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Nutrition and Physiology

Optimization of formulations with balanced biochemical composition and possibilities for their extrusion

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Abstract. Combinations of different raw materials (beans, einkorn wheat, and buckwheat) for obtaining formulations with high protein content and balanced amino acid composition were studied using simplex centroid design. The target functions for optimization were content of protein, sulfur-containing amino acid methionine and cysteine, lysine, and tryptophan. The optimal area of combinations of raw materials in the food formulas with balanced biochemical composition was obtained. Optimized ternary mixture consisting of 50% bean, 40% einkorn wheat, and 10% buckwheat with different moisture content (16, 22, and 28%) has been extruded in a laboratory single screw extruder (Brabender 20 DN, Germany). Extrusion parameters were as follows: feed screw speed 50 rpm, die diameter 3 mm, screw compression ratio 2:1, temperature profile 100/140/160°C, screw speed 160 rpm. The three extrudates with different initial moisture were evaluated by sectional expansion index, water absorption index, water solubility index, and density, with the aim of choosing the best treatment. The results demonstrated that the 22% initial moisture content yielded an extrudate with good physicochemical characteristics overall but an optimization study is needed to confirm this.

Keywords: beans, einkorn wheat, buckwheat, protein, amino acids, extrusion

Introduction

It is known that proteins of legume seeds are largely balanced in terms of essential amino acids (Comai et al., 2007; Iqbal et al., 2006). They are rich in lysine, arginine and leucine. The sulphur-containing amino acids (methionine and cysteine) are determinative amino acids (Montoya et al., 2008; Shekib et al., 1986; Wang and Daun, 2006). The content of lysine and tryptophan are determinative in the wheat (Abdel-Aal et al., 1995). That's why the shortage of incoming essential amino acids in legumes can be compensated by combining them with cereals.

Extrusion processing is a modern, highly efficient method, with proven technical and economic advantages for processing raw materials. It is used in seeds of legumes, because of the understanding that they are not expanded well with extrusion. In recent years, the expanding of the raw material base and the assortment of extruded food product increases, their consumption also increases (Penov and Petrova, 2014). The expansion index is an important parameter determining the extrusion process. It characterizes the structural-mechanical changes which occur as a result of the extrusion processing of the material and can be controlled by changing the type and origin of the components, and by varying the process conditions in the extruder.

Recovery and raised interest in legumes has been recently observed, which on the one hand is due to their nutritional value, and on the other – the unused opportunities to create new products with them. The increasing popularity of buckwheat will result in a greater variety of products thereof. One possible solution is to extrude buckwheat (Edwardson, 1996), yielding new, ready-to-eat products

such as extruded chips and many others (Petrova, 2012; Petrova and Desev, 2012). To obtain extrudates with balanced biochemical composition proper selection of the raw materials and the ratio between them is required. New in the study is the combination of legumes with other crops of plant origin, which is an expedient approach for obtaining raw material with very good functional characteristics. The main hypothesis of the research is absence of data in the literature on extrusion processing of ternary mixture from legumes, einkorn wheat and buckwheat.

The aim of the study is optimization of formulations with balanced biochemical composition and investigation of the possibility for their extrusion processing.

Material and methods

Representative samples of commercial raw materials einkorn wheat (*Triticum monococcum*) and buckwheat (*Fagopyrum*) were provided and delivered from the Experimental station, village of Lomets, municipality of Troyan, Bulgaria. Bean seeds (*Phaseolus coccineus* L.), cultivar "Bivolare", were grown and supplied from the Experiment station in the Rhodope Mountains. The proximate composition and amino acid profile for each of the raw materials were provided by suppliers or sourced from the literature as shown in Table 1.

Einkorn wheat, buckwheat, and bean seeds were ground using a hammer mill (SWH 20, Glen Creston LTD, UK) and passed through standard sieves. The prepared particle size of einkorn wheat, buckwheat, and bean grits was in the range of 0.4 – 0.5 mm. The

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Table 1. The information of the components used for formulation

Composition, %	Raw material		
	Bean	Buckwheat	Einkorn wheat
Protein	24.8	13.25	16.7
Methionine	1.1	0.19	1.75
Lysine	6.5	0.67	2.60
Cysteine	1.1	0.23	2.86
Tryptophan	1.3	0.19	2.59

bean, einkorn wheat, and buckwheat grits were mixed in the ratio of 50:40:10. Water was added slowly to the samples to obtain various moisture contents (16, 22 and 28%). The wet materials were placed and kept in sealed plastic bags for 12 h in a refrigerator at 5°C. The samples were tempered for 2 h at room temperature prior to extrusion. They were extruded in a laboratory single screw extruder (Brabender 20 DN, Germany). The extruder barrel (476.5 mm in length and 20 mm in diameter) contained three sections and independently controlled die assembly electric heaters. Extrusion parameters were as follows: feed screw speed 50 rpm, die diameter 3 mm, screw compression ratio 2:1, temperature profile 100/140/160°C, screw speed 160 rpm. The obtained extrudates were air-dried at ambient temperature for about a week. Sectional expansion index (*SEI*) was measured as the ratio of the diameter of the extrudate to that of the die. The diameter of extrudate was determined as the mean of 10 random measurements using a Vernier caliper. *SEI* is dimensionless.

Density (ρ , g/cm³) of the extrudates was determined by measuring the weight and the diameter of the same amount of extrudates (10 pieces) of the same length at each point of the experiment. The sample was weighed using a balance while its diameter and length were measured using a Vernier caliper. Ten replications were done for each sample. The volume of each extrudate was calculated by assuming that its shape approximates cylindrical. The density was calculated by the formula:

$$\rho = \frac{M \left(1 - \frac{W}{100} \right)}{V - M \frac{W}{100}} \quad (1)$$

where *M* is the weight of the sample (g), *V* is the volume of the sample (cm³), and *W* is moisture content of the sample (%). The moisture contents of the extrudates were determined as described by (AOAC, 1984). Triplicate determinations were carried out and the result averaged. Variation in the product density of the product is largely dependent on the proportion of voids or bubbles that were trapped in the extruded products as they solidified from the extruder. The product density is expected to be correlated with the void fraction of the products, hardness and general consumer acceptability of the product.

To determine water absorption and solubility indices, the extrudates were finely ground using a laboratory hammer mill and sieved through a 500 μ m sieve. A 0.2 g sample was placed in a tared centrifuge tube and 5 ml distilled water added. After standing for 30 min at 30°C (with intermittent shaking every 5 min), the sample was centrifuged at 3000 rpm for 20 min using a centrifuge CH 90-2A. The supernatant was decanted into a tared aluminium pan and weight

gain in the gel was noted. The supernatant was evaporated to dryness at 105°C until constant weight.

Water absorption index (*WAI*, g/g) and water solubility index (*WSI*, %) were calculated as:

$$WAI = \frac{m_g}{m_o} \quad (2)$$

$$WSI = \frac{m_{ds}}{m_o} \cdot 100 \quad (3)$$

where *m_g* is the weight gain of the gel (g), *m_o* is the weight of the dry sample (g), *m_{ds}* is the weight of the dried supernatant (g). Triplicate determinations were carried out and the result averaged.

A three-component, constrained simplex centroid mixture design was used for the development of formulations with a balanced biochemical composition (high protein content and markedly good participation of essential amino acids). The mixture components consisted of bean grit (*X₁*), buckwheat grit (*X₂*), and einkorn wheat grit (*X₃*). Component proportions were expressed as fractions of the mixture with a sum (*X₁* + *X₂* + *X₃*) of one (Table 2). The

Table 2. Mixtures composition in formulations with a balanced biochemical composition in a three-component constrained simplex centroid design

Formulation ^a	Ingredients proportion		
	<i>X₁</i> (Bean)	<i>X₂</i> (Buckwheat)	<i>X₃</i> (Einkorn wheat)
1	1	0	0
2	0	1	0
3	0	0	1
4	0.5	0.5	0
5	0.5	0	0.5
6	0	0.5	0.5
7	0.33	0.33	0.33

^a Formulation numbers correspond to Figure 1

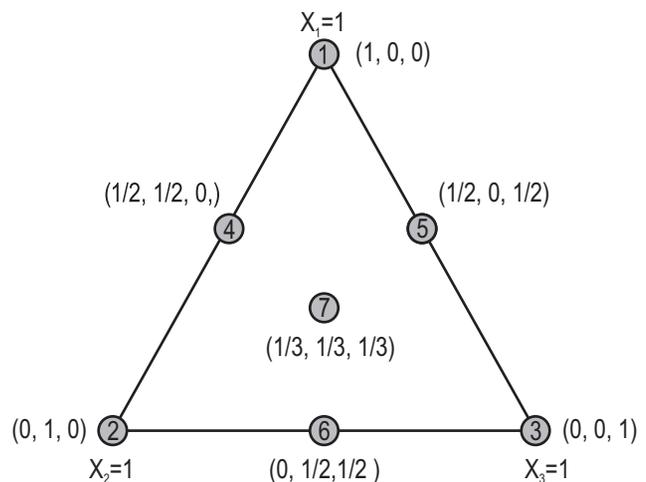


Figure 1. Seven point simplex-centroid design for the interaction of bean (*X₁*), buckwheat (*X₂*), and einkorn wheat (*X₃*) grit in formulations with a balanced biochemical composition

seven points were three single components, three two-component mixtures and one three-component mixture (Figure 1).

Scheffe's canonical special cubic equation for three components was fitted to data collected at each experimental point using backward stepwise multiple regression analysis as described by Cornell (1980). This canonical model differs from full polynomial models in that it does not contain a constant term (intercept equal to zero). Variables in the regression models, which represent two-ingredient or three-ingredient interaction terms, were referred to as "non-linear" terms. The postulated canonical special cubic equation was:

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 \quad (4)$$

where Y is a predictive dependent variable (protein, sulfur-containing amino acids methionine and cysteine, lysine, tryptophan content), $\beta_1, \beta_2, \beta_3, \beta_{12}, \beta_{13}, \beta_{23},$ and β_{123} are the corresponding parameter estimates for each linear and cross-product term produced for the prediction models for bean, buckwheat, and einkorn wheat grit, respectively. An analysis of variance was performed on the data and response surfaces were generated for each response using predictive models. The fitted model for protein, methionine and cysteine, lysine, and tryptophan content was used to optimize the formulations with balanced biochemical composition.

The terms in the canonical mixture polynomial have simple

interpretations which can be found in specialized texts (Myers and Montgomery, 2002). The usual way to summarize mixture proportions is via triangular (ternary) graphs. One can add a fourth dimension to the triangle, perpendicular to the first three, to plot the value for the dependent variable or it can be indicated, as is more usual, in a two-dimensional plot where the contour of constant height is graphed on a triangle.

Results and discussion

Using the simplex method and procedures of modeling related to it and optimization after treatment of the results equations for protein, sulfur-containing amino acids methionine and cysteine (Met + Cys), lysine (Lys) and tryptophan (Trp) content have been obtained, as follows:

$$\text{Protein} = 24.8X_1 + 16.7X_2 + 13.25X_3 + 0.02X_1X_3 + 0.02X_2X_3 - 0.12X_1X_2X_3, \% \quad (5)$$

$$\text{Met + Cys} = 2.2X_1 + 4.61X_2 + 0.42X_3 + 0.02X_1X_2 + 0.04X_1X_3 + 0.02X_2X_3 - 0.24X_1X_2X_3, \% \quad (6)$$

$$\text{Lys} = 6.5X_1 + 2.6X_2 + 0.67X_3 + 0.02X_1X_3 + 0.02X_2X_3 - 0.03X_1X_2X_3, \% \quad (7)$$

$$\text{Trp} = 1.3X_1 + 2.59X_2 + 0.19X_3 + 0.02X_1X_2 + 0.02X_1X_3 - 0.12X_1X_2X_3, \% \quad (8)$$

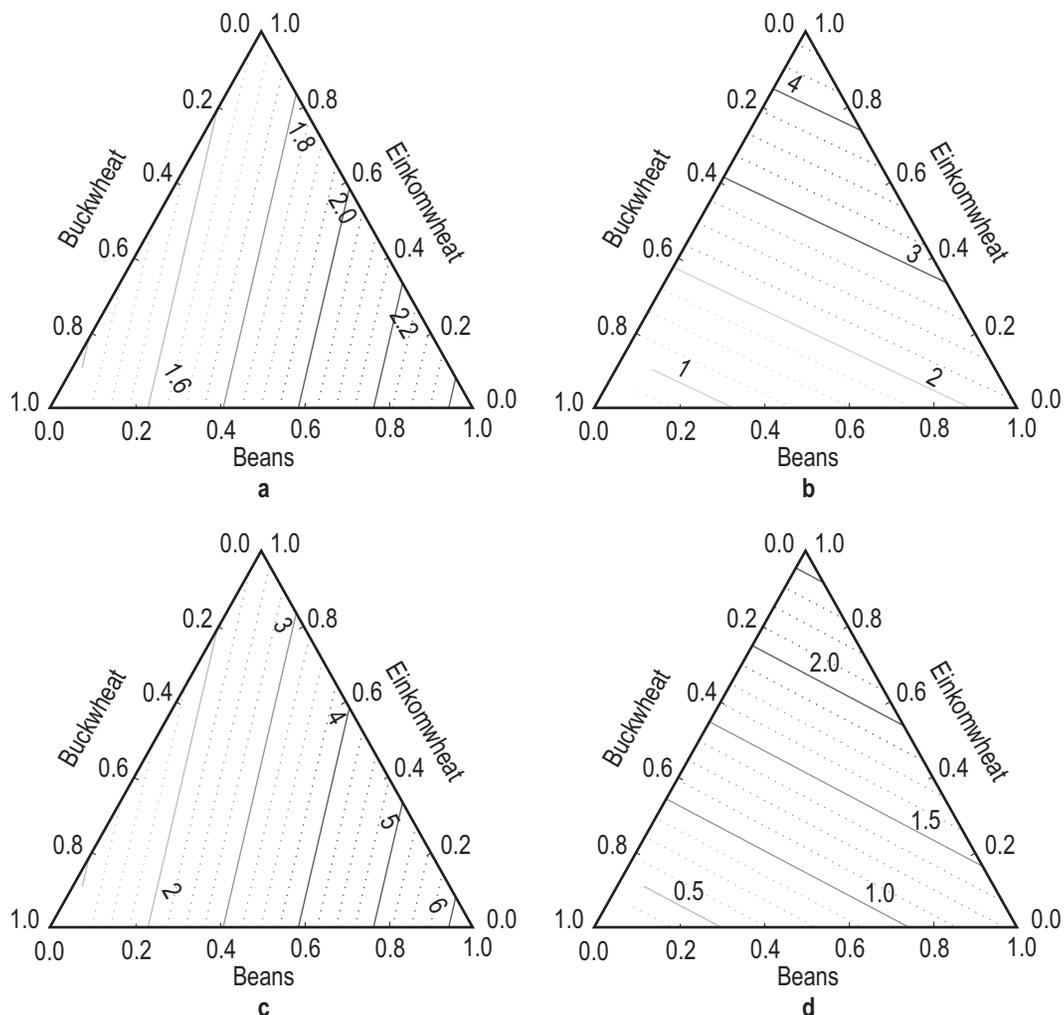


Figure 2. Two-dimensional triangular coordinate system showing the protein content (a), Met+Cys (b), Lys (c) and Trp (d)

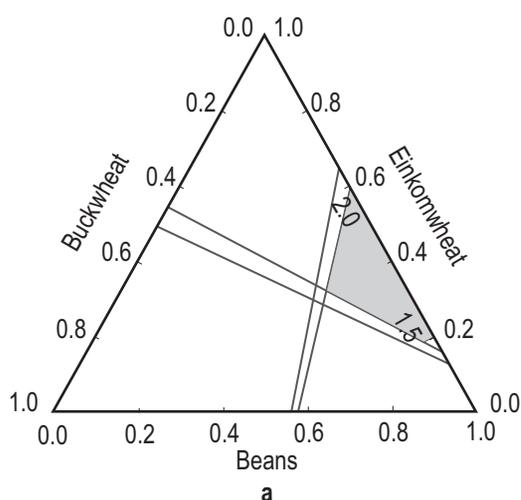


Figure 3. Graphical optimization of the ingredients in the mixture of formulations with balanced biochemical composition

The resulting equations with high accuracy describe the change of the contents of the dependent variable ($R^2 > 0.9$). The two-dimensional contour plots are shown in Figure 2.

For optimization of the ternary mixture obtained from beans, einkorn wheat, and buckwheat the following limited conditions have been accepted: protein content $> 20\%$, methionine and cysteine content $> 2.5\%$, lysine content $> 4\%$, and tryptophan content $> 1.5\%$. Optimization has been carried out by the superposition of the contour plots for predicted protein, methionine and cysteine, lysine, and tryptophan content of the formulations with a balanced biochemical composition. The optimum area for the ingredients in the mixture of the formulations is presented on Figure 3 (the darkened area). To each point of this area correspond ingredients in the mixture whose values with respect to the content for protein, sulfur-containing amino acids methionine and cysteine, lysine and tryptophan are optimized.

Based on the definite optimal area and a result of the obtained mathematical models the following composition of a ternary mixture of bean, einkorn wheat and buckwheat is selected: protein (20.40), methionine and cysteine (2.99), lysine (4.36), tryptophan (1.71).

Optimized ternary mixture obtained from bean, einkorn wheat, and buckwheat with different moisture content (16, 22, and 28%) has been extruded in a laboratory single screw extruder. The three extrudates with different initial moisture were evaluated by sectional expansion index (*SEI*), water absorption index (*WAI*), water solubility index (*WSI*), and density (ρ), with the aim of choosing the best treatment. The results of this evaluation are presented in Table 3.

Expansion, as measured by expansion index and density, is used to express product characteristics after extrusion. The values of the expansion index and density are dependent on the feed moisture, screw speed, and temperature of extrusion (Balandran-Quintana et al., 1998; Dogan and Karwe, 2003; Gujska and Khan, 1990). These values are also dependent on all ingredients contained in the extruded foods, such as starch, protein, fat, sugar, fibers, and so on. All these ingredients have different effects on extrudate expansion (Mercier and Feillet, 1975; Faubion and Hosney, 1982; Launay and Lisch, 1983). The extrudates from beans have different appearance from the extrudates from cereals. Overall, the extrudates from beans have significantly lower expansion indices compared to corn flour (Gujska and Khan, 1990). A high expansion ratio at low feed moisture content for extruded products is typical for cereals and their expansion depends on the degree of gelatinization

Table 3. Functional properties of an extruded mixture of bean, einkorn wheat, and buckwheat with different moisture content

Parameter	Moisture content, %		
	16	22	28
<i>SEI</i>	1.51 ± 0.07	1.74 ± 0.09	1.54 ± 0.11
ρ , g/cm ³	0.739 ± 0.05	0.571 ± 0.04	0.721 ± 0.07
<i>WAI</i> , g/g	7.615 ± 0.09	7.822 ± 0.16	10.11 ± 0.11
<i>WSI</i> , %	14.53 ± 0.12	14.33 ± 0.09	11.75 ± 0.19

**SEI* sectional expansion index, ρ density, *WAI* water absorption index, *WSI* water solubility index

of starch (Repo-Carrasco-Valencia et al., 2009). Faubion et al. (1982) reported a decrease in expanded volumes of cereals with increasing amounts of protein, and Linko et al. (1981) with increasing amounts of lipids. Table 3 shows that the sectional expansion index of extrudates increased with the feed moisture content before it reached a critical level (22%) after which *SEI* declined. *SEI* exhibited the highest value when extrusion was carried out at moisture content 22%. These changes in product characteristics are a result of modification of the starch and protein components under high temperature and pressure in the extruder barrel. An increase in *SEI* when moisture content went from 16 to 22% may be due to a reduction in viscosity, which resulted in less mechanical damage to starch, thus enabling dough to expand more and faster (Harper, 1986; Colonna et al., 1989). Similar finding has also been reported by others (Balandran-Quintana et al., 1998; Gujska and Khan, 1991). Balandran-Quintana et al. (1998) have extruded pinto bean flours at three different die temperatures (140, 160, and 180°C), feed moisture content (18, 20, and 22%), and screw speeds (150, 200, and 250 rpm). They have reported that the expansion index increased with increasing the feed moisture from 18 to 20%. At 180°C and 22% moisture, the expansion index decreased, probably because at high temperatures starch dextrinization occurred.

The density has been linked with the expansion ratio in describing the degree of puffing in extrudates (Asare et al., 2004) and the high density is associated with a low expansion index. The density of the extrudate decreased with increasing moisture content from 16 to 22%, then it increased. This could be due to starch degradation at high temperatures resulting in less expansion (Kokini et al., 1992). Similar results were shown by Balandran-Quintana et al. (1998) for extruded whole pinto bean meal.

Water absorption index depends on the availability of hydrophilic groups and on the gel formation capacity of the macromolecules (Gomez and Aguilera, 1983). It is a measure of damaged starch together with protein denaturation and new macromolecular complex formations (Dogan and Karwe, 2003). The water absorption index increased when the initial moisture increased. Our results show that *WAI* of the extruded mixture of bean, einkorn wheat, and buckwheat increases from 7.62 to 10.11 g/g with raising the moisture content from 16 to 28%. Similar results have been observed by Dogan and Karwe (2003) and Repo-Carrasco-Valencia et al. (2009).

The water solubility index decreased with increasing moisture content. Similar results have been observed by Repo-Carrasco-Valencia et al. (2009). This fact can be explained by the greater rupture of starch granules at the lower initial humidity. Low moisture content in the raw material in extrusion enhances the friction and the energy dissipation to the product, causing the dextrinization of the

starch and, at the same time, improving the *WSI* (Repo-Carrasco-Valencia et al., 2009). According to Gutkoski and El-Dash (1999) *WSI* is a parameter that indicates the degradation of starch granules.

Regarding the sectional expansion index, water absorption index, water solubility index, and density, the results demonstrated that the 22% initial moisture content yielded an extrudate with good physicochemical characteristics overall but an optimization study is needed to confirm this.

Conclusion

The determination of functional characteristics such as bulk density, sectional expansion index, water absorption index and the water solubility index allow to do optimization as regards the content of protein, sulfur-containing amino acids methionine and cysteine, lysine and tryptophan. An optimized ternary mixture consists of 50% bean, 40% einkorn wheat, and 10% buckwheat with different moisture content (16, 22, and 28%). Formulations with high protein and balanced amino acid composition have been obtained. The possibility for single screw extrusion of the optimized ternary mixture has been investigated and the parameters bulk density, sectional expansion index, water absorption index and the water solubility index have been determined. There is inverse relationship between the bulk density and the sectional expansion index and the correlation is a linear one ($R = 0.98, p < 0.05$).

The most important consequences for the science and practice resulting from the conducted research are that the resulting extrudates after grinding can be successfully used for the preparation of an instant product with balanced biochemical composition and good characteristics, such as bulk density, sectional expansion index, water absorption index and water solubility index.

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Review

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Thesis:

Hristova D, 2013. Investigation on genetic diversity in local sheep breeds using DNA markers. Thesis for PhD, Trakia University, Stara Zagora, Bulgaria, (Bg).

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