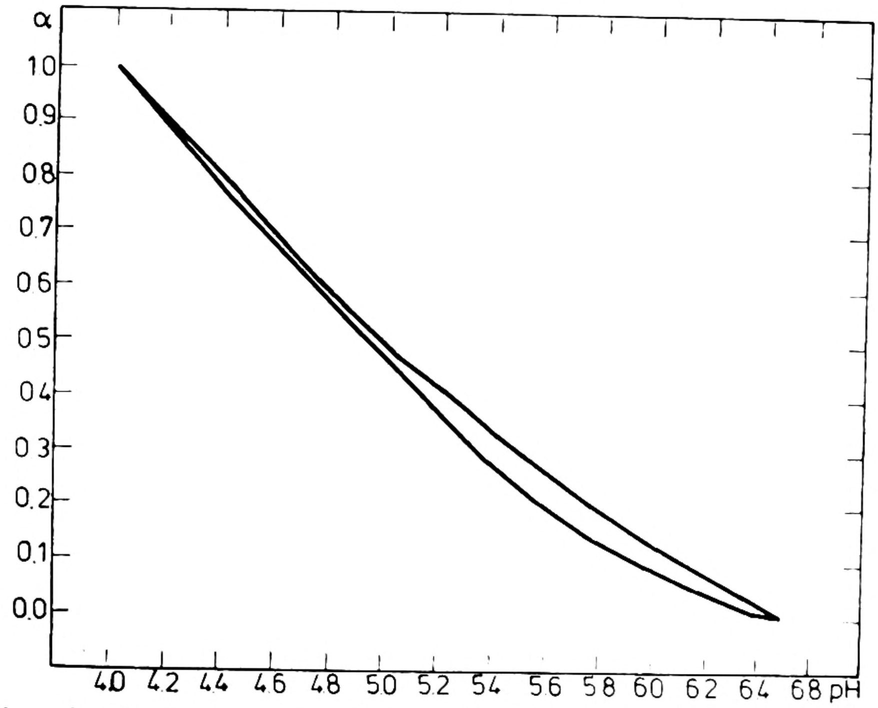


Fig. 2. Hysteresis of titration of 0.5% solution of proteins of the extruded product obtained from buckwheat flour. Temperature of extrusion 130°C

An equally strong effect of the extrusion process (temp. 130°C) on the solubility of nitrogen compounds was found in case of obtaining a product extruded from buckwheat groats. The above results show clearly that an extrusion temperature of 130°C has the strongest effect on the solubility of nitrogen compounds of the mixture of buckwheat flour and milk proteins and intensifies changes caused by torrefaction of buckwheat grain if such grain is used to obtain extruded products.

The influence of the extrusion process on the properties of proteins is also confirmed by the decrease (Figs. 1 and 2) or increase (Figs. 3 and 4) of the surface of hysteresis fields. This dependence was more distinct, the

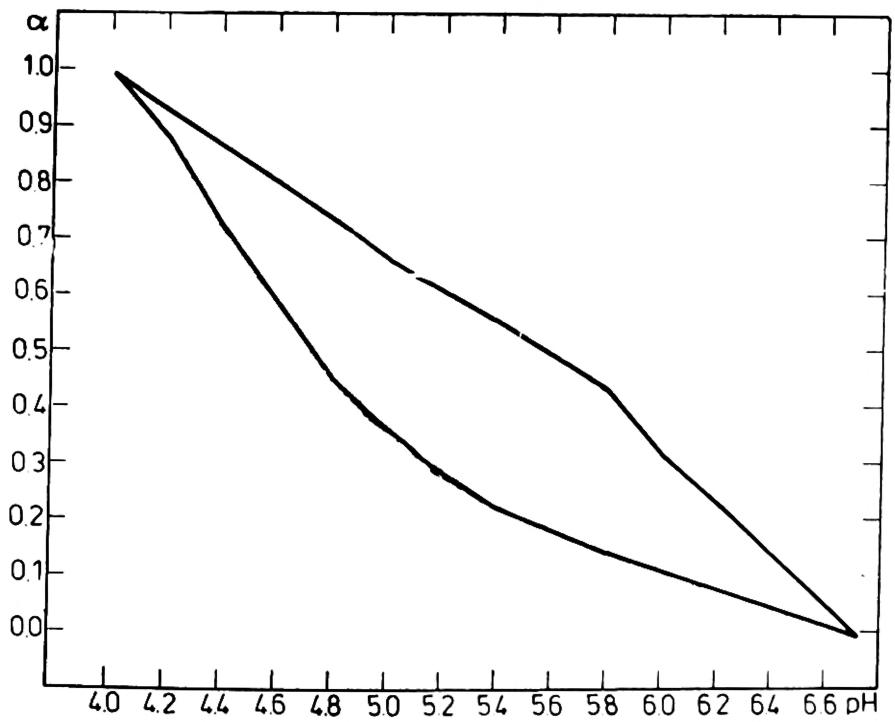


Fig. 3. Hysteresis of titration of 0.5% solution of proteins of mixture of buckwheat flour with 25% addition of milk proteins

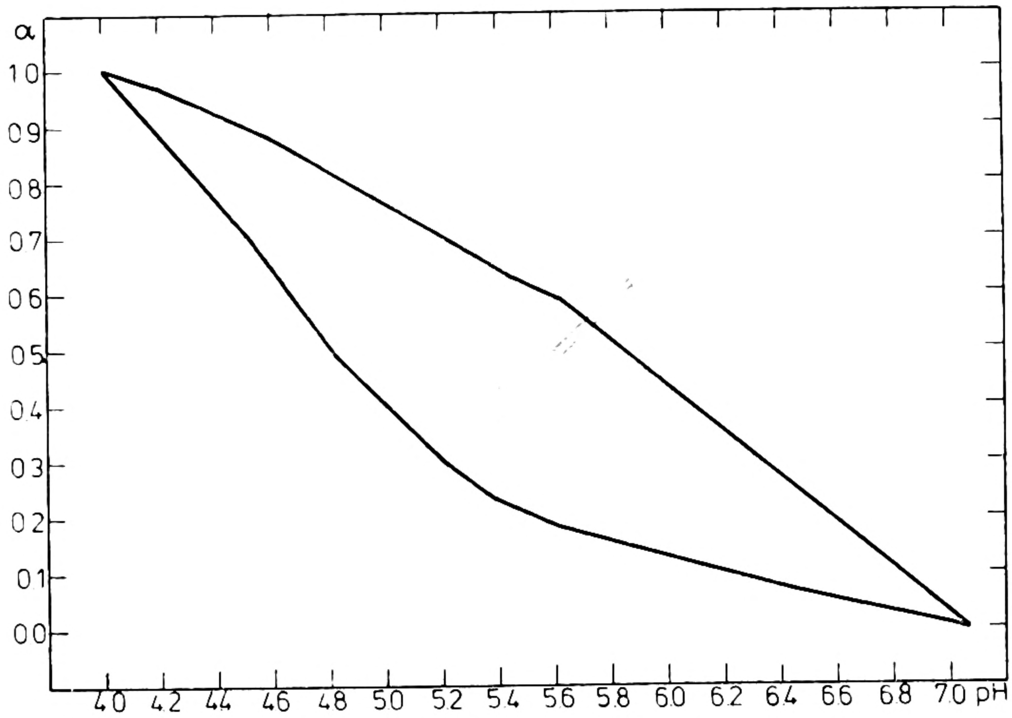


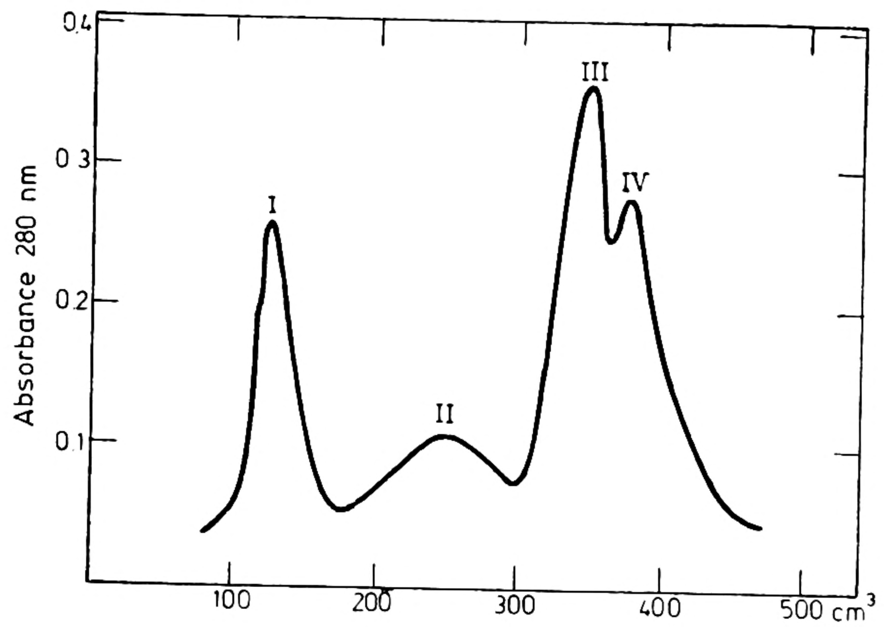
Fig. 4. Hysteresis of titration of 0.5% solution of proteins of buckwheat flour with 25% addition of milk proteins, extruded at 130°C

higher the temperature of the process (Table 2). According to Kirchmeier [10] titration curves may characterize the degree of aggregation of particles, accessibility of functional groups and buffering properties of proteins. It may then be supposed that an extrusion of 100-130°C causes an association of low-molecular nitrogen compounds and casein fractions. Wiechen and Knoop [21] demonstrated that the addition of a negligible amount of plant proteins containing polar and non-polar groups with various charges in the presence of calcium ions led to the formation of polymers and protein aggregates. Also Śmietana [19] found an interaction of casein during heating not only with other milk proteins but also with plant proteins. These statements may explain the increase of hysteresis field. On the other hand, the reduction of hysteresis field of buckwheat proteins may be an evidence of their thermolability, the consequence of which may be their degradation or participation in reactions of forming complexes with carbohydrates and lipids.

Table 2. Value of quotient of surface of hysteresis field and surface of unitary field, d integral of pH calculated for extruded products and raw materials

Type of raw material	d integral of pH				
	before extrusion	after extrusion			
		100°C	110°C	120°C	130°C
Buckwheat flour	0.24	—	0.09	—	0.09
Buckwheat flour with an addition of 25% milk proteins	0.53	0.34	0.52	0.56	0.64

The chromatographic characteristic of proteins of the examined products extruded at 130°C from buckwheat flour and a mixture of flour with milk proteins is presented in Figs. 5, 6, 7 and 8. The process of extrusion did not affect in a significant degree the pattern of the fractionated proteins. Among the buckwheat proteins, an increase of the share of fraction III and lowering of the content of fractions I, II and IV, was noted. The addition of milk proteins did not cause any significant changes



No of fraction	$\frac{\text{Eluated N}}{\text{Transferred N}} \times 100$
I	22.70
II	19.12
III	31.27
IV	26.90

Fig. 5. Chromatographic separation of proteins of buckwheat flour on Sephadex gel G-100 (Proteins were extracted with the use of 2 and 10% solution of NaCl)

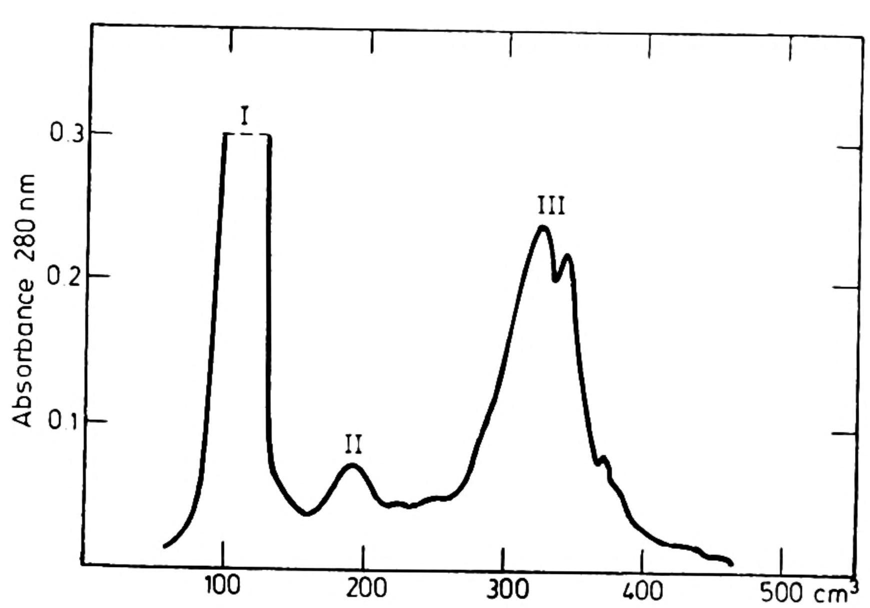
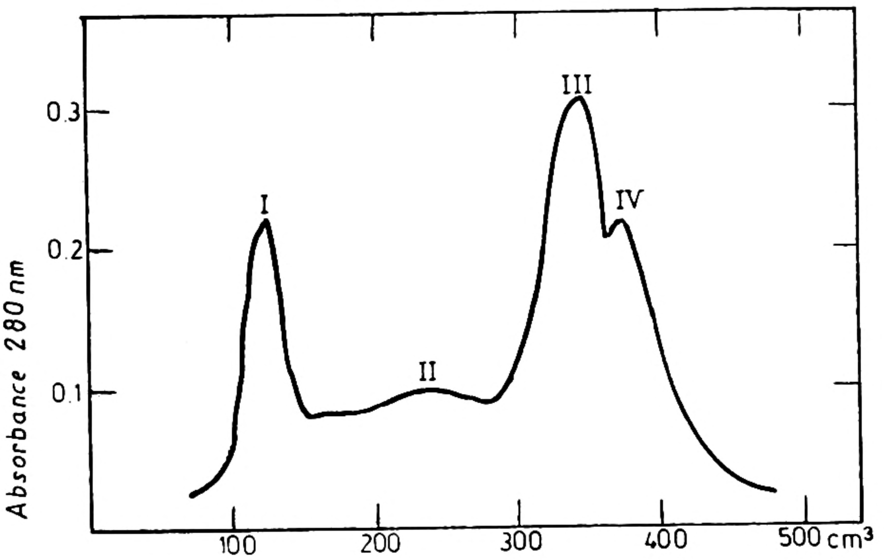


Fig. 6. Chromatographic separation of proteins of mixture of buckwheat flour and 25% milk proteins (Sephadex G-100)



No of fraction	$\frac{\text{Eluated N}}{\text{Transferred N}} \times 100$
I	18.90
II	14.17
III	44.61
IV	22.30

Fig. 7. Chromatographic separation of proteins of product extruded from buckwheat flour (Sephadex G-100)

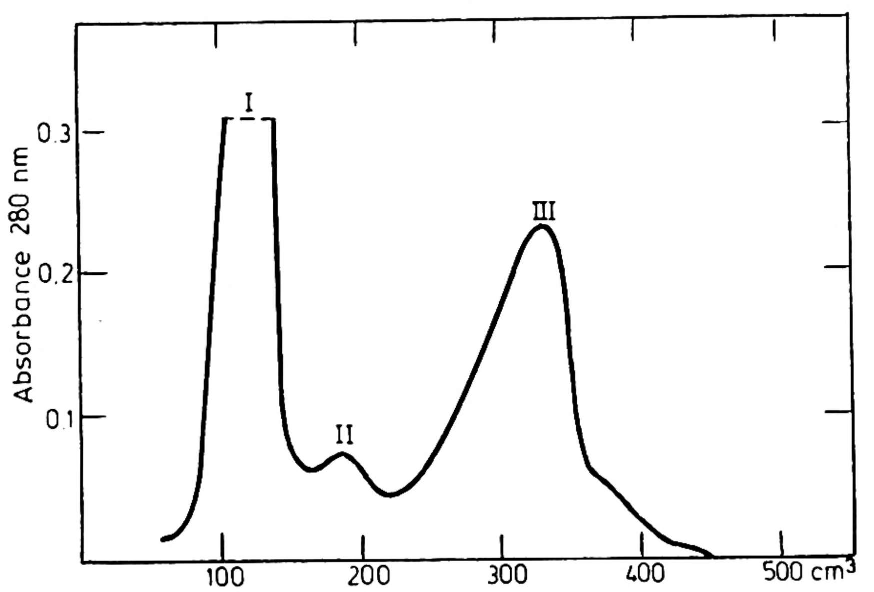


Fig. 8. Chromatographic separation of proteins of extruded product obtained from a mixture of buckwheat flour and milk proteins (Sephadex G-100)

either. Differences were only found in quantities of protein of fraction I and III.

Chromatographic patterns, in spite of small differences, show that in the extrusion process it is possible to combine certain protein fractions with a similar molecular weight and a similar dispersion in buffer solutions.

The simiar pattern of fractionation of proteins after extrusion does not provide an univocal explanation of the mutual interactions of protein molecules and influence on the physico-chemical properties of the product. It may be presumed that the extruded product obtained from a mixture of buckwheat proteins and milk proteins is a fairly stable system, created in the environment of dissociated calcium-phosphate salts of milk a fact which also confirm the results of earlier studies [19].

EFFECT OF THE EXTRUSION CONDITIONS ON CARBOHYDRATES

In the examined products extruded from buckwheat flour and its mixture with milk proteins, the temperature of the process was found to have the greatest influence on the level of starch and oligosaccharides (Table 3). The reduction of starch content points to the possibility of thermal dextrination which has been already clearly stated in studies on the influence of hydrothermal treatment of buckwheat grain [18]. In turn, it is probable that oligosaccharides participated in reactions of forming complexes with amino acids.

Table 3. Quantitative characteristics of carbohydrates

Type of product	Content % DM			
	starch	reducing sugars	oligosaccharides	cellulose
Buckwheat flour	81.25	0.41	1.21	1.20
extruded at 100°C	75.45	0.56	0.66	1.18
extruded at 120°C	77.12	0.57	0.57	1.25
extruded at 130°C	77.49	0.44	0.65	1.15
Mixture of 75% buckwheat flour and 25% milk protein	61.00	0.75	1.25	1.05
extruded at 100°C	57.93	1.11	0.56	1.18
extruded at 130°C	62.13	0.85	0.57	1.10
Disintegrated buckwheat groats	82.74	0.56	1.05	1.45
extruded at 130°C	81.64	0.20	0.34	1.48

In the chemical composition of starch isolated from the extruded products, an increase was recorded in the content of total nitrogen, free and bound lipids (Table 4). The level of total nitrogen in isolated starch depended both on the temperature of extrusion and type of raw material. These results may point to the formation of starch-protein complexes.

Special attention is paid to studies on the formation of lipid-starch complexes during the extrusion of starch raw materials [3, 13]. It is believed that these complexes have a defined meaning in preventing the dezorganization of starch grains and they are something like a cementing material, preserving the structure of glued starch.

The results given in Table 4 not only showed the possibility of forming starch-lipid complexes but also that the latter are more easily formed in a lower extrusion temperature. It is additionally interesting that

Table 4. Chemical composition of starch isolated from the extruded products

Product	Content, % DM			
	total nitrogen	free lipids	bound lipids	ash
Buckwheat flour	0.24	0.37	0.80	0.55
extruded at 100°C	1.79	8.48	3.20	2.39
extruded at 120°C	1.27	6.81	2.95	2.02
extruded at 130°C	0.51	4.97	2.63	2.49
Mixture of 75% buckwheat flour and 25% milk proteins	0.24	0.57	0.88	0.72
extruded at 100°C	1.04	8.49	4.07	2.86
extruded at 130°C	3.19	1.93	3.59	1.74
Disintegrated buckwheat groats	2.05	0.49	1.56	0.66
extruded at 130°C	0.95	6.14	3.14	0.82

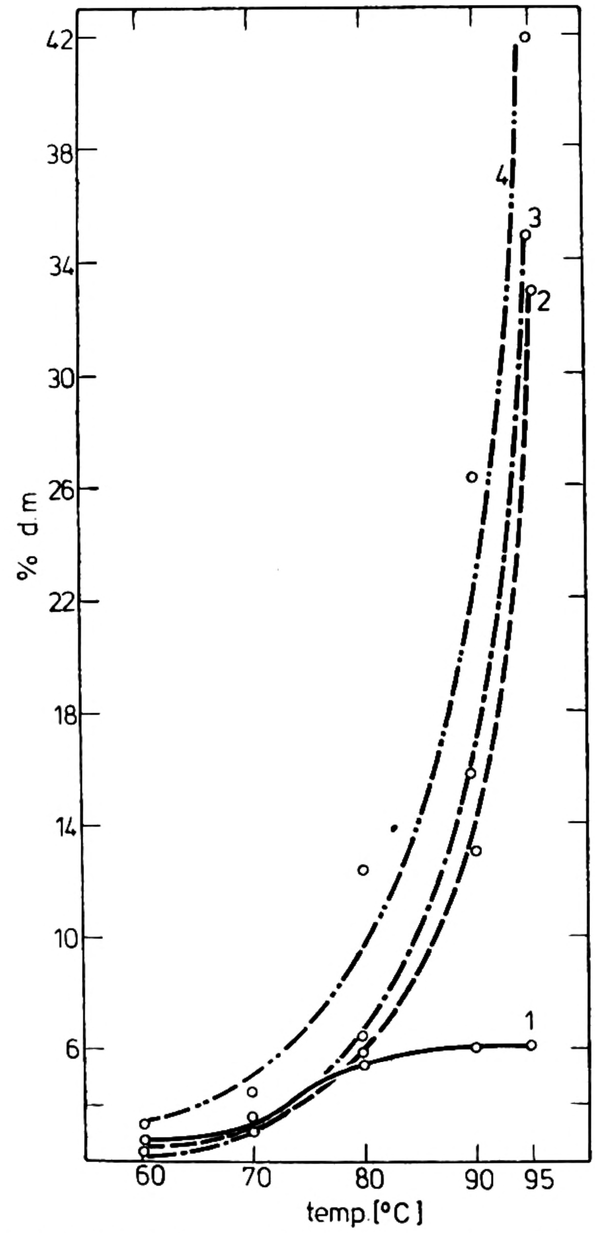


Fig. 9. Solubility of starch isolated from buckwheat flour and extruded products, depending on temperature; 1 — buckwheat flour, 2 — temperature of extrusion 100°C, 3 — temperature of extrusion 120°C, 4 — temperature of extrusion 130°C

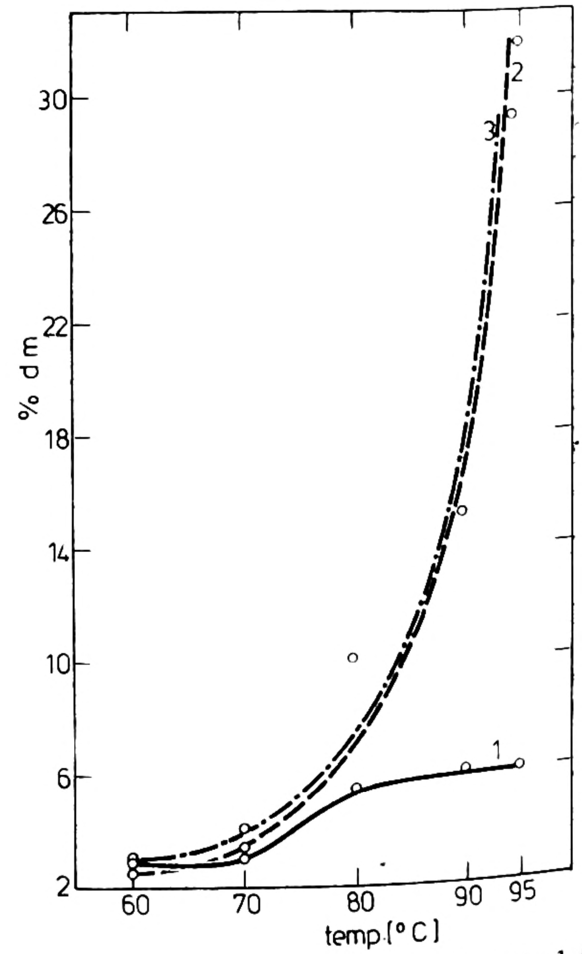


Fig. 10. Solubility of starch isolated from a mixture of buckwheat flour with 25% milk proteins and extruded products depending on temperature; 1 — mixture, 2 — temperature of extrusion 100°C, 3 — temperature of extrusion 130°C

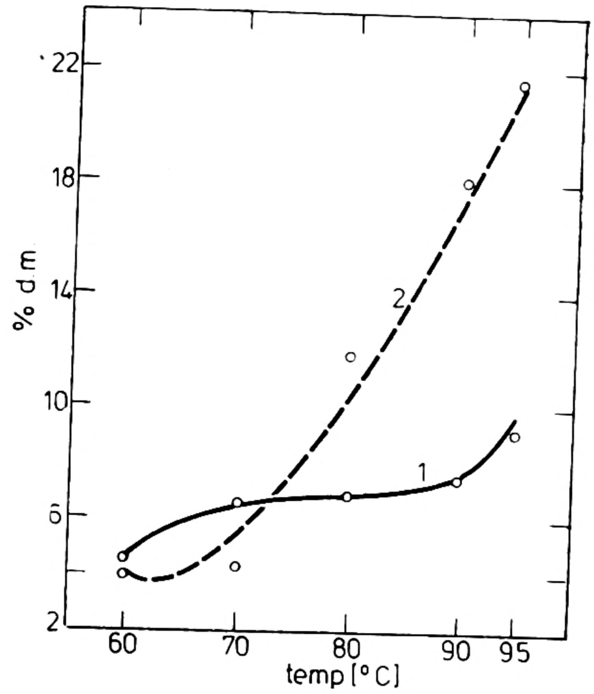


Fig. 11. Solubility of starch isolated from buckwheat groats and extruded product at 130°C; 1 — buckwheat groats, 2 — temperature of extrusion

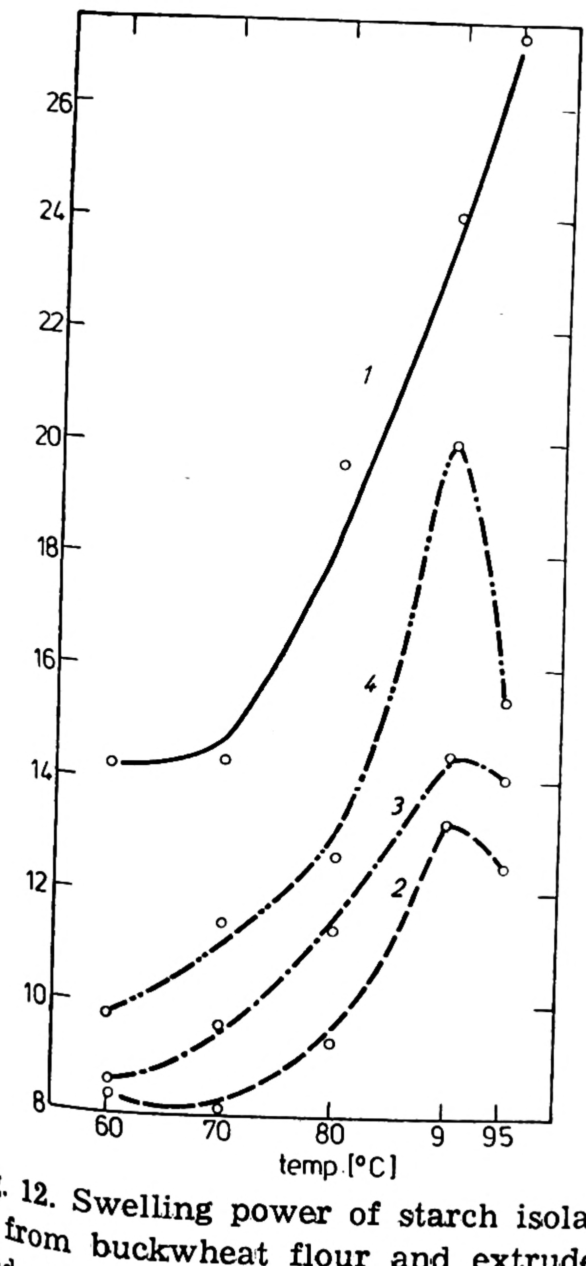


Fig. 12. Swelling power of starch isolated from buckwheat flour and extruded products depending on temperature; 1 — buckwheat flour, 2 — temperature of extrusion 100°C, 3 — temperature of extrusion 120°C, 4 — temperature of extrusion 130°C

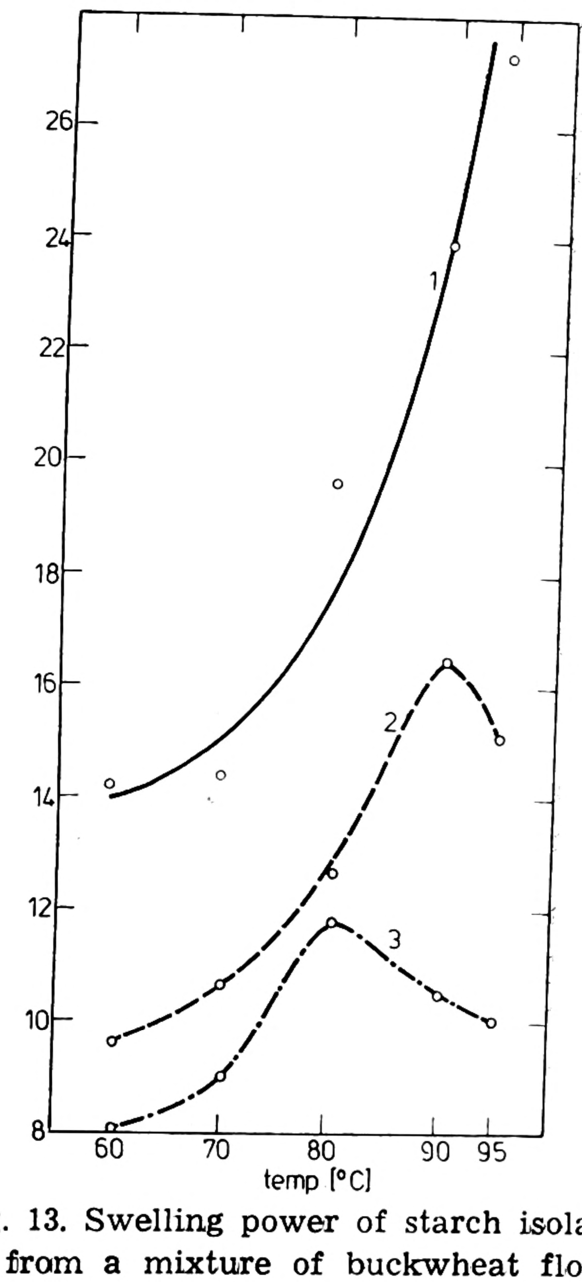


Fig. 13. Swelling power of starch isolated from a mixture of buckwheat flour and 25% milk proteins and extruded products depending on temperature; 1 — mixture, 2 — temperature of extrusion 100°C, 3 — temperature of extrusion 130°C

they are also formed when the raw material for extrusion is buckwheat groats in which the degree of starch gelatinization is about 80% [18].

Extrusion of buckwheat flour at 100°C caused gelatinization of starch in 84% which corresponds to its gelatinization after steam torrefaction of buckwheat grain during the process of obtaining buckwheat groats [18]. Application of higher temperatures of extrusion increased the degree of gelatinization to 91%. A high degree of gelatinization of starch was also found in products obtained from a mixture of buckwheat flour and milk proteins. Extrusion of buckwheat groats did not have such an influence on the increase of gelatinization degree.

The results of the degree of starch gelatinization are confirmed by curves of solubility and swelling power (Figs. 9, 10, 11, 12, 13, 14). The solubility of starch isolated from the extruded products obtained from

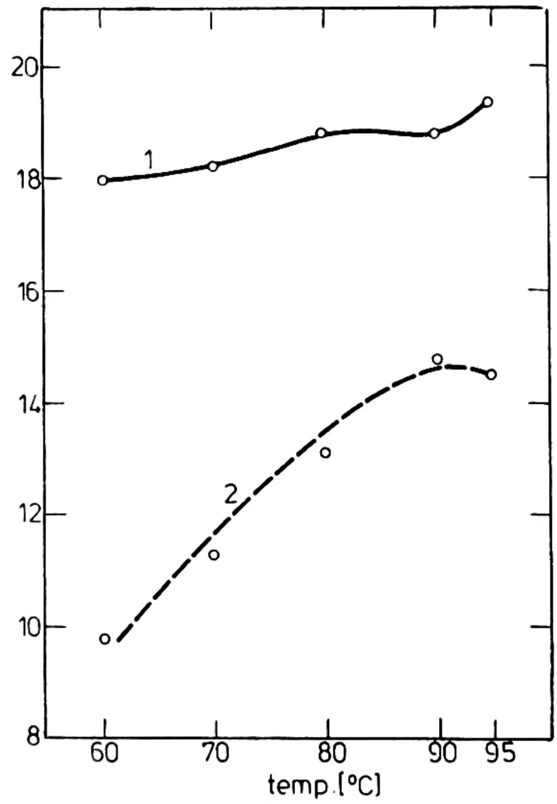


Fig. 14. Swelling power of starch isolated from buckwheat groats and product extruded at 130°C; 1 — buckwheat groats, 2 — temperature of extrusion 130°C

buckwheat flour with and without an addition of 25% milk proteins was the highest at 90-95°C and it was higher in comparison with starch isolated from raw material and product extruded from buckwheat groats. In turn, the swelling capacity of the examined starches was lower in comparison with the raw material and the changes caused by the extrusion temperature were more marked.

Effect of extrusion conditions on lipids

The level of free and bound lipids in the extruded products depended on the raw material and temperature of extrusion (Table 5). It may be supposed that the action of heat and water causes two phenomena: release of certain groups of lipids and creation of bounded forms.

Table 5. Level of free and bound lipids in the extruded products

Product	Content, % DM	
	free	bound
Buckwheat flour	2.56	1.28
extruded at 100°C	3.61	2.19
extruded at 120°C	4.62	2.34
extruded at 130°C	4.53	2.83
Mixture of 75% buckwheat flour and 25% milk proteins	3.83	1.44
extruded at 100°C	3.81	2.92
extruded at 130°C	3.69	3.14
Disintegrated buckwheat groats	2.81	0.56
extruded at 130°C	4.25	3.69

Similar dependences as a result of hydrothermal steam treatment of buckwheat grain were stated by Zalesskaya [22] and Yakovyenko [15] who concluded that the formation of bounded forms may favourably affect the stability of the product during its storage.

CONCLUSIONS

1. The applied parameters of extrusion affect the chemical changes and physico-chemical properties of proteins and starch of buckwheat flour and its mixtures with milk proteins.

2. In respect to nitrogen compounds the total content of which was not changed in the examined products, an influence of the process on their physico-chemical properties such as solubility and buffer capacity, was found. Chemical changes consist in a higher share of nitrogen fraction with lower molecular weight.

3. In respect to carbohydrates, the most important quantitative changes include lowering of the level of starch and oligosaccharides. Chemical composition of isolated starch as a result of higher content of nitrogen compounds, free lipids, bound lipids and ash points to the possibility of formation of complexes with these compounds.

4. Extrusion of buckwheat flour and its mixture with a 25% addition of milk proteins causes a high degree of starch gelatinization, increase of its solubility and reduction of its swelling power.

5. The stated chemical changes and physico-chemical properties of starch proteins are not unfavourable, therefore it may be concluded that buckwheat grain is suitable in obtaining extruded products.