Original scientific paper
UDC 631.164.27:634/635

EXAMINATION OF QUALITY AND HYGIENIC CORRECTNESS OF THE BY-PRODUCTS OBTAINED IN MANUFACTURING VEGETABLES AND FRUITS

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Abstract

The subject of this work is of special interest for the Republic of Macedonia because the by-products obtained from agro industry range from 5 to10% for tomatoes, 25 to 30% for peppers and 20 to 25% for grapes. To examine the by-products quality and hygienic correctness were taken the food samples who origin from different regions of Macedonia.

In the food samples basic chemical composition was established by standard methods. The calcium content was established by spectrophotometric method (ISO 6490/2:1983), and the total amount of phosphorus by colorimetric method. On the basis of the results obtained on the nutrients content, applying suitable formula, the metabolic energy was counted (Alderman [4] and Grbesha [15]). To establish the mycotoxins content in samples of by-products the thin layer chromatography method was performed, and the standard multimycotoxicologyc method according to Balzer *et al*. [8]. The heavy metals content (Pb, Hg, As, Cd) was established by flaming atomic absorption spectrophotometry on the apparatus Perkin-Elmer 3300. The content particular of amino-acids in proteins was established by chromatographic method on automatic aminoanalizer according AOAC. Food samples for microbiological examination were inoculated in tiogluconant mix and physiologic solution from which further series dilution was made. Enumeration and identification of present bacteria and fungae was performed by standard microbiological methods.

The chemical analysis of the by-products obtained in manufacturing grapes from vine production, peppers from ajvar production and tomatoes from ketchup production, indicates that they contain great amounts of proteins (grapes - 12.66%, peppers - 18.77%, tomatoes - 21.15%), fats (grapes - 10.60%, peppers - 13.20%, tomatoes - 8.18%) and cellulose (grapes - 39.16%, peppers - 39.31%, tomatoes - 37.78%) where the proteins have an unfavourable amino-acid composition. Results from microbiological analyses (number and presence of: *Bacillus* spp., *Staphylococcus* spp., *E. coli, Salmonella* spp., *Clostridium* spp., *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp.), heavy metals examinations (As, Pb, Hg, Cd) and mycotoxins content (aflatoxin B1, ohratoxin A, zearalenone, T-2 toxin) showed that the by-products obtained in the manufacturing vegetable and fruits are hygienically correct and safe food.

***Key words**: By-products obtained in manufacturing tomatoes, Peppers and grapes, Quality, Hygienic correctness, Animal nutrition.*

1. Introduction

One of the basic scientific tasks concerning nutrition is to contribute to finding best productive solutions for livestock production, which means that an adequate choice of food should provide intensive production with optimal results. Except for quality, the intensive production requires, above all, economic nutrition, especially for animals concurrent with humans in the choice of food. The solution of this complex and extraordinarily important issue lies in the usage of new sources of nutrients, and it is certain that the potentials and resources lie in the usage of by-products from agro-industrial complexes.

The international association "New sources of food" under the auspices of FAO has been stressing the importance of introduction of new nutrients in the animal nutrition since 1976 when attention was paid, due to roughage food of bad quality, to the by-products from agro-industry, by-products in manufacturing wood and cellulose, as well as recycling by-products of animal origin and communal disposals. At the symposium data were presented (Chenost and Mayer [11]; Devendra [14]; Chicco and Schultz [12]; Adegbola [3]; Scouri [24]; Vlitos [35]; Arora [6]; and Balch [7]) on the quantifiers, chemical composition, nutritive value and the possibility for their practical application in the nutrition with various by-products from the food industry, especially the possibility of reducing the deficit of fodder balance in some species of livestock production.

Fodder balance analysis in the existing livestock production conditions in this area also point out a significant deficit of feedstuffs necessary for producing food of animal origin. According to Zlatich [38], the annual need of animals on the territory of former Yugoslav Republic rises to about 9.479.060 tons of starch units and 1.441.377 tons of crude proteins. On the other side the production of animal feed provides about 10 million tons of starch units and about 1.9 million tons of crude proteins. Of the total production of animal feed, about 22% of the energetic base consist of by-products from food industry, i.e. about 3.5 million tons of starch units. However, about 75% of the total production is from wheat and maize straw.

Similar results have been obtained by examination of the fodder balance in Macedonia (Shokarovski *et al.,* [31]). The results show that 30% of the energy is from by-products from food industry. It is certain that dominant nutrients are cereal straw (barley, oats, maize) except wheat whose major part is used for covering the floor, but it is interesting that in manufacturing tomatoes, peppers and grapes there is 5 to 10, 25 to 30, and 20 to 25% by-products respectively.

For good assessment of the possibilities, as well as the right use of nutrients afterwards, it is indispensable to make a detailed analysis, in fact to assess the nutritional value of the feedstuffs. The basic thing is to know the chemical composition which, although incomplete, is the basic information about the examined food. When speaking about by-products obtained in manufacturing tomatoes, peppers and grapes, it should be emphasized that there is little information in specialized literature. In addition, the information about the chemical composition differs, and is even contradictory, which shows that the processing is not standardized.

It is certain that most information about the basic chemical composition refers to by-products obtained in manufacturing grapes, in fact grape skin (Sinovec and Shevckovich [25]; Radovanovich and Rajich [23]; Stojanovich *et al.* [29]). The grape skin is primarily rich in water but in order to preserve it longer and to prevent it form spoiling it is dried and processed so that it can be used as industrial mixture for animal nutrition. The water content rises from 8.00 to 10.00%, ash from 4.00 to 4.38% whereas the phosphorus content (about 0.95%) is higer than the calcium content (0.15%), however (Stojanovich *et al.* [29]) the information quite contradictory (0.63% Ca and 0.24% P). The content of proteins rises from (10 to 10.62%), and there is very little information about the amino-acid content (NRC, 1998 [20]). Generally, the lyzin (0.39) methonine and cystine (0.13), threonine (0.30) and tryptophane (0.25) content is very low. The fats content rises from 8.00 to 10.00% and the non-nitrogen extracted materrs content from 31.00 to 47.20%. On the other hand the fibers content is high (23.20 to 25.00, but also 31.60%). So the energetic value (ME) is relatively satisfactory and rises from 7.67 to 8.01 MJ/kg (Todorov [34]; Shokarovski and Cilev [32]). However, because of the relatively variable composition other information can be found in literature which is very similar (Smilevski *et al.* [26], [27], [28]; Damjanovska *et al.* [13]; Shokarovski *et al.* [30]) or significantly different from the above mentioned (Jurgens [17]; Bath *et al.*. [9]). Regarding the chemical, and specially the amino-acid composition Vlahovich *et al.* [36] report that in processing fresh grape skin different products can be obtained (standard flour, fine and rough fractions). The water content in standard flour is about 10.52%, the ash content 4.80%, proteins 11.64%, fats 9.95%, fibers 24.86 % and NEM 49.85%. In a fine fraction the nutrients content is 9.64, 5.17, 14.20, 11.28, 17.26 and 42.45%, and in a rough fraction 11.03, 4.01, 6.20, 7.12, 32.45 and 39.22%. Similarly the above authors have established difference in the amino-acid composition. In the fine fraction the lyzins content is 0.84%, methionines 0.35% and threonines 0.78% while in the standard flour it is 0.39, 0.13 and 0.30%, respectively.

Relatively little information regarding basic chemical composition refers to by-products obtained in manufacturing tomatoes (Sinovec and Shevkovich [25]; Bath *et al*. [9]). The tomato pulp is primarily rich in water (> 60%) so, in order to preserve it longer and prevent it from spoiling, it is dried and processed so that it can be used in industrial production of mixtures for animal nutrition. The water content rises from 6.00 to 7.50% and ash 7.00 to 8.00% whereas the phosphorus content (about 0.57%) is a little higher than the calcium content (0.40%). The proteins content rises from 17.05 to 23.50% and there is no information about the amino-acid composition (Bogdanov [10]). The fats content rises from 10.00 to 12.30%, and the non-nitrogen extracted materrs content 24.30 to 31.00% (Allen [5]). On the other side the fibers content is relatively high (24.80 to 26.40%) so the energetic value (ME) is relatively satisfactory and rises from 10.29 to 10.94 MJ/kg. However, on the relatively variable composition other information can be found in literature, very similar

(Todorov [34]) but significantly different in particular parameters (Jurgens [17]; Shokarovski and Cilev [32]).

Relatively little information regarding basic chemical composition refers to by- products obtained in manufacturing peppers (Shevkovich *et al.* [33]; Todorov [34]; Shokarovski and Cilev [32]). The peppers pulp is primarily rich in water (> 60%) thus, in order to preserve it longer and prevent it from spoiling, it is dried and processed so that it can be used in industrial production of mixtures for animal nutrition. The water content rises about 10.00%, and ash 6.75 to 7.03%. The proteins content rises from 15.21 to 17.94% and there is no information about the amino-acid composition. The fats content rises from 9.00 to 9.66%, and the non-nitrogen extracted materrs content 19.62 to 36.54%. On the other side, the fibers content is relatively high (22.50 to 35.76%) so the energetic value (ME) is relatively satisfactory and rises from 8.46 to 9.66 MJ/kg.

For good assessment of the possibilities and usage of by-products from agro-industry, as well as the right use of the nutriments afterwards, it is indispensable to make a detail quality analysis, in fact to assess the nutritional value and the hygienic correctness of the feedstuffs.

2. Materials and Methods

Having in mind the supposition that the usage of by-products from the food industry is beneficial from nutritive, hygienic, sanitary, ecologic and economic aspect, this assignment required quality examination, that is, nutritional value and hygienic correctness of by-products obtained in manufacturing tomatoes, peppers and grapes. Consequently, the following parameters were observed and obtained:

1. The nutrition value of by-products from food industry
   - basic chemical composition
   - particular amino-acids content
2. Hygienic correctness of by-products from food industry
   - number and kind of bacteria and mould
   - micotoxins content
   - heavy metals content

In the food samples basic chemical composition was established by standard methods (Sinovec and Shevkovich [25]). The calcium content was established by spectrofotometric method (ISO6490/2:1983 [16]), and the total amount of phosphorus by colorimertric method (Regulation book [21]). On the basis of the results obtained on the nutrients content, applying suitable formula, the metabolic energy was counted (Alderman [4]; Grbesha [15]).

To establish the micotoxins content in samples of by-products the thin layer chromatography method (AOAC, [1]) was performed, and the standard multimicotoxicologyc method according to Balzer *et al.* [8].

The heavy metals content (Pb, Hg, As, Cd) was established by flaming atomic absorptional spectrophotometry on the apparatus Perkin-Elmer 3300, (Vukashinovich [37]). The content particular of amino-acids in proteins was established by chromatographic method on automatic aminoanalizator (AOAC, [2]).

The food samples for microbiological examination were inoculated in tiogluconant mix and physiologic solution from which further series dilution was made (Medanich and Zakulja [19]). 0.5 mL solution was spread on selective ground for determining the number and kind of the bacteria and mould.

3. Results and Discusion

The chemical composition of by-products obtained in manufacturing grapes, tomatoes, peppers and maize is presented in Table 1.

Table 1. Chemical composition of researched by-products and maize [%]

Chemical composition	By-products			Maize
	Grapes	Tomatoes	Peppers	
Moisture	8.40	8.18	8.61	13.00
Ash	4.36	3.38	6.15	1.20
Protein	12.66	21.15	18.77	8.00
Fat	10.60	13.20	8.18	4.00
Fibre	39.16	39.31	37.78	2.10
NFE	24.82	14.78	20.51	71.70
Calcium	0.64	0.41	0.56	0.02
Phosphorus	0.41	0.36	0.82	0.30
ME, MJ/kg	8.99	8.61	8.50	13.97
Lysine	0.33	0.31	0.29	0.20
Methionine + Cystine	0.13	0.11	0.15	0.26
Threonine	0.18	0.02	0.10	0.10
Tryptophane	0.35	0.22	0.25	0.40

We can see from the table that the examined products contain significantly larger amounts of proteins and fats in comparison to corn, whereas proteins have less satisfactory amino-acid composition. On the other hand, because of a high level of cellulose and a very low level of carbohydrate, they represent a significantly pure source of energy in comparison to maize.

In Table 2 the results of bacterial analysis of sample by-products obtained in manufacturing grapes, tomatoes and peppers are presented. We can see from the table that the sample by-products from food industry contain acceptable amount of bacteria and mould, that is, they can be used in animal nutrition.

Table 2. Determination of bacteria and mould in samples of by-products [CFU/g]

Type of microorganisms	By-products		
	Grapes	Tomatoes	Peppers
Bacillus spp.	+	+	+
Staphylococcus spp.	+	+	+
E. coli	+	+	+
Salmonella spp.	-	-	-
Clostridium spp.	200	250	150
Total number of bacteria	**5×10^6**	**4×10^6**	**7×10^6**
Aspergillus spp.	+	+	+
Penicillium spp.	+	+	+
Fusarium spp.	+	+	+
Total number of moulds	**3×10^3**	**5×10^3**	**4×10^3**

In Table 3 the heavy metals content in sample by-products obtained in manufacturing grapes, tomatoes and peppers is shown. We can see that particular heavy metals in sample by-products from food industry are below acceptable maximum quantity, that is, they can be used by animal nutrition.

Table 3. Heavy metals content in samples of by-products [mg/kg]

By-products	Heavy metals			
	As	Pb	Hg	Cd
Tomatoes	0,018	0,786	0.003	0,028
Peppers	0,007	0,435	0.002	0,016
Grapes	0,012	0,226	0.001	0,015

In Table 4 the micotoxins content in sample by-products obtained in manufacturing grapes, tomatoes and peppers is presented. We can see from the table that the sample by-products from food industry contain acceptable amount of micotoxins, that is, they can be used in animal nutrition.

Table 4. Micotoxins content in samples of by-products [mg/kg]

Type of micotoxins	By-products		
	Grapes	Tomatoes	Peppers
Aflatoxin B_1	-	-	-
Ohratoxin A	0.16	0.10	0.21
Zearalenone	0.35	0.27	0.40
T-2 toxin	0.10	0.05	0.07

3.1. Chemical composition

Chemical composition i.e. nutritional value, represents the basic parameter which can indicate the degree of possible usage of some feedstuffs in animal nutrition. The basic step is knowing the chemical composition which, although incomplete information, gives basic information about the examined feedstuffs. On the basis of the chemical analyses results, the obtained amount can be established and the meal can be optimized as well.

The chemical composition of by-products obtained in manufacturing grapes is characterized by a low level of water (8.40%) which is in accordance with the information from literature (Sinovec and Shevkovich [25]; Radovanovich and Rajich [23]; Stojanovich *et al.* [29]). The low level of water enables usage in industrial production of mixtures for animals, thus providing longer life and prevention of deprivation.

The ash content in the feedstuffs is satisfactory (4.36%), which is in accordance with the information from literature. Accordingly, it can be noted that besides the calcium and phosphorus content the relation between the mentioned macroelements is satisfactory. However these values are only partially in compliance with the information from literature (Radovanovich and Rajich [23]), but there is completely contradictory information as well (Stojanovich *et al.* [29]).

The examined feedstuffs are characterized by a relatively high level of proteins from 12.66%, which is a little more than reported in literature as average amount (Sinovec and Shevkovich [25]; Radovanovich and Rajich [23]). This is quite understandable having in mind the discoveries of Vlahovich *et al.* [36], who report that by processing grape skin different products can be obtained (standard flour, fine and rough fraction) which differentiate, hence the reported rise in the proteins content from 6.20 to 14.20%.

While investigating the proteins content attention should be paid to the amino-acid composition. Although the examined feedstuffs contain particular amino-acids (lyzine, threonine), more than maize, it should be emphasized that their relation is unsatisfactory, refering most of all to lysine, which contains more than maize, and methionine, which contains less than maize. However, having in mind that these feedstuffs would be used in pig nutrition in smaller amounts firstly as energy source, it can be concluded that the amino-acid composition will not affect negatively the meal nutrition value.

The determined fats content (10.60%) and extracted materials with no nitrogen (24.82%) is in compliance with the information from literature (Smilevski *et al.* [27], [28]), but it should be emphasized that bigger differences were observed in the extracted materials content with no nitrogen, which is probably in relation to the above mentioned variable other nutrients content. Having in mind the significant presence of these nutrients, whose presence is larger in maize when speaking about fats it should be expected that energetic value is similar or higher than the energy content in maize.

However the exceptionally high level of fibrous materials (39.16%) significantly reduces the usefulness of nutrients, consequently the feedstuffs energetic value. The metabolic energy content of 8.99 MJ/kg is mainly in accordance with the literature information.

Generally, the examined feedstuffs are characterized by a high level of fats and proteins, whereas proteins have a less satisfactory amino-acid composition. On the other side, because of the high level of cellulose the examined feedstuffs are a poor source of energy from maize. However, despite differences in chemical composition of by-products obtained in manufacturing grapes in reference to maize, it can be concluded that using these feedstuffs in a smaller amount will not affect negatively the meal nutritive value.

The chemical analyses results of by-products obtained in manufacturing tomatoes are mainly in accordance with the literature, but significantly differentiate in particular parameters. The water content (8.18%) is in accordance with the literature (Sinovec and Shevkovich [25]; Bath *et al.* [9]) while the established ash content (3.38%) is below the amount reported in literature. Also the amount of calcium and phosphorus is partially in accordance with the literature (Allen [5]).

The examined feedstuffs are characterized by a high level of proteins (21.15%) which is in accordance with the amount reported in literature (Bogdanov [10]). When examining the protein content the attention should be paid to amino-acid composition for which no information was found in literature. Although the examined feedstuffs contain a significantly larger amount of proteins than maize, the particular amino-acids content, except for lysine, is very low. However, having in mind that these feedstuffs would be used in pigs nutrition in a smaller amount, it can be concluded that using the established amino-acid composition will not affect negatively the meal nutritive value.

The established fats content (13.20%) and materials with no nitrogen (14.78%) is in accordance with the literature (Todorov [34]), but it should be emphasized that bigger differences are observed in the extracted materials with no nitrogen content which is probably in relation to the possible of other variable nutrients. Taking into consideration the significant presence of fats, higher in maize, it should be expected that the energetic value is relatively high. However, the high level of fibrous material (39.31%) significantly reduces the usefulness of the nutrient, and consequently the feedstuffs nutritive value. The metabolic energy content of 8.61 MJ/kg is mainly in accordance with the literature (Jurgens [17]; Shokarovski and Cilev [32]).

Generally, the examined feedstuffs are characterized by a high level of fats and proteins, whereas proteins have a less satisfactory amino-acid composition. On the other side, because of the high level of cellulose the feedstuffs represent a poor source of energy from maize. However, despite differences in the chemical composition of by-products obtained in manufacturing tomatoes in reference to maize, it can be concluded that using these feedstuffs in smaller amounts will not affect negatively the meal nutritive value.

It is difficult to compare the results of the chemical analysis of by-products obtained in manufacturing peppers with similar ones because of a very limited information (Shevkovich *et al.* [33]; Todorov [34]; Shokarovski and Cilev [32]).

The water content (8.61%) and the ash content (6.15%) are in accordance with the literature information, whereas the phosphorus content (about 0.82%) is higher than the calcium content (0.56%).

The examined feedstuffs are characterized by a relatively high level of proteins (18.77%), which is a little more than the amount reported in literature. When examining the protein content attention should be paid to amino-acid composition for which no information was found in literature. Although the examined feedstuffs contain a significantly larger amount of proteins than maize, the methionine and tryptophane content is significantly low. Having in mind that these feedstuffs will be used in pigs nutrition in smaller amounts, it can be concluded that using the established amino-acid composition will not affect negatively the meal nutritive value.

The established fats content (8.18%) is a little lower than information found in literature, but exceptionally higher than maize so be a relatively high energetic value should be expected. However, the high level of fibrous material (37.78%) significantly reduces the usefulness of the nutrient, and consequently the feedstuffs nutritive value, which is about 8.50 MJ/kg.

Generally, the examined feedstuffs are characterized by a high level of fats and proteins, whereas proteins have a less satisfactory amino-acid composition. On the other side, because of the high level of cellulose the examined feedstuffs represent a poor source of energy from maize. However, despite differences in the chemical composition of by-products obtained in manufacturing peppers with reference to maize, it can be concluded that using these feedstuffs in smaller amounts will not affect negatively the meal nutritive value.

Finally, on the basis of the chemical composition, it can be concluded that by-products obtained in manufacturing grapes, tomatoes and pepper are feedstuffs which are characterized by a high level of proteins and fats, whereas the proteins have a non satisfactory amino-acid composition. However, despite differences in the chemical composition with reference to maize, it can be concluded that the examined by-products can be used in smaller amounts in the pigs nutrition.

3.2. Hygienic correctness

When speaking about quality and possibility for using particular feedstuffs in animal nutrition, alongside with knowing the chemical composition, it is indispensable to assess the hygienic correctness, that is, possible dangerous effects.

There are no exact data on the degree of contamination of the examined by-products obtained by manufacturing grapes, tomatoes and peppers in the literature. However, in the conducted analysis presence of saprophyte bacteria was established (*Staphylococcus* spp., *E. coli*, *Bacillus* spp.) and bacteria poisoning food (*Clostridium* spp.), but no *Salmonella* spp. The number of saprophyte bacteria found in the examined by-products meets the legal regulation regarding the nutrition of young animals (Regulation book [22]). Additionally, in the examined samples presence of saprophyte mould was established (*Asspergillus* spp., *Penicillium* spp., *Fusarium* spp.) characteristic for the pigs nutrition in this region. (Markovich *et al*. [18]), but the total amount of saprophyte phungi meets the legal regulation regarding the nutrition of young animals (Regulation book [22]).

During the micotoxic examination of sample by-products obtained in manufacturing grapes, tomatoes and peppers the presence of ohratoxin A, zearalenon and T-2 toxin was established in minimal quantities, but no aflotoxin in either of the samples. Considering the kind and amount of micotoxins, as well as the fact that these feedstuffs are used in small amounts and in combination with other ingredients on the mixtures it can be concluded that the examined feedstuffs meet legal regulations regarding pigs nutrition (Regulation book [22]).

The heavy metals content (As, Pb, Hg and Cd) in the examined by-products was within the limits of maximum amount of dangerous materials in animal nutrition established in the Regulation book [22]. The obtained results show that the examined by-products can be limitlessly used as food in pigs nutrition.

Overall, it can be concluded that, on the basis of the conducted analysis, the by-products obtained in manufacturing grapes, tomatoes and pepper represent safe food which can be used in the pigs nutrition without affecting their health condition.

4. Conclusions

On the basis of the obtained results from the conducted examinations we can draw the following conclusions:

- By-products obtained in manufacturing grapes, tomatoes and peppers represent feedstuffs which are characterized by a high level of proteins, fats and cellulose, whereas proteins have non satisfactory amino-acid composition.

- On the basis of the bacterial analysis and examinations of the heavy metals and micotoxins content it can be concluded that the examined by-products obtained in manufacturing grapes, tomatoes and peppers represent hygienically correct and safe food that can be used in the pigs nutrition without affecting their health condition.

5. References

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