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# Simple utilization of lactic acid whey in dairy processing

J. Csanádi

e-mail: csanadi@mk.u-szeged.hu

G. Szász e-mail: tabrika88@hotmail.com O. H-Bara

e-mail: otti@mk.u-szeged.hu

University of Szeged, Faculty of Food Engineering, Department of Food Engineering, 6724 Szeged, Mars Sq. 7. Hungary

Abstract. The use of ultra-filtered lactic acid whey retentate was investigated for the making of sour cream. The utilization of lactic acid whey is limited due to its special properties, so the logical utilization way is to use it in fermented products. First, we concentrated lactic acid whey collected from cottage cheese making by ultrafiltration (UF), then UF Whey Retentate (UFWR) was added (by 2, 5, and 10%) into fat standardized cream for sour cream making. We investigated the texture and sensory properties of the sour cream samples compared with the industrial products. Generally, we can state that the use of small portion of UF whey retentate did not result noticeable changes and did not reduce the sensory value of sour creams. Higher UF whey retentate addition improved some texture properties of experimental samples, but the summarized evaluation of UFWR addition was not unequivocal. Control samples showed better results. Based on our results, the sample, which contained 5% UF whey retentate, had good texture and acceptable sensory properties. Furthermore, more than 5% UF lactic acid whey retentate (coming from our own ultrafiltration process) resulted remarkably

Keywords and phrases: whey utilization, sour cream, ultrafiltration

worse sensory properties than the other samples. Further investigation is needed to find the optimal composition and sensory properties of UFWR. Furthermore, we have to perform technological investigation to reach a higher concentration factor using pre-treatment of whey and to avoid the precipitation of whey proteins during the high temperature pasteurization of cream, cream mixed with UFWR or diafiltered whey retentate. We guess that the use of one-stage diafiltration would already decrease the unfavourable sensory properties of lactic acid whey retentate.

### 1 Introduction

A major part, actually 65–90%, of milk becomes side products (or waste) in cheese and casein production. By some estimation, 62 billion litres of whey are produced each year world-wide (*Malkovic*, 2015), causing significant problems for producers considering environmental aspects. But whey contains valuable organic compounds (as proteins, lactose, and fat) and minerals. Actually, the beneficial effect of whey proteins and other compounds on human health are well-known (*Squadrito et al.*, 2000; *Ha & Zemel*, 2003; *Bhathena & Velasquez*, 2002; *Seppo et al.*, 2003; *Hayes & Cribb*, 2008; *Fanti et al.*, 2006; *Chen & Reimer*, 2009; *Tahavorgar et al.*, 2015).

Therefore, this extremely huge amount of whey is a big problem and there is need to reduce its polluting effect and to produce valuable products. By today, the utilization of whey has arrived to the separation and purification of different special compounds from the simple use as the feed for animals. Whey proteins and serum proteins seem to be the most valuable components of whey, but the other compounds are also good raw materials (e.g. lactose) for further utilization. The simplest utilization is to produce flavoured whey drinks as "Riska Shake" produced by Alföldi Tej Ltd., Hungary. But, usually the sensory properties, low pH and the low solid content make whey difficult to use it in other products. Consequently, the taste and smell ameliorate, and the reducing of moisture content can open up new perspectives for the lactic acid whey utilization.

Many researchers published results of the different membrane filtration techniques used for whey concentration (*Brennan et al.*, 2006; *Walstra et al.*, 2006; *Román et al.*, 2009; *Hodúr et al.*, 2010; *Szélpál et al.*, 2014; *Schwinden Prudencio et al.*, 2014). Very important results were published related to the enhancement of membrane techniques to reach higher efficiency and to the removal of lactic acid from lactic acid whey (*Chen et al.*, 2016). This objective can be reached with the use of vibration (*Kertész et al.*, 2010), with sonication

(Gajendragadkar & Gogate, 2016), with electrical methods (Almecija et al., 2009: Corbatón-Báquena et al., 2016), with diafiltration, or with optimization of the condition of the filtration (Román et al., 2011). As a result of the development of whey membrane filtration process, many forms of utilization can be adopted in the food and medicine industry. There are many results published in different area (including the use of lactic acid whey) such as the purification of whey proteins (*Chandrapala et al.*, 2016), fractionation of bioactive peptides from whey (Baldasso et al., 2011), the enzyme recovery from solutions (Arrutia et al., 2016; Lemmer et al., 2015), the production of lactic acid (Kececi et al., 2015), and the stabilization of different emulsions as well (Wojtyniak et al., 2016; Ruttarattanamongkol et al., 2015). Other applications are as follows: improvement of foaming properties (Sun et al., 2015), stabilization of different whey protein drinks (Le et al., 2016), bacterial production of hyaluronic acid (Wronkowska et al., 2015), improvement of bread properties (Lőrincz et al., 2011), not to mention the traditional possibilities such as the production of ordinary whey powder and different whey cheeses (e.g. Ricotta). A complex utilization system of acid whey was presented in the work of Al-Khajafi et al. (1977).

Our objective was the investigation of ultra-filtered lactic acid whey retentate (UFWR) use with low concentration factor in the production of sour cream in order to reduce the amount of whey as wastewater. The low concentration factor of whey can be justified by lower expenses comparing to ultrafiltration with two or three stages of diafiltration or reverse osmosis. Another aim was to explore the effect of UFWR addition (as milk substitute) on the texture and the sensory properties.

# 2 Materials and methods

### 2.1 Ultrafiltration

A simple ultrafiltration was performed in batch system without diafiltration, using VSEP-LP vibration ultrafilter (New Logic Research, Inc., Emeryville, California 94608, USA). Conditions: pressure: 8.0 bar; temperature: 40 °C; membrane: PAN (poli-akril-nitril); cut-off value: 10 kDa (*Figure 1*). Ultrafiltration was performed using a simple batch system without special pretreatment of lactic acid whey; so, the concentration factor was fairly low (1.8).



Figure 1: VSEP ultrafilter

### 2.2 Chemical investigation

A Bentley 150 milk analyser (Bentley Instrument Inc. Chaska, Minnesota 55318 USA) was used for the determination of whey composition, ultrafiltered retentate, and permeate at 40 °C. Data were expressed in m/m%. The composition of the different experimental media is presented in *Table 1*. We did not reach remarkable protein increment and lactose was enriched, probably due to the different complex molecule formation.

Table 1: Composition of whey, permeate, and UF retentate (m/m%; concentration factor: 1.8)

	Fat	True protein	Crude protein	Lactose	Total solids
Whey (Feed)	0.15	0.59	0.90	3.67	6.49
Permeate	0.40	0.49	0.63	3.27	5.61
Retentate	0.44	0.88	1.32	5.52	8.66

### 2.3 Experimental sour cream making

After the addition of different percentages (2%, 5%, 10%) of UFWR into milk (without pH buffering) and after fat standardization to 16 m/m%, the cream was heated to 60 °C for homogenization at 150 bar pressure with Gaulin Lab 60 homogenizer (Graafdijk-Oost 23, 2973 XB, Molenaarsgraaf, Netherlands) (*Figure 2*). Then, the homogenized cream was pasteurized at 72 °C with one-minute holding time, and then it was cooled to 26 °C for inoculation.



Figure 2: The homogenizer (A) and the packaging machine (B)

The gentle pasteurization condition is explained by the heat sensitivity of whey proteins fortified in cream. Inoculation rate was 2 ml/3000 ml cream, from a 20% culture solution using DI-PROX<sup>®</sup> M 272 freeze-dried starter (Bioprox Ltd. 92532 Levallois-Perret Cedex, France). Then, different samples were packed in cups with a semi-automatic packaging machine (Junior Handy type, Zootechnika Ltd. Gödöllő, Hungary). After packaging, the samples were fermented in a thermostat (Labor MIM, Hungary) at 26 °C for reaching 4.6 pH measured with Orion 4Star instrument (Thermo Fischer Scientific Inc., Singapore). Then, samples were cooled in a refrigerator and were stored until the investigations.

### 2.4 Texture properties

Brookfield LFRA CT3 texture analyser (Brookfield Engineering Laboratories, Inc., Middleboro, Massachusetts, USA, 02346) was used for the determination of hardness, adhesiveness, and adhesive force of sour cream samples. Measuring conditions: simple compression test was used with a 12-mm-diameter plastic cylinder (penetration target: 20 mm; penetration force: 50 mN; speed:

0.5 mm/s). Measures were performed in three replicates using five parallel samples. Every sample was measured in three places of the surface.

#### 2.5 Whey leakage ratio

Whey leakage was determined with the method published in the work of Al-Khajafi et al. (1977). First, a 40-mm-diameter semi-sphere was cut into the surface of the curd; then, after one hour, the weight of the accumulated whey was measured. Less amount of whey (higher water-binding capacity) means better texture.

#### 2.6 Sensory evaluation

Sensory evaluation was performed by ten persons using Hungarian Standard 12253-84. Part of the sensory evaluation was the comparison of experimental samples to the control samples -20% fat content – made in a dairy firm.

# 3 Results and discussions

#### 3.1 Whey leakage

The measured whey leakage values (*Table 2.*) were high, considering the classification method in *Al-Khajafi et al.* (1977) (<0.5 ml, 0.5-1.0 ml, >2.0 ml). E.g. the low pasteurizing temperature and the slow heating rate can be explained with the experimental condition of making sour cream samples.

Whey ratio		Average		
(%)	First	Second	Third	8-
2	5.35	5.62	5.47	5.48
5	2.09	2.83	2.38	2.38
10	4.08	4.12	4.08	4.09

Table 2: Whey leakage of sour cream samples (means; g; n=45)

The explored differences demonstrate that whey proteins can play an important role in the water-binding capacity of lactic acid gels such as sour cream. Interestingly, the higher whey retentate ratio reduced the whey leakage first, while a more than 5% ratio yielded worse result. This is explained by the higher dilution ratio of cream with UF whey retentate, maybe because of its lower crude protein content compared to cream (1.94–2.01%). Higher ratio of UF whey retentate resulted lower protein content in the cream-UF retentate mixture than the dilution in lower ratio. The 5% UFWR ratio was the optimal in our experiments.

### 3.2 Texture analysis

Significant difference ((p  $\leq 0.05$ ); using Statistica 12 for MS Windows software) was revealed between the samples made with 2% and 5% added UF whey retentate and also between the samples' 2% and 10% UF retentate-added samples (*Figure 3*).

But the difference was not significant between the samples made with 5% and 10% UFWR addition. The higher values of investigated texture properties are explained by the higher amount of denatured whey proteins built into the casein protein matrix during the lactic acid clotting.

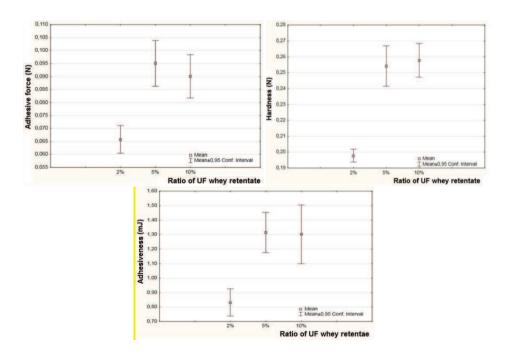


Figure 3: Texture properties of different samples made with UF whey retentate (n = 45)

The lack of further improvement in these properties can be explained by the lower relative ratio of casein in the mixture containing a higher ratio of UF whey retentate. As the aggregation between denatured whey proteins and casein micelles can improve texture properties, the lower casein content in the mixture can compensate this beneficial consequence. As *Figure 4* shows, the control sample gave slightly better results. It is explained by higher fat content (20%) and the industrial processing condition. However, the results of samples made with the addition of 5% and 10% UF whey retentate were close to those of the control ones.

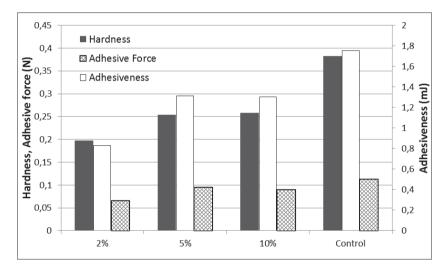


Figure 4: Comparison of texture properties of experimental and control samples (n=10)

#### 3.3 Sensory evaluation

The sensory evaluation showed markedly different results compared to the results from texture analysis. Higher UFWR addition resulted worse judgement of experimental samples (*Figure 5*). Especially samples made with the highest UFWR addition got unsavoury characteristics.

We can confirm that the special sensory property of whey, especially of lactic acid whey, limit further direct utilization. Higher ratio of UFWR addition resulted worse scores in all replicates, except for smell. The sample made with 10% UFWR addition yielded the worst score (12.5 points), so this experimental product cannot be marketed.

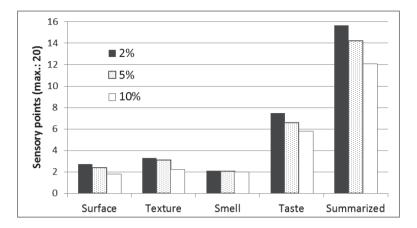


Figure 5: Sensory evaluation of samples with UF whey retentate addition

# 4 Conclusion

The addition of UFWR into cream can be a form of utilization of this dairy side-product. We investigated the use of UFWR from a single-batch ultrafiltration in order to limit further processing costs. We did not achieve enough high protein content in retentate and because of mild sensory properties of experimental samples our mixtures did not result a real, satisfactory solution. Although the texture properties of experimental samples were improved (5%)UFWR addition gave the best result), the sensory scores decreased with the increase of UFWR addition. Based on the sensory evaluation, the limit of the addition of UF lactic acid whey retentate from a single-batch ultrafiltration is 5%. Further investigation is needed to find the optimal composition and sensory properties of whey UFWR using diafiltration. Furthermore, we have to perform a technological investigation to reach a higher concentration factor using pre-treatment of whey and to avoid the precipitation of whey proteins during the high temperature pasteurization of cream, cream mixed with UFWR, or diafiltered whey retentates. We guess that the use of onestage diafiltration would already decrease the unfavourable sensory properties of lactic acid whey retentate.

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# Market orientation of the Hungarian SMEs working in the meat processing and dairy industries

Zs. Polereczki e-mail: polereczki.zsolt@econ.unideb.hu Debrecen University, Faculty of Economic Sciences Marketing and Trade Institute H-4032 Debrecen, Böszörményi u. 138. Hungary

Sz. Vukoszávlyev e-mail: szlobodan@gmail.com Hiperon Genetics Kft. H-1111 Budapest, Karinthy Frigyes u. 27. IV. em. 2. Hungary

M. Véha

e-mail: veha.miklos@naturtrade.hu Naturtrade Hungary Kft. H-6725 Szeged, Szabadsajtó u. 54. Hungary

> Z. Szakály e-mail: szakaly.zoltan@econ.unideb.hu

Debrecen University, Faculty of Economic Sciences Marketing and Trade Institute H-4032 Debrecen, Böszörményi u. 138. Hungary

**Abstract.** We are looking for the answer as to what tendencies were indicative of the future development of required marketing activity of the SMEs in the article dealing with the marketing activity of the SMEs working in the food industry. The article is based on a nationwide survey among 200 SMEs working in the food processing industry. In this

Keywords and phrases: market orientation, food industry, SME  $\,$ 

article, we focus on the SMEs working in the dairy and meat processing industries. The results of the nationwide research and some domestic references refer to that there is a latent demand of effective marketing activity among small and medium-sized enterprises. It manifests itself in specifying marketing-related fields to be improved in the future. The marketing itself is believed not to be an important field at the same time. This apparent opposition is the small enterprise marketing paradox in the background of which is the lack of knowledge about the marketing instruments. It can be stated that these small businesses collect mainly general market information and have no information about particular products. Therefore, the presence of marketing planning is really rare and where there is some kind of planning it is not connected to available funds and follow-up control. The marketing strategy can be characterized by products processed mainly at low or medium level. Therefore, market position is defined by "lower price-good quality". They mainly use the traditional distribution channels and their communication is accidental and has a low level.

The marketing-oriented way of thinking still exists among the factors affecting entrepreneurial behaviour, which cannot be found at the level of clusters, according to our results. We could identify 8.3% of the enterprises as having satisfactory marketing activity.

# 1 Introduction

The operation of smaller enterprises diverges in several ways from the logic of the general market orientation models. These are worth calling attention to, and we must assess the research results dealing with them. In their case, personal attitudes can have a significant influence on the culture, operation, and learning abilities of the organization in terms of marketing and market orientation knowledge (*Cegarra-Navarro & Rodrigo-Moya*, 2007).

In the case of small companies, the level of market information collection is inadequate; they characteristically do not have an independent marketing expert. Their marketing activity relies on secondary data such as scientific papers, sectoral analyses, or information acquired through personal channels (consumers, suppliers, business partners). If the enterprise sells its product in a small geographic area or region, it is able to use the different data collection methods more efficiently. The communication of market information within the corporation is less important in the case of SMEs since the owner is often also the decision-maker (*Werhees & Meulenberg*, 2004). However, effective communication of market information is linked to the staff's satisfaction and increases in their loyalty, since *Ruekert's* results (1992) suggest that an increase in market orientation is connected to satisfaction with the workplace.

Tajeddini et al. (2006) in Sweden researched 650 companies with between 5 and 200 employees, applying the MKTOR scale. Their research suggests that there is a positive correlation between consumer orientation and market share, returns on investments (ROI), and the rate of new products in total sales. According to their results, properly operating competitor orientation also has a positive influence on the market share and the rate of new products in total sales. Coordination between functional areas could have a positive influence on the returns on investments (ROI), while it did not have a statistically justifiable influence on the other mentioned areas. Their research focused on the degree of companies' market orientation and the connection with innovativeness. In this respect, they also stated the importance of market orientation, since all the three areas of the MKTOR scale are statistically justifiable and have a positive connection with the enhancement of a company's innovation abilities.

In Spain, Armario et al. (2008) surveyed the characteristics of the market orientation of small- and medium-sized enterprises together with their increased international presence. They analysed the area, involving 112 SMEs and applying the MARKOR scale. Their results supported the idea that with an increase in market orientation the performance of a company improves. They pointed out that companies with more efficient marketing systems are more successful competitors compared internationally. They also confirmed that with stronger marketing orientation the examined companies could develop better corporate education abilities, and through this they could respond faster and more efficiently to the market.

Baker & Sinkula (2009) studied the behaviour of American small companies, involving 88 companies in the research. Applying the MORTN scale created by Desphande & Farley (1998), they aimed to find out the common influence of market orientation and entrepreneurial orientation on entrepreneurial profitmaking ability. Their results show that an increase in market orientation has a significant positive impact on financial success. However, they call attention to the fact that marketing orientation means not only the tracing of product preferences but also the monitoring of the external market environment and the division of consumer information. In companies of this size, it must also include the manager's total commitment to maximizing consumer satisfaction.

Siu & Liu (2005) studied 307 small- and medium-sized Chinese enterprises. They carried out their research with a logical model established by themselves; its fields were corporate philosophy, strategic analysis, marketing aims, marketing strategy, marketing organization, and marketing control. Their results show that those small companies perform better where marketing takes a central place in the company's philosophy.

However, they also call attention to the fact that in their case it is advertising and sales elements that are important, while market analysis is pushed into the background. The pretence of strategic planning was observed at a higher rate among those enterprises with a better than average performance. These companies put a high emphasis on a wide-scale position analysis. They also found significant differences in the marketing strategic aims of companies with a higher marketing orientation level compared to the other companies. Marketoriented enterprises with better performance characteristically have longerterm profit aims, which means that the time horizon of their planning is much longer than that of their competitors. In terms of marketing strategies and their control, some other differences have been noted among those performing better or worse than the competition. Companies with a better than average result in each case had a marketing strategic plan, while those with worse performance tried to advance by reducing their costs and increasing their productivity. The existence of strategy within these companies had a definite link with the differences in their corporate structure. The more successful companies had an independent marketing unit.

From the body of research surveying market orientation, we would highlight the research of Verhees & Meulenberg (2004), who studied market orientation in relation to innovation ability. Their research is mentioned here because the authors, going beyond the models investigating market orientation, tried to work out a logical frame adjusted to the peculiar operation of SMEs applying the experiences coming from these conceptions. Their starting point was Kohli & Jaworsky's model (1990), but they significantly reinterpreted it. They reduced its definition of market information collection to obtaining consumer information. They omitted the communication between corporate units since in their interpretation data collection, strategic planning, and decision-making are concentrated in the hands of one person, the owner. Besides this, they restricted responsiveness to conformity in the field of innovation. According to their results, the positive influence of improved market orientation in small companies is clear. They revealed a positive correlation between consumer information and innovation ability, which has a favourable influence on product quality, the range and level of connecting services, and proper timing.

This last study reminds us that in the case of small- and medium-sized enterprises the usefulness of the results revealed by general market orientation models is restricted, since several of their elements (e.g. information flow between organizational units, coordination between functions, responsiveness) have a totally different meaning for SMEs than they do for bigger companies.

# 2 Materials and methods

The two fundamental methods of marketing research – primary and secondary data collection – were used to collect results that are introduced in this article.

### 2.1 Data collection

Primary data collection can be separated into two phases: the phase of preparation and that of quantitative data collection.

The field work of the national in-hall test was carried out in June and July in 2010 with the help of the countrywide network of Szocio-Gráf Market and Public Research Institute seated in Pécs. 200 enterprises were examined in this research, but we focus in this article on the SMEs working in the meat processing and dairy industry (*Table 1*).

Filling in the questionnaires was the task of previously prepared questioners, who filled them personally with marketing leaders of enterprises or, if there were not any, with a leader of each enterprise who had a full view to the whole activity of the enterprise.

Industry	0–9 heads	10–19 heads	20–49 heads	50–249 heads	Altogether
Meat processing, production of meat products	27	14	20	15	76
Production of dairy products and ice creams	9	1	3	7	20
Altogether	36	15	23	22	96

Table 1: Composition of the sample

The applied questionnaire can be separated into two blocks of questions that are followed by background variables. Altogether 61 questions were put to respondents and as a last group of questions statements were created, in which the agreement of respondents was examined. This question group helped form clusters. At the end of the questionnaire, 7 background variables took place. Characteristically closed type questions were applied with two or more possible answers. However, respondents were allowed to give opinions different from the given ones in the "Others" answer category. In the questions – where scales had to be used –, Likert scales ranging from 1 to 5 were applied in all cases. In these scales, only the first and the last points were named.

The first group of questions examined the information collection habits of the respondents, the second one studied the existence of the enterprise's planning, the third one considered the leading structure, and the fourth one examined the expansion conceptions and cooperation willingness of enterprises. The next four groups of questions analysed the marketing tools according to the 4P. At the end of the questionnaire – as a ninth question group –, 44 statements are suitable to form clusters. With these statements, the respondents' agreement was analysed.

### 2.2 Methods of analysis

The data received from the research were analysed with the help of SPSS 13.0 statistical software package. Frequency superscripts from univariate statistical analyses, arithmetic average from medium values, connected to this, standard deviation (square average mutation) from deviation superscripts were applied (*Sajtos & Mitev*, 2007; *Molnár*, 2007). These methods are widely known and their application is a daily practice in market research tasks, which is why we do not consider necessary to introduce them.

# 3 Results

### 3.1 Marketing situation report on food industrial SMEs

As an element of the examined enterprises' information collection habits, I wanted to know to what extent enterprises do market research/data collection activity. 69.8% of the respondents do some kind of information collection in order to meet their potential target markets. This rate can be regarded quite favourable until we take into consideration the channels used to get information, which are summarized in *Table 2*.

It can be seen that these information sources are usually suitable to obtain knowledge about general market tendencies (acquaintanceship, public databases). Those channels that could give concrete consumer information about the enterprises' products (own research on enterprises, market research company) fell rather behind on the list. According to this, the target mar-

Angeven estamony	Division of answers		
Answer category	Respondents	%	
Acquaintanceship	55	82.1	
Database, available in public	29	43.3	
Own research of enterprises	19	28.4	
Market research company	3	4.5	
Others	3	4.5	

Table 2: Information sources used to meet the potential target markets  $(N = 67)^*$ 

\* Respondents could give more than one answer

ket selection based on market information is characteristic in only 3.1% of the respondents. Nevertheless, the respondents judge the level of information supply as sufficient.

Thus, it is not surprising that planned marketing activity is characteristic in only a very small proportion. 29.2% of the respondents claimed to make a marketing plan, which seems a good rate at first. Examination of the further elements of the group of questions focusing on planning became more and more detailed; e.g.: presence of marketing budget, its controlling and lifecycle analyses. The former determined number began to decrease dramatically following the detailed questions. Only 15.6% of them have a marketing budget, which is controlled regularly by 14.6% of them (the way of control is not mentioned yet), and only 9.4% follow and evaluate their products' lifecycle. So, the former 29.2% decreased quite a lot as the questions got more detailed.

These results forecast the level of the enterprises' marketing knowledge. One element of the question group examining the management of the company searched its source. Table 3 contains the results of this question group.

Probably the following tendency can be found in the background of the not too favourable results concerning the situation of marketing: enterprises consider the experiences obtained in the course of business satisfactory for marketing activity. A question arises here: what kind of knowledge can these enterprises receive from each other if none of them have marketing knowledge? Altogether 9.4% of the enterprises have a colleague with – in most cases, a medium-level – marketing qualification.

Analysing product policy, it has been found that the examined enterprises try to compete on the market with their products' good quality. However, they do not possess any information about their consumers' judgement. The product's pretended consumer judgement coincides with the respondents' admittedly own opinion in this question. Moreover, they are convinced that the product's quality would be the most important factor of competition in the food industry nowadays, with an average rate of only 3.64 on a one-tofive scale. Some 65.6% of the respondents deal with the production of mass products that provide the majority of their income.

Answer category	Division of an	swers %
	Respondents	
Past experiences	76	79.2
From journals and special books	13	13.5
Through participation in professional		
conferences	10	10.4
The enterprise has a leader with		
marketing qualification	9	9.4
Others	3	3.1

Table 3: Source of marketing knowledge  $(N=96)^*$ 

\* Respondents could give more than one answer

Analysing pricing strategy together with product policy, it can be demonstrated that the enterprises believe that the basis for positioning is obviously in the dimension of lower price and good quality. The basis of the respondents' pricing is firstly formed by the consumers' acknowledged market value, secondly, by the production costs, and thirdly by profit maximization. Among pricing points of view, there was not observed any consideration resulting from marketing aims (e.g. positioning considerations).

Enterprises do not carry out a conscious channel policy: 60.4% of the respondents do not select their markets, they try to be present everywhere where they can. 58.3% of the respondents use a channel with one or two elements in order to pass their products to consumers, but in spite of this the average rate for this question is 3.47 on a one-to-five scale (1 means "I cannot follow it at all" and 5 means "I am completely able to follow it").

An important character of their communication is that 53.1% of them do not possess a brand name. Those who do some kind of planned communication, characteristically target the end-users, and they target intermediate persons at a lower rate, or a group with 15.6% of respondents wants to send messages to both groups. The typically applied tools are consumption inspiration and direct selling, while intermediate persons are mostly motivated with gifts. Both communication forms are done at a low intensity.

### 3.2 The appearing latent demand

Several Hungarian researches (*Sajtos*, 2004; *Szabó*, 2009; *Polereczki & Szabó* 2005; Józsa, 2004) show the same unfavourable picture analysing the small enterprises' marketing activity as the above mentioned situation. A question arises here whether the judgement of marketing as a tool is so unfavourable among Hungarian small enterprises or not. In order to give an answer, we need to analyse what companies think about future, which fields they expect development in.

In 2003, Nyers & Szabó asked enterprises in their survey about which fields they see the main future success factors. The answers are shown in Table 4.

	The rate of h	The rate of high $(5)$ agreement			
Main success factors	Firms in	Firms in			
Main success factors	foreign	Hungarian			
	property	property			
Better service of clients' needs	39.3	44.8			
Occupation of new markets	26.2	26.3			
Introduction of international quality assurance	31.1	24.3			
Forming individual markets	13.1	16.2			
Introduction of a new product or service	21.3	15.1			
Improvement of employees' qualification	9.8	13.7			
Introduction of modern information system	13.1	12.9			
Developing new technology	8.2	11.7			
Development of cooperation	13.1	11.5			
Service providing	16.4	10.3			
Opening export markets	11.5	8.1			

Table 4: The main success factors determined by SMEs

Source: Nyers & Szabó (2003)

If we examine the answers written in bold letters in *Table 4*, then we can see that respondents named fields in big proportion that are in strict connection with marketing. Forming new markets, a better understanding of the clients' needs, creating individual (niche) markets are all tasks that can be developed classically with the help of marketing tools.

The results of our research also show a similar tendency. The results that can be seen in *Table 5* show respondents' ideas about future development. Respondents also named several fields that can be covered with marketing tools to a large proportion, such as expansion of customer base, or product innovation.

Table 5:	Possible	future	development	directions	according	$\operatorname{to}$	respondents
(N=96)*							

Δ	Division of answers		
Answer category	Respondents	%	
Expansion of customer base	45	46.9	
Product innovation in the field of mass			
products	26	27.1	
Product innovation on the market of			
niche market products	26	27.1	
Development of marketing activity	10	10.4	
Forming horizontal integrations	5	5.2	
Forming vertical integrations	2	2.1	
Other	4	4.2	

\* Respondents could choose from more than one answer

In another question, respondents stated that they would be able to make an acceptable profit by an average of 18.23% price-level increase. The tools of reaching a higher price level according to enterprises are summarized in *Table 6*.

The fields that also need an important marketing activity were also emphasized in this case. Another important aspect of the results is that respondents ranked marketing activity behind in both *Table 5* and *Table 6*; only about 10-11% of them think it could be important in the future. It can be stated that enterprises do not consider marketing a key-factor from the point of view of their future, but at the same time they name such directions important that definitely belong to this field.

The apparent contradiction can be resolved as follows. It is well known that these enterprises' real marketing knowledge is very low. In other words, we can say that these enterprises do not really know what activities marketing really contains. In spite of this, they instinctively see their way out of this more and more difficult market fight in such fields that are in strong connection with

	Division of answers		
Answer category	Respondents	%	
Development of a relations system	36	37.5	
Product innovation on the market of			
niche market products	27	28.1	
Product innovation in the field of mass			
products	22	22.9	
Development of marketing activity	11	11.5	
Horizontal cooperation with other processors	10	10.4	
Application of brand names	6	6.3	
Vertical cooperation	3	3.1	
Others	10	10.4	

Table 6: The tools of reaching a higher price level according to respondents  $({\rm N}{=}96)^*$ 

\* Respondents could choose from more than one answer

marketing. This is nothing else but a latent demand for an effective marketing activity present in enterprises.

At this point, it is worth mentioning the work of *Chikán & Czakó* (2002), in which they explain that the position of marketing has become stronger in enterprises during the past few years, but, at the same time, it is well behind the level of western countries. According to the results of *Achrol & Kotler* (1999), this tendency can be reckoned as natural because it is observed in their research that the marketing orientation of enterprises operating under hard competition conditions was getting stronger. So, enterprises exposed to hard competition consider marketing more and more as a strategic tool. It can be concluded that with a stronger competition in Hungary a stronger marketing orientation can be expected.

We can state that with a fiercer market competition among the investigated enterprises such field is expected to develop in the future that might lead to an improvement in their marketing orientation.

### 3.3 The appearance of marketing orientation

In the followings, a question arises: if this latent demand really exists among the analysed enterprises, then does it appear at the level of real activity and can it be identified?

In order to clear up this question, factor and cluster analyses (K-means) were carried out on the basis of agreement of 44 statements appearing at the end of the questionnaire regarding the enterprise's way of thinking and activity. The results of the research showed the following: 4 different characteristic behavioural models were successfully identified during factor analysis, among which the marketing-oriented way of thinking could already be found. These are those who consider it necessary to pay particular attention to understanding consumer needs. They try to form product features according to these needs at an economically justifiable level, considering sustainability point of views. However, it has to be noted that this factor could barely be identified. In the next step, cluster analysis was carried out based on certain factors. It can be seen from the results that none of the cluster groups are characteristically marketing-oriented. The presence of marketing could be found only in one group, but even there marketing got a very subordinated and executive function with no noticeable effect on the enterprise's strategy. So, the presence of market orientation at the level of factors is not strong enough to form a market-oriented group among the clusters.

Although a marketing-oriented way of thinking could not be proved at the level of the enterprise cluster, a further examination was carried out in order to try to find an enterprise group with marketing-oriented attitudes. For this reason, four fields were stressed upon from the question block connected to marketing information collection and marketing planning. Answers given to these questions gave the basis for determining sufficient marketing activity. These four fields are the following: market information collection serving the basis for product features, an existing marketing plan with own budget, control of amounts spent on marketing as well as analysis and use of product life cycle. Thus, the rate of enterprises that carry out market information collection as a basis of marketing planning for concrete products, which possess formerly determined own marketing budget, control the utilization of this money, and set their planning to the current life cycle of their products/product groups, amounts to 2.3% of the whole sample. This rate increases to 8.3% in case the proper operation of three of the listed four elements is considered sufficient.

Testing these enterprises in terms of enterprise size, two poles can be found. Their common feature is that all but one of them pertain to the meat industry, all of them operate in the form of an Ltd., and they do not show local uniformity. A smaller group of them belong to small enterprises of 0–20 employees and maximum of Ft 250 M net income. Enterprises with 20–100 employees and more than Ft 750 M net income form a bigger part. These are the firms that can be called innovators – examples of consumer novelty acceptance – in

the examined group of small enterprises.

According to the results, it can be stated that the marketing-oriented way of thinking already exists among the enterprises of both examined industries, although it has only a small effect on SMEs operating in the sectors.

# 4 Conclusions

As to the results, information collection pertaining to the general market tendencies is typical of the respondents and few of them have particular information about their own product. Otherwise, they believe that their market research activity is at the required level. It shows that they do not perceive the lack of information and it is not believed as an obstacle from the view of their market success.

Therefore, the presence of marketing planning is low in the sample, while, on the other hand, it is not linked to budget and subsequent supervision. The tested enterprises usually do not have an employee with marketing qualification, and therefore there is not a permanently responsible person for marketing activities. This kind of behaviour is behind the lack of strategic approach. It causes the disability of setting up long-term conceptions. It also results in low marketing efficiency even among companies that have some kind of planned marketing activity.

An overwhelming majority of the companies believe that the way to future is the improvement of mass products. On the other hand, more and more of them want to produce special products as well. It is mainly caused by the lack of alternative future perspectives, which is due to the lack of information about consumer demands, and the production- and sale-oriented approach. They do not have the abilities to plan the company's activities on a consumeroriented way or to reach the target groups effectively, and no more than 8.3% of them have the adequate marketing skills. It means that a marketing-oriented way of thinking can be measured among the factors, while at the same time it is characteristic of such a few enterprises that an independent behaviour group (cluster) cannot be found. It also means that a market-oriented way of thinking is getting stronger among SMEs in the food industry, which is defined as one of the side effects of stronger market competition by the literature.

Their product policy focuses on producing mass products, which is linked to a lower price position. 72.9% of them are able to make profit at present prices, but they would like an average price rise of 16.7%.

The classical channels (retail trade, wholesale trade, HORECA) dominate

their sales. Their communication is accidental and the market position of their products is the lower price-proper quality. 53.1% of them do not have an own brand name and 60.4% of them have no web page. Their communication strategy is built up according to the pull strategy; therefore, the target of their messages are the end-consumers. On the other hand, the push strategy is less wide-spread. It can be stated that the push strategy is more useful for them regarding the characteristics of their products and the features of these two strategic options.

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# Flour quality and kernel hardness connection in winter wheat

B. P. Szabó e-mail: szpb@mk.u-szeged.hu E. Gyimes e-mail: gyimes@mk.u-szeged.hu

A. Véha e-mail: veha@mk.u-szeged.hu Zs. H. Horváth

e-mail: horvatzs@mk.u-szeged.hu

University of Szeged, Faculty of Engineering, H-6725 Moszkvai krt. 5–7., Szeged, Hungary

**Abstract.** Kernel hardness is controlled by friabilin protein and it depends on the relation between protein matrix and starch granules. Friabilin is present in high concentration in soft grain varieties and in low concentration in hard grain varieties. The high gluten, hard wheat flour generally contains about 12.0-13.0% crude protein under Mid-European conditions. The relationship between wheat protein content and kernel texture is usually positive and kernel texture influences the power consumption during milling. Hard-textured wheat grains require more grinding energy than soft-textured grains.

The aim of our research was to determine the possible relationship between kernel hardness and various other parameters of the flour (dough visco-elastic characteristics, wet gluten, water absorption, flour recovery, alveograph). We used Perten SKCS 4100 to determine the kernel hardness, while the Perten 3303 mill was used to establish Particle Size Index (PSI). Registered and widely used Hungarian wheat varieties (7 of HRWW and 4 of SRWW) were applied in the study. Twin correlations were used to determine the relationship among the various traits.

According to the results, there is a very strong correlation between milling energy and kernel hardness (r = 0.99). The correlation between

Keywords and phrases: wheat kernel, flour parameters, SKCS 4100

hardness index and the examined flour parameters was also significant (r = 0.81-0.87). We found strong correlation between the milling energy and water absorption (r = 0.88) of flour. The associations found in this study will help the better understanding of the technological aspects concerning wheat grain and flour quality.

# 1 Introduction

Kernel hardness has a profound effect on the resulting flour's baking properties. The flour which is made from hard wheat has a medium to high protein content and contains stronger gluten-forming proteins than the flour which is made from soft wheat.

Kernel texture is very strongly heritable in wheat. Friabilin protein determines kernel hardness. When the amount of friabilin is high, kernel hardness is low and when the amount of friabilin is low kernel hardness is high. We can classify kernel hardness in these two groups (*Greffeuille et al.*, 2006). Hardness in wheat is largely controlled by genetic factors, but it can also be affected by the environment and other factors such as lipid, moisture, and pentosan content. Friabilin, a marker protein for grain softness, consists of two proteins, puroindoline a and b (*Martin et al.*, 2006). Lipid-binding proteins puroindolines a (PINA) and b (PINB) have been identified as responsible for determining differences between hard- and soft-textured wheat (*Gyimes*, 2004; *Gyimes et al.*, 2001).

The high gluten, hard wheat flour generally contains about 12.0-13.0% crude protein under Mid-European conditions. The relationship between wheat protein content and kernel texture is usually positive and kernel texture influences power consumption during milling. Hard-textured wheat grains require more grinding energy than soft-textured grains (*Békési*, 2001).

Good mill and baker quality wheat belong to the hard grain type. Both the milling industry and the baking industry (making of bread) prefer this type. The hard endosperm composition is in close relationship with the large flour yield (greater ratio of the valuable fraction), the flour's greater water consumption, the volume of the bread, the bread's quality parameters (e.g. inner height), and the protein content ( $V\acute{e}ha$ , 1999).

For the determination and measuring of the endosperm structure, kernel hardness indicators were made, which measure the power needed to snap a seed. With this method, they determine a factor: Hardness Index (HI), which is one of the bases of mill crop's acceptance qualification.

# 2 Objectives

Our aim is to determine kernel hardness. In our investigation, we have used the Perten SKCS 4100, the Perten 3303 mill. We used Hungarian samples. Registered and widely used Hungarian wheat varieties (7 of HRWW and 4 of SRWW) were applied in the study, which were assigned code numbers.

# 3 Measurement methods

The Perten SKCS 4100 instrument (*Figure 1*) is one of the well-known machines which examine kernel hardness. This device measures kernel texture by crushing the kernels and recording the force required to crush the kernel. This machine reports the average force required to crush 300 kernels, in terms of a hardness index (HI). The SKCS-4100 can complete a test in about 3 minutes and it simultaneously reports mean and standard deviation data for diameter, kernel weight, moisture content, and the HI. (*Szabó*, 2006).



Figure 1: Perten SKCS 4100 instrument

We used Perten 3303 mill (*Figure 2*) to determine the Particle Size Index (PSI). This involves grinding a sample, and sieving a weighed amount through a standard screen for a standard time. Particle size index (PSI) helps us to determine the texture of a wheat kernel. We determine the specific grinding energy demand ( $e_f$ ). All measurements were repeated 3 times.

We measured the dough visco-elastic characteristics, water absorption, wet gluten, and alveograph. Milling test: we used Brabender R Quadrumat R Senior (*Brabender GmbH* & Co. KG, Duisburg, Germany) laboratory mill to

check the milling properties of different types of grain and determining the flour yield (FL) of the wheat sample.



Figure 2: Perten 3303 laboratory mill

Gluten index: gluten index (GI) was examined by Glutomatic 2200 (Perten Instruments AB Huddinge, Sweden). Dry gluten content was measured after drying with Glutork 2020 (Perten Instruments AB Huddinge, Sweden) automatic gluten dryer. Farinograph test: we used the Brabender  $\widehat{B}$  farinograph (*Brabender GmbH & Co. KG, Duisburg, Germany*). The farinograph determines dough and gluten properties of a flour sample by measuring the resistance of dough against the mixing action of blades.

Alveograph characteristics: Chopin Alveograph NG (CHOPIN Technologies, Villeneuve-la-Garenne Cedex, France). The alveograph test was performed according to the EU standards. The results include P Value, L Value, P/L Value, and W Value.

# 4 Results

The SKCS 4100 compartmentalizes the results in two groups: under 50 is soft grain (the hardness index was between 27 and 36) and above 50 is hard grain (the hardness index was between 57 and 81).

We use twin correlation to determine the relationship between the results. Table 3 shows the result of the analysis.

Sample	Moisture	Milling	Water	Wet		Alveo	ograph	
code	(%)	(%)	absorbent	$_{(\%)}^{ m gluten}$	Р	L	P/L	W
II.	13.27	71.88	54.8	21.58	42.40	65.5	0.65	102.06
III.	13.86	71.79	57.3	27.48	63.49	93.8	0.68	204.54
VI.	14.01	74.01	54.0	16.85	45.72	51.5	0.89	103.99
IX.	14.00	68.33	56.6	25.30	49.99	67.3	0.75	123.80
IV.	13.90	72.89	60.9	28.13	88.25	70.0	1.26	251.35
VII.	13.85	71.28	61.4	22.88	105.5	43.0	2.45	195.84
VIII.	13.58	70.16	63.2	33.68	87.95	75.5	1.14	226.64
Х.	13.37	70.96	67.9	31.70	93.18	59.9	1.56	178.48
XI.	13.15	67.94	66.8	35.60	100.3	47.0	2.16	189.91
XII.	12.82	70.46	63.0	29.68	103.9	61.5	1.69	252.19
XIII.	12.92	69.66	56.9	31.08	54.85	66.0	0.83	148.09

Table 1: Flour parameters

Table 2: Results of SKCS 4100 and Perten 3303 mill

Samples	Perten SKCS 4100 (HI %)	Perten 3303 mill $(e_{\rm f} {\rm mWh/cm}^2)$
II.	27	0.235
III.	36	0.245
VI.	20	0.215
IX.	29	0.255
IV.	61	0.440
VII.	57	0.435
VIII.	67	0.465
Х.	81	0.555
XI.	81	0.545
XII.	81	0.535
XIII.	68	0.470

Table 2 shows also the Perten-HI and grinding energy values in the tests. The SKCS 4100 compartmentalizes the results in two groups. Under 50, the entries belong to the Soft Wheat category, while entries above values of 50 are considered as a Hard Wheat category. The average HI was 55.2 with values between 20 (min) and 81 (max).

		Perten Hardness	Grinding	Moisture	Milling	Water	Wet		Alveogra	ıph	
		Index HI (%)	energy (e <sub>g</sub> mWh/cm <sup>2</sup> )	(%)	extraction (%)	absorption (ml)	gluten (%)	Р	L	P/L	W
Perten Hardne Index (%) Grindi	iess HI	1									
energy mWh/	y (e <sub>g</sub>	0.991	1								
Moistu (%)	ure	-0.637	-0.60	1							
Millin extract (%)	tion	-0.437	-0.417	0.417	1						
Water absorp (ml)		0.876	0.878	-0.346	-0.402	1					
Wet gl (%)	luten	0.833	0.781	-0.531	-0.660	0.756	1				
	Р	0.816	0.826	-0.244	-0.224	0.873	0.560	1			
A L	L	-0.217	-0.320	0.141	0.096	-0.260	0.171	-0.325	1		
V E	P/L	0.640	0.687	-0.187	-0.240	0.724	0.300	0.875	-0.691	1	
E O.	W	0.675	0.634	-0.151	-0.055	0.623	0.582	0.808	0.209	0.468	1

Table 3: Correlation matrix for the technological traits and grinding energy of wheat entries in Szeged, Hungary

According to the results, there is a very strong correlation between milling energy and kernel hardness (r = 0.99) (*Figure 3*). The correlation between hardness index and the examined flour parameters was also significant (r = 0.81 - 0.87). We have found strong correlation between milling energy and water absorption (r = 0.88) of the flour. For example: hardness index – wet gluten r = 0.83; hardness index – water absorption r = 0.88 (*Figure 4*), hardness index – P value of alveograph r = 0.82. There is a correlation between the ef and water absorption r = 0.88. We have found correlation between water absorption and P value of alveograph, r = 0.87.

Sample set "B" showed a very close correlation with the Hardness Index, measured by SKCS 4100, the water absorbance capacity of the flour (*Figure* 4), made out of the crops, and obtained an acceptable correlation with the flour's wet gluten content, and the alveograph deformation work as well.

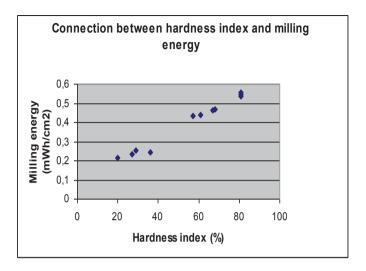


Figure 3: HI and milling energy (ef) connection

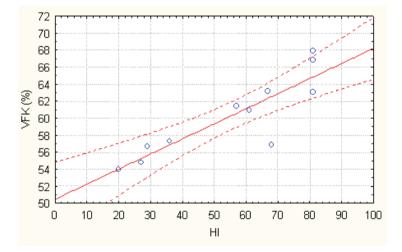


Figure 4: Connection between water absorbance capacity and Hardness Index

The associations found in this study will help to better understand the technological aspects of wheat grain and flour quality as well as provide useful information to breeders to develop new, high-quality hard and soft wheat varieties.

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# Investigation of wheat grits during storage

Zs. H. Horváth e-mail: horvatzs@mk.u-szeged.hu B. P. Szabó

e-mail: szpb@mk.u-szeged.hu

## A. Véha

e-mail: veha@mk.u-szeged.hu University of Szeged, Faculty of Engineering, 6725 Moszkvai krt. 9., Szeged, Hungary

**Abstract.** The change of the quality of wheat milling products was investigated in our work. We analysed different types of wheat grists that are used in household (BL-55, BL-196, BFF-55 and AD). The grists were stored in three type of packages (paper bag, transparent PE bag, and woven PP bag) and in two different places (bright/warm and dark/cool place) for 6 months. The titre and colour characteristics of samples were measured monthly. Colour measurements were performed with a Hunter MiniScan colour-measuring instrument. The CIELab colour system was used for colour characterization. The values of titre were analysed using ANOVA. The type of package did not have significant influence on the titre. In the case of the BL-55, BL-196, and BFF-55 type of flours, the storage conditions had a significant effect on titre: it was smaller for samples that were stored in the dark/cool place. The value of titre rose significantly during storage for all samples.

To determine the change of colour, we calculated the  $\Delta E_{ab}^*$  colour differences between colour coordinates measured at the beginning and during storage. The colour of the BL-55 and BL-196 flour samples did not change perceptibly. The variation of colour of the BFF-55 and AD type of flours was imperceptible for samples stored in the dark/cool place. The changing of the colour was well perceptible in the case of samples stored in the bright/warm place using paper bag or PP bag.

Keywords and phrases: wheat milling product, colour, storage, titre

## 1 Introduction

Wheat grindings are one of the most important and most frequently used raw materials. As for every alimentary product, also for wheat grindings, the colour is an important parameter, which gives a primary image of it – especially for durum wheat pasta since it does not contain eggs. This explains the fact that instrumental colour measurements are applied on durum semolina also in industrial practice. In literature, various research results report on colour measurements of wheat grindings. Oliver et al. (1997) showed during the qualification in 1993 already that the ash content influences the colour of the flours. Further research on this topic by *Horváth et al.* (2004) proved that flours prepared from harder grain have lower L<sup>\*</sup> coordinate and higher a<sup>\*</sup> coordinate, and thus they are darker and have browner tone; besides that, the L\* lightness coordinate shows good correspondence with the whiteness index of the flours. Halászné et al. (1995) proposed a qualification system based on the colour measurements of durum semolina. D'eqido & Paqani (1997) compared the colour characteristics of pasta made of durum flour obtained by different grinding procedures. During the product manufacturing, the colour characteristics were mainly used to determine the appropriate roastedness (Hotti et al., 2000). Humphries et al. (2005) found a correlation between CIE  $b^*$ and the lutein concentration of wheat. Konopka et al. (2004) established a relation between the colour characteristics of the flours and their lipid and colorant content. Gökmen & Senyuva (2006) investigated the effect of heating on the colour parameters of wheat flour. László et al. (2008) examined the effects of ozone, UV, and combined ozone-UV treatment on the colour of wheat flour. Lamsal & Faubion (2009) studied the effect of an enzyme preparation on wheat flour and dough colour, and pointed out that enzyme preparation did not improve lightness  $(L^*)$  and yellowness  $(b^*)$  of the flour system, but benzoyl peroxide sharply reduced b<sup>\*</sup>.

The titre of wheat milling products is an important attribute, too. The high titre values of a wheat product cause failure in their quality. We investigated how the titre and colour characteristics of a wheat milling product change during storage.

## 2 Materials and methods

#### 2.1 Materials

Different types of wheat milling products used in household were investigated:

- BL-55 wheat flour;
- BL-196 whole wheat flour;
- BFF-55 pastry flour;
- AD semolina.

The samples were stored in three different types of packages:

- paper bag;
- transparent PE bag;
- woven PP bag.

The wheat products were stored in two different places, a bright/warm and a dark/cool place. The titre and colour coordinates of samples were measured monthly for 6 months.

#### 2.2 Measurement of titre

Measurement of titre was performed according to MSZ 6369-11:1987 (*Hun-garian Standard Library*). Suspension was made of 20 g flour and 200 cubic centimetre of water. This suspension was titrated to pH 8.4 using 0.1 mol/l NaOH. The titre (T) was calculated using the following formula:

$$T = L/2,$$

where: L - the quantity of the used 0.1 mol/l NaOH in cubic centimetre.

#### 2.3 Measurement of colour

Colour measurements were performed with a HunterLab MiniScan colourmeasuring instrument. The CIELab colour system was used for colour characterization. In this colour space, the colour points are characterized by three colour coordinates. L\* is the lightness coordinate ranging from no reflection for black (L\* = 0) to perfect diffuse reflection for white (L\* = 100). a\* is the redness coordinate ranging from negative values for green to positive values for red. b\* is the yellowness coordinate ranging from negative values for blue and positive values for yellow.

The total colour change is given by the colour difference  $(\Delta E_{ab}^*)$ , in terms of the spatial distance between two colour points interpreted in the colour space (*Hunter*, 1987):

$$\Delta \mathbf{E}_{\mathrm{ab}}^* [(\mathbf{L}_1^* - \mathbf{L}_2^*)^2 + (\mathbf{a}_1^* - \mathbf{a}_2^*)^2 + (\mathbf{b}_1^* - \mathbf{b}_2^*)]^{1/2}.$$

If  $1.5 < \Delta E_{ab}^* < 3$ , then the colour difference between samples can hardly be visually distinguished; if  $\Delta E_{ab}^* > 3$ , then the colour difference between two samples can be visually distinguished.

The chroma  $(C_{ab}^*)$  was used to determine the change of colour.

$$C_{ab}^*((a^*)^2 + (b^*)^2)^{\frac{1}{2}}$$

The chroma represents colour saturation, which varies between dull at low chroma values and vivid colour at high chroma values (*Hunter*, 1987).

#### 3 Results

#### 3.1 Variation of the titre of wheat milling products

The values of titre were analysed using analysis of variance (ANOVA) (*Rice*, 1995). The Shapiro–Wilk test was used to control the conformance of data to the Gaussian distribution. The homogeneity of variances in the different groups was checked using Cochran test and Bartlett test. The results of ANOVA can be seen in *Table 1*.

Type of		Factor								
milling	Storage time		Storage place		Type of package					
product	F-	p (level of	F-	p (level of	F-	p (level of				
	value	significance)	value	significance)	value	significance)				
BL 55	132.47	0.000	78.05	0.000	1.56	0.221				
BL 196	97.47	0.000	54.24	0.000	0.36	0.691				
BFF 55	37.46	0.000	12.63	0.001	1.25	0.302				
AD	59.72	0.000	0.02	0.876	2.92	0.073				

Table 1: Results of ANOVA for values of the titre

The results of ANOVA demonstrate that the titre of the milling product was significantly influenced by factor storage time (p<0,001), whereas the type of package did not influence the titre significantly. In the case of BL-55, BL-196, and BFF-55 types of flour, the storage conditions had significant effect on titre (p<0,001). The value of titre was smaller for samples stored in the dark/cool place.

Detailed analysis of changes in the averages of titre values measured during storage are presented with a confidence interval at a level of 95% in figures 1–4 for the different types of milling products.

In *Figure 1*, we can see that the titre of BL55 type of wheat flour was dynamically increasing for two months, but after the rise it became slower.

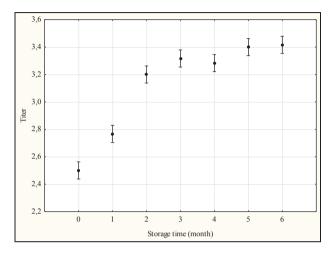


Figure 1: Results of ANOVA for titre in the case of the BL55 type of wheat milling product (average with confidence interval at a level of 95%)

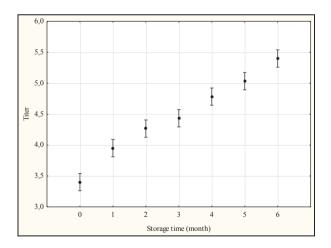


Figure 2: Results of ANOVA for titre in the case of the BL196 type of wheat milling product (average with confidence interval at a level of 95%)

In the case of the BL196 flour, the titre increased permanently during storage, from 2.5 units to 5.4 units. In the case of the BFF 55 product, the titre

increased permanently during storage too, from 2.1 units to 2.7 units. The titre of AD wheat milling product did not change significantly for four months, after which it rose powerfully, from 1.8 to 2.5 units.

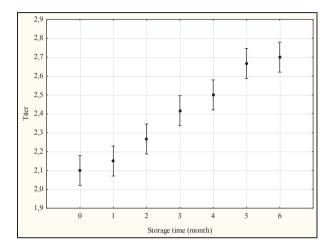


Figure 3: Results of ANOVA for titre in the case of the BFF55 type of wheat milling product (average with confidence interval at a level of 95%)

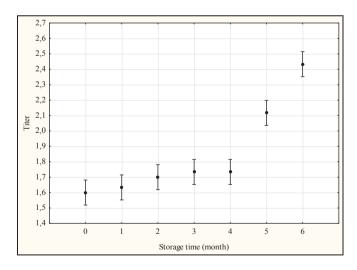


Figure 4: Results of ANOVA for titre in the case of the AD semolina type of wheat milling product (average with confidence interval at a level of 95%)

#### 3.2 Change of the colour of wheat milling products

The colour differences of colour coordinates measured at the beginning and during storage were calculated to determine the changes in colour for all the different types of samples. The values are presented in *Table 2*. Colour difference values are usually higher in the case of samples stored in the bright/warm place, but values are higher than 3 units only for five samples.

Type of	Storage	Type of		Stor	age tin	ne (mor	nth)	
$\operatorname{milling}$	place	package	1	2	3	4	5	6
product				$\Delta \mathrm{E}^*_\mathrm{al}$	, Colou	r differ	ence	
		paper bag	0.45	0.36	0.65	1.24	0.64	0.88
	dark/cool	PE bag	0.43	0.37	0.24	1.04	0.75	0.87
BL55		PP bag	0.39	0.34	0.62	0.93	0.52	1.07
2200		paper bag	0.20	0.62	1.21	1.05	1.43	1.51
	bright/warm	PE bag	0.39	0.31	0.48	1.07	0.57	1.14
		PP bag	1.09	0.69	1.21	1.40	1.15	1.80
		paper bag	0.42	0.45	0.47	1.41	0.66	0.86
	dark/cool	PE bag	0.48	0.29	0.51	0.85	0.59	1.16
BL196		PP bag	0.66	0.14	0.61	1.47	0.67	0.98
DII00	bright/warm	paper bag	0.53	0.21	0.63	1.24	0.98	0.70
		PE bag	0.77	0.95	0.36	1.42	0.89	0.96
		PP bag	0.73	0.33	0.75	1.46	1.27	1.04
		paper bag	0.81	0.73	0.72	1.01	0.86	1.37
	dark/cool	PE bag	0.84	0.66	0.66	0.75	0.65	1.01
BFF55		PP bag	0.85	0.64	0.74	1.33	0.93	1.36
DII 00		paper bag	0.81	1.42	2.38	1.66	2.76	3.63
	bright/warm	PE bag	0.77	0.49	0.51	1.18	0.66	1.44
		PP bag	0.78	1.08	1.79	1.69	2.63	3.59
		paper bag	1.53	1.89	1.55	1.86	1.49	1.83
	dark/cool	PE bag	1.31	1.48	1.47	0.78	1.29	1.70
AD		PP bag	1.38	1.45	1.24	0.89	0.88	2.07
		paper bag	1.41	1.27	1.46	1.81	3.19	4.33
	bright/warm	PE bag	1.28	1.23	0.72	0.51	1.56	2.40
	·	PP bag	1.23	1.31	1.34	1.56	2.74	4.29

Table 2:  $\Delta E_{ab}^*$  colour differences calculated between colour coordinates measured at the beginning and during storage

The colour of BL-55 and BL-196 flour samples did not change perceptibly. The variation of colour of the BFF-55 and AD type of flours was imperceptible for samples stored in the dark/cool space.

The changing of the colour was well perceptible in the case of samples stored in the bright/warm place using paper bag or PP bag, after 5 or 6 months. The C\*ab chroma values of the initial samples and stored samples indicate that the colour of samples has become less saturated. In the case of the BFF-55 pastry flour, the chroma decreased from 8.37 units to 6.82 and 6.62 units; for AD semolina, the rise was from 7.73 units to 5.89 and 5.76 units.

To summarize, we can state the following:

The type of package did not influence the titre significantly. In the case of BL-55, BL-196, and BFF-55 types of flours, the storage conditions had a significant effect on titre: it was smaller for samples stored in the dark/cool place. The value of titre rose significantly during storage for all samples.

The colour of BL-55 and BL-196 flour samples did not change perceptibly during storage.

The variation of colour of the BFF-55 and AD type of flours was imperceptible for samples stored in the dark/cool space. The changing of the colour was well perceptible in the case of samples stored in the bright/ warm place, using paper bag or PP bag, after 5 or 6 months – the colour became less saturated.

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# The effect of storage on the colour of paprika powders with added oleoresin

Zs. H. Horváth

e-mail: horvatzs@mk.u-szeged.hu University of Szeged, Faculty of Engineering, 6725 Moszkvai krt. 9., Szeged, Hungary

Abstract. The use of natural food colours is preferred to that of artificial dyestuffs for modern alimentary purposes. Paprika is a spice plant grown and consumed in considerable quantities worldwide and also used as a natural food colour, so the colouring power of powders is very important. The colour of paprika powder is highly relevant too because the consumer concludes its colouring power based on its colour. The colouring power of paprika powders is directly determined by the quality and quantity of the colouring agent of paprika. The paprika oleoresin, that is an oil soluble extract from the fruits of Capsicum Annum Linn or Capsicum Frutescens, is suitable to raise the colour agent content of paprika powders. We investigated how the colour and the characteristics of paprika powder samples with added oleoresin change in the course of storage. The colour agent content of 7 different quality powders was increased with 7–75% using oleoresin. The initial colour agent content of samples changed between 41 and 169 ASTA units. The powders were made from Chinese, Peruvian, and Hungarian paprika. Colour measurements were performed with a HunterLab MiniScan colour-measuring instrument. The CIELab colour system was used for colour characterization. The colour agent content and the colour coordinates of samples were measured throughout 9 months. The decrease of colour agent content varied between 22 and 51 percent, while the average reduction was 33 percent. The quantity of added oleoresin did not influence the colour agent content decrease significantly.

Keywords and phrases: paprika powder, colour, storage

The values of colour difference changed between 2 and 4.5 units. The initial paprika powder influenced the variation significantly, but the quantity of added oleoresin did not have a significant effect.

# 1 Introduction

The use of natural food colours is preferred to that of artificial dyestuffs for modern alimentary purposes. Paprika is a spice plant grown and consumed in considerable quantities worldwide, and also used as a natural food colour. The colouring power of paprika powders is directly determined by the quality and quantity of the colouring agent of paprika. The colour agent content of powders decreases during storage time and it is influenced by the stages of the processing. Dehydration is the most critical step of the processing. The effect of the heat impairs the colour agent, aroma, and flavour substratum of paprika. Several researchers investigated the optimal parameters of dehydration (Minquez-Mosquera et al., 2000; Ramesh et al., 2001; Shin et al., 2001; Doymaz & Pala, 2002; Kim et al., 2004; Perez-Gamez et al., 2005; Simal et al., 2005). Topuz et al. (2011) compared the Refractance Window (RWD) method to dry paprika in comparison with freeze-drying, hot-air oven drying, and natural convective drying methods. It was pointed out that the least colour agent content decrease was in the case of the natural convective drying method. Colour agent content reduction is effected by storage conditions. There are many studies on the changes in the colour agent content of the paprika storage processes (Park et al., 2007, Banout et al., 2011, Topaz et al., 2011, Chetti et al., 2012).

The colour of the powder is influenced by many factors besides the colouring agent content. The colour of the powder is influenced by its particle size, oil and moisture content, but first of all by the colour agent content. The instrumental colour measurement is not used in industrial practice – the development of the colour of the paprika powder is made based on the empirical facts; therefore, the quantity of the colour of the end-product is often not correct.

Since the 1970s, a number of papers have been published on measurements of the colour of paprika powders (*Horváth & Kaffka*, 1973; *Drdak et al.*, 1989). Measurements have been performed relating to the correlation between visual sensing and the instrumentally measured colour characteristics (*Huszka et al.*, 1985, *Horváth*, 2007). The effects of ionizing irradiation on the colour of paprika powder were investigated by *Fekete-Halász et al.* (1996). *Minguez et al.* (1997) analysed how the colour of the powder is changed by the ratio of the yellow and red pigments within the total colouring agent content. There are many papers about the changes in the colour characteristics of the paprika during different drying and storing processes (*Park et al.*, 2007, *Banout et al.*, 2011, *Topaz et al.*, 2011, *Chetti et al.*, 2012). In the case of the Korean cultivars, no significant change in colour characteristics was detected when the moisture content varied between 10% and 15% (Chen et al., 1999). Horváth & Hodúr (2007) investigated Hungarian paprika powders, and pointed out that the colour of the powder was observed to be turning into darker and deeper red while increasing moisture content. Various investigations have been made on the connection between the colouring agent content of the powder and the colour characteristics measured by different techniques (*Navarro et al.*, 1993, *Nieto-Sandoval et al.*, 1999). Such investigations have yielded partial results, but there is no formula that describes the correlation between the colouring agent content and the colour characteristics.

The paprika oleoresin, that is an oil soluble extract from the fruits of Capsicum Annum Linn or Capsicum Frutescens, is often used to increase the colour agent content of paprika powders. We investigated how the colour agent content and the colour of paprika powder samples with added oleoresin change during storage time.

# 2 Materials and method

#### 2.1 Materials

The colour agent content of 7 different quality powders was increased. The initial colour agent content of the samples changed between 41 and 169 ASTA units. The powders were made from Chinese, Peruvian, and Hungarian paprika. The colour agent content was increased using 0.5–3.0 g oleoresin added to 100 g paprika powder.

#### 2.2 Measurement of colour agent content

After homogenization of powders, the colour agent content of samples was measured. The ASTA (American Spice Trade Association) unit was used to measure the colour agent content of paprika powders according to MSZ EN ISO 7541. The acetone extracts of paprika powder were measured by photometer at 460 nm. The ASTA unit was calculated using the following formula:

$$ASTA = \frac{Absorbance \cdot 16, 4 \cdot \mathbf{f}}{\text{weight of sample (g)}},$$

where  $\mathbf{f}$  is a correction factor for the used photometer.

#### 2.3 Measurement of colour

Colour measurements were performed with a HunterLab MiniScan colourmeasuring instrument. The CIELab colour system was used for colour characterization. In this colour space, the colour points are characterized by three colour coordinates. L\* is the lightness coordinate ranging from no reflection for black (L\* = 0) to perfect diffuse reflection for white (L\* = 100). a\* is the redness coordinate ranging from negative values for green to positive values for red. b\* is the yellowness coordinate ranging from negative values for blue and positive values for yellow.

The total colour change is given by the colour difference  $(\Delta E_{ab}^*)$  in terms of the spatial distance between two colour points interpreted in the colour space (*Hunter*, 1987):

$$\Delta E^*_{ab} = \left[ (L^*_1 - L^*_2)^2 + (a^*_1 - a^*_2)^2 + (b^*_1 - b^*_2) \right]^{1/2} .$$

If  $1.5 < \Delta E_{ab}^* < 3$ , then the colour difference between two paprika grists can hardly be visually distinguished; if  $\Delta E_{ab}^* > 3$ , then the colour difference between two paprika grists can be visually distinguished (*Horváth*, 2007).

The chroma  $(C_{ab}^*)$  was used to determine the change of colour:

$$C_{ab}^* = ((a^*)^2 + (b^*)^2)^{\frac{1}{2}}.$$

The chroma represents colour saturation, which varies from dull at low chroma values to vivid colour at high chroma values (*Hunter*, 1987). The samples were stored at room temperature, protected from light. The colour coordinates and colour agent content were measured monthly for 5 months, and after 7 months and 9 months.

## 3 Results

#### 3.1 Changes of the colour agent content

To evaluate the changes of colour agent content, we calculated the value of the decrease of colour agent content measured at different times, correlated to the initial value. The values were given in percentage. First, we analysed how the colour agent content decreased throughout 9 months, influenced by the initial paprika samples and the quantity of added oleoresin. The ANOVA was applied. The results of ANOVA is shown in *Table 1*.

Table 1: Variance table in the case of colour agent content decrease throughout 9 months

Factor	F value	Significant level
Quantity of added oleoresin	0.72	0.54
Initial paprika powder	31.99	0.00

It can be established that the quantity of added oleoresin did not influence the colour agent content decrease significantly, but the initial paprika powder affected it significantly. In *Figure 1*, we can see the averages decrease with confidence interval at a level of 95%.

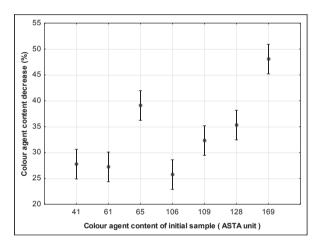


Figure 1: Results of ANOVA for colour agent content decrease throughout 9 months (average with confidence interval at a level of 95%)

The decrease of the colour agent content varied between 22 and 51 percent. It can be seen well that the decrease was valid mostly in the case of Hungarian paprika powders (65 ASTA, 128 ASTA, and 169 ASTA) and the loss was small for Peruvian powders.

#### 3.2 The change of the colour characteristics

To evaluate the change of colour, we calculated the  $\Delta E_{ab}^*$  colour difference values between colour coordinates measured at first and measured during storage. The values for the different samples are shown in figures 2–4. We can see that the values of colour difference for initial samples (0 g) and the values of colour difference for samples with added oleoresin do not differ significantly.

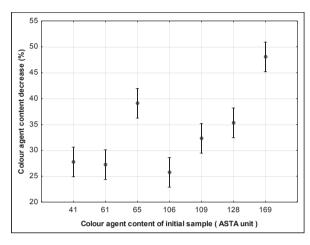


Figure 2:  $\Delta E_{ab}^*$  colour differences calculated between colour coordinates measured at first and during storage time in the case of Hungarian samples

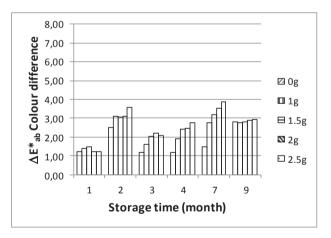


Figure 3:  $\Delta E_{ab}^*$  colour differences calculated between colour coordinates measured at first and during storage time in the case of Chinese samples

Colour difference values exceeded the perceptible 3 units after 7 months in the case of samples made of Hungarian paprika and in the case of Chinese paprika samples. Colour differences were less than 3 units for all samples in the case of Peruvian paprika.

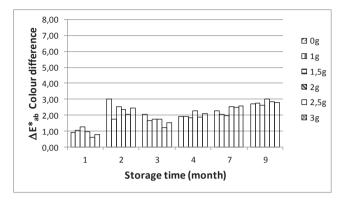


Figure 4:  $\Delta E_{ab}^*$  colour differences calculated between colour coordinates measured at first and during storage time in the case of Peruvian samples

	Storage time (month)								
Initial sample	Added oleoresin (g)	1	2	3	4	5	7	9	
	0.0	-1.12	-1.04	-1.15	-2.36	-1.87	-2.64	-3.52	
	0.5	-0.83	-1.57	-1.91	-2.67	-1.69	-3.2	-3.58	
Hungarian	1.0	-0.82	-1.56	-2.06	-2.36	-1.36	-3.12	-3.52	
	1.5	-1.17	-1.86	-2.12	-2.51	-1.46	-3.25	-3.45	
	2.0	-1.22	-1.51	-2.30	-2.78	-1.62	-3.53	-3.70	
	0.0	0.25	-0.47	0.15	-0.15	-0.06	0.71	-1.23	
	1.0	-0.18	-0.40	-0.33	-0.45	-0.63	-0.27	-0.80	
Chinese	1.5	-0.47	-0.78	-0.54	-0.60	-0.85	-0.39	-1.04	
	2.0	-0.80	-0.94	-0.80	-1.20	-1.27	-1.05	-1.25	
	2.5	-1.09	-0.90	-0.55	-1.04	-1.31	-1.00	-1.30	
	0.0	-0.47	-1.87	-1.32	-0.93	-1.40	-1.81	-1.47	
	1.0	-0.75	-1.06	-1.07	-1.53	-1.60	-1.76	-1.90	
Dominian	1.5	-0.58	-1.15	-1.15	-1.21	-1.29	-1.80	-1.86	
Peruvian	2.0	-0.89	-1.80	-1.44	-1.98	-2.10	-2.18	-2.43	
	2.5	-0.54	-1.62	-1.08	-1.69	-2.24	-2.23	-2.40	
	3.0	-0.73	-1.91	-1.40	-1.83	-2.33	-1.96	-2.34	

Table 2:  $\Delta L$  lightness coordinate differences calculated between colour coordinates measured the first time and during storage

In *Table 2*, we present  $\Delta L$  lightness coordinate differences calculated between colour coordinates measured at first and during storage. The values are negative, so the colour of powders became brighter during storage. In *Table*  $\beta$ , we can see  $C_{ab}^*$  chroma calculated between colour coordinates measured at first and during storage time. The values are positive, so the colour of powders became less saturated during storage.

		Storag	ge time	(mont	h)			
Initial	Added							
sample	oleoresin	1	2	3	4	5	7	9
sample	(g)							
	0.0	1.58	0.78	0.72	1.89	0.83	2.29	1.17
	0.5	0.62	0.82	0.81	1.31	-0.41	1.95	0.31
Hungarian	1.0	0.49	0.64	0.66	1.34	-0.74	1.34	-0.09
	1.5	0.06	0.79	0.80	1.16	-1.01	1.38	0.07
	2.0	-0.02	0.81	0.76	0.95	-0.50	1.32	0.13
	0.0	1.07	2.47	0.93	0.72	1.40	2.67	1.68
	1.0	1.29	3.08	1.57	1.88	2.68	2.13	2.35
Chinese	1.5	1.35	2.95	1.97	2.34	3.01	2.17	2.35
	2.0	0.85	2.95	2.05	2.18	3.22	1.94	2.49
	2.5	0.58	3.40	2.02	2.55	3.57	1.89	2.55
	0.0	0.80	2.28	1.41	1.52	1.67	2.32	1.63
	1.0	0.72	1.37	1.16	0.85	1.02	1.39	0.96
Peruvian	1.5	1.07	2.23	1.31	1.33	1.46	1.38	1.22
	2.0	0.33	1.48	0.75	0.95	1.38	1.05	0.69
	2.5	0.28	1.28	0.51	0.77	1.06	0.57	0.33
	3.0	0.27	1.51	0.30	0.92	1.08	1.18	0.45

Table 3:  $C^*_{ab}$  chroma differences calculated between colour coordinates measured the first time and during storage time

In summary, we can state the following: The colour agent content decreases for initial samples (0 g), and does not differ significantly for samples with added oleoresin. The decrease of colour agent content varied between 22 and 51 percent; the average reduction was 33 percent. The values of colour difference for initial samples and those for samples with added oleoresin do not differ significantly. During storage time, the colour of paprika samples became brighter and less saturated.

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# Effect of drying methods for inner parameters of red beetroot (*Beta vulgaris L.*)

D. Székely e-mail: babinszky-szekely.dora@etk.szie.hu B. Illés e-mail: gitta0814@gmail.com

M. Stéger-Máté

J. Monspart-Sényi

e-mail: stegerne.mate.monika@etk.szie.hu e-mail: monspart.elemerne@etk.szie.hu

Department of Food Preservation, Faculty of Food Science, Szent István University, 1118, Villánvi út 29–43., Budapest, Hungary

**Abstract.** In compliance with consumer expectations, careful processing and preservation are increasingly used with fruits and vegetables. The aim is that during these treatments the valuable nutritional characteristics of the raw materials change as little as possible. Drying has been used for the preservation of raw materials for a long time, which can distinguish two different groups based upon pressure. These are the atmospheric and the more careful vacuum drying.

During the research, Alto F1 beetroots were being dried in vacuum and under atmospheric pressure at different temperatures. Vacuum drying took place at 40, 50, and 60 °C, while atmospheric drying at 60, 70, and 80 °C. All drying processes lasted 150 minutes. During drying, changes of moisture content and water activity were monitored. After drying, colour measurement was realized and the inner parameters were investigated, such as polyphenol, betalain, and antioxidant capacity. These measured parameters were compared in the case of atmospheric and vacuum drying.

Keywords and phrases: beetroot, atmospheric drying, vacuum drying

## 1 Introduction

Although the consumption of vegetables is becoming more and more prevalent, the consumption of beetroot in our country is very low despite that numerous researches demonstrate its cancer-preventive, antioxidant, and antiinflammatory effect. In addition, its nutritional content is very favourable; besides the high fibre and mineral content, vitamin C and folic acid are substantial. Especially in fresh or in bottled form, it is applied to colour yogurts and cookies because of its large amount of pigments (betanin, vulgaxantin I-II). The consumption of dried red beet is becoming more and more common. Beetroot (*Beta vulgaris L. ssp. esculenta Gurke var. rubra L.*) has already been grown since antiquity. The wild ancestor of beetroot is *Beta vulgaris var. maritima*, which can be found mainly around the Mediterranean Sea. The Romans, Greeks, and Egyptians were familiar with its wild variety used for direct consumption – this has been known only since the XIX–XX. centuries (*Hájas*, 1976).

Although healthy lifestyle receives more and more emphasis, so the beneficial effects of the consumption of beetroots is well-known, the consumption per capita is only 0.5 kg a year. This amount is higher in other European countries. People in Northern Europe, Japan, the UK, and Germany show a preferance for consuming beetroots (*Balázs*, 2004).

Beetroot has excellent physiological properties. Its macro- and micronutrient content is remarkable and its vitamin content is high. Its vitamin A and C content is substantial and its vitamin B is outstanding. Vitamin B1 (thiamine), vitamin B2 (riboflavin), and vitamin B3 (niacin) can be found in most root vegetables with dark green leaf, such as in beetroot. Beetroots play a vital role due to their remarkable folate content. Folic acid helps to prevent cancer and in cooperation with vitamin B contributes to the proper functioning of the nervous system (*Takácsné*, 2002).

Betalain – water-soluble, nitrogen-containing plant pigments – gives the red colour of red beet (*Ravichandran et al.*, 2013). Beetroot has a lot of positive properties for the human body. It has antioxidant, anti-inflammatory, hepato-protective, and anticancer effects (*Nemzer*, 2011). The colouring effect of betalain shows similarity with the anthocyanin compounds (*Csapó & Csapóné*, 2003). Betalains can be divided into two groups based on their colour: redpurple betacyanin and yellow betaxanthin (*Georgiev et al.*, 2010).

### 2 Materials and methods

#### 2.1 Materials

The Alto F1 beet variety was grown in the Experimental Plant of Tan's economy at the Faculty of Horticultural Sciences, Szent István University. During cultivation, beetroots were treated with Genesis fertilizer, which contains the followings: 27% nitrate, 5% MgO, and 7% CaO. Beetroots used in the experiment were harvested in July 2014. After harvesting, they were washed, peeled, and sliced into 2 mm slices, and then they were stored in frozen state at 18 °C for 2 weeks.

#### 2.2 Methods

Drying lasted 150 minutes. The following drying instruments were used: MEMMERT-VO vacuum drying and LP-322 type drying oven. Based on the experimental plans, drying was done in vacuum at 40, 50, and 60 °C (sample codes: 40V, 50V, 60V) and in drying oven at 60, 70, and 80 °C (sample codes 40L, 50L, 60L). After drying, beetroots were extracted. Nutrition characteristics were assessed in the dried and extracted beetroots.

Sampling was done every 30 minutes in each case. Subsequently, moisture content – Kern DBS 60-3 – and water activity – LabMaster-aw – of the samples were determined. The chemical tests (polyphenol content (*Singleton & Rossi*, 1965), antioxidant capacity (*Benzie & Strain*, 1996), betacyanin (*Castellar et al.*, 2003) and betaxanthin content (*Stinzinget et al.*, 2005), and the physical measurements (rewetting, colour measurement – Minolta CR 200) were carried out only in the case of dried beetroots. During rewetting, the absorption index, or rehydration ratio (RR) is the ratio of rehydrated product weight (Wr) and the dry weight of the product (Wd) of the dried beetroot samples.

## 3 Results and discussion

The aim of my work was to compare the nutritional characteristics of the dried beetroots using vacuum drying and drying oven. During the evaluation of the results, the received values were compared.

Figure 1 shows the changes of the moisture content. During the same drying time, the moisture content of the 60V, 60L, 70L, and 80L samples are almost identical. Vacuum drying at 40 and 50 °C would require a longer drying period to reach a moisture content under 10%.

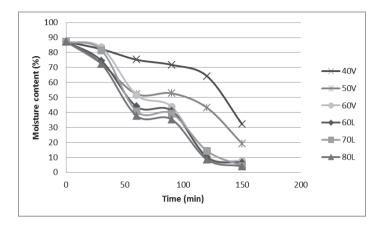


Figure 1: Moisture content in the case of different drying parameters

Table 1 presents the final moisture content after drying.

	Moisture content (%)	Water activity
40V	32.000	0.791
50V	19.100	0.567
60V	7.557	0.400
60L	6.416	0.453
70L	4.262	0.405
80L	4.023	0.310

Table 1: Moisture content and water activity

Figure 2 shows the water activity of the samples during drying. Sampling was taken every 30 minutes. The initial water activity of the raw material was the same ( $a_w = 0.892$ ) in all cases.

Among the samples, the 40V shows the smallest change. In the case of vacuum drying, a substantial reduction of water activity occurred only after 90 minutes of drying, but with the drying oven this set in after 60 minutes of drying.

Figure 3 contains the rewetting rate of the dried samples. It can be observed that the 60V shows the highest rewetting rate, which is due to mild drying. In the case of the 50V (RR = 2,8) and 40V (RR = 2,272), rewetting rate becomes increasingly smaller as in these samples the final moisture contents were 19.1% and 32%; so, during rewetting, these samples absorbed less water.

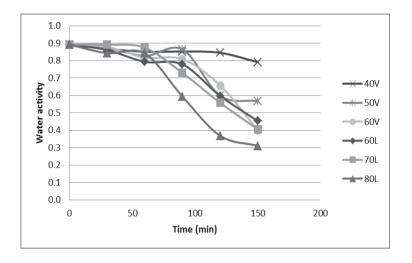


Figure 2: Water activity in the case of different drying parameters

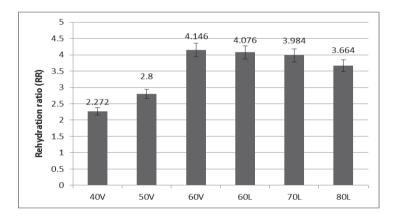


Figure 3: Rehydration ratio of the dried samples

As the drying temperature is raised, the colour difference values ( $\Delta E$ ) of the consecutive samples are compared to each other in *Table 3*. Between the 40 V and 50V, 60V and 60L, and 70L and 80L samples, the deviation is clearly visible, while the difference between the 50V and 60V and 60L and 70L samples is barely noticeable.

Parameters	40V	50V	60V	60L	70L	80L
$a^*$	7.46	5.23	4.46	1.38	1.49	1.28
$b^*$	1.87	1.46	1.23	0.61	0.60	0.54
L *	21.42	18.72	19.48	20.17	20.33	22.17

Table 2: Results of the colour measuremnets in the case of dried samples

Table 3: Values of the colour differences between each sample pair

Samples	$\Delta E$	Differences perceptible to the eye
40V-50V	3.52	Clearly visible
50V-60V	1.10	Barely noticeable
60V-60L	3.22	Clearly visible
60L-70L	0.19	No noticeable
70L-80L	1.86	Noticeable

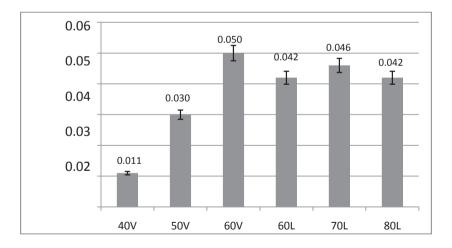


Figure 4: Antioxidant capacity of the dried samples

Figure 4 contains the antioxidant capacity of the dried samples. The highest antioxidant capacity (0.05 mg/100g) can be seen in the 60V sample, but the antioxidant capacity of the 70L samples (0.046 mg/100g) is slightly smaller.

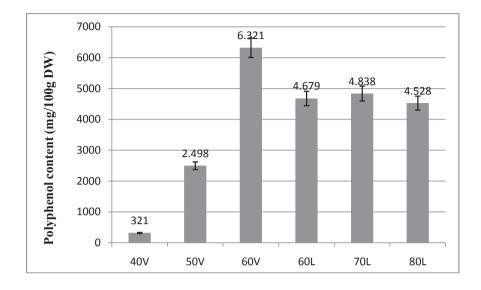


Figure 5: The polyphenol content of the dried samples

Figure 5 shows the polyphenol content of the dried samples. The 60V sample shows the highest polyphenol content. Polyphenol contents yield almost the same values in the case of atmospheric drying.

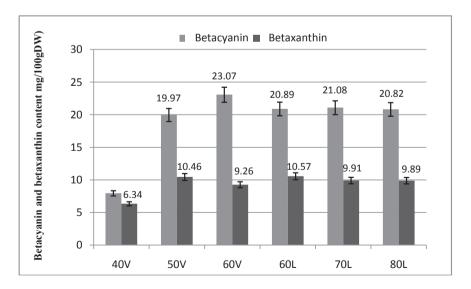


Figure 6: Betacyanin and betaxanthin contents of the dried samples

Based on *Figure* 6, there is a substantial difference between the 40V and the other samples. Betacyanin values do not show remarkable deviation, except for the 40V sample. Betaxanthin content is the highest in the case of the 60V sample.

# 4 Conclusions

Based on the measurements, vacuum drying is a more favourable method to preserve the nutritional characteristics, but it is necessary to dry the samples for the same moisture content and water activity because high moisture content and water activity affect the extraction and stability of betalain. In the future, it is worth drying samples with same moisture content and investigating the effects of more drying methods and temperatures.

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# Alternative grains in nutrition

Sz. Jevcsák e-mail: jevcsak@agr.unideb.hu P. Sipos

e-mail: siposp@agr.unideb.hu

University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Food Technology, H-4032 Debrecen, Böszörményi u. 138. Hungary

**Abstract.** Many people suffer from gluten sensitivity or gluten intolerance. They have to avoid or limit their gluten intake. Sorghum and millet are gluten-free cereals, wherefore persons with gluten sensitivity or gluten intolerance could consume them. Moreover, they have a lot of positive effects due to their phenolic compounds as phenol acid or flavonoid. Antioxidant activity in sorghum is especially high in comparison with other cereals. Our aim was to compare literature data about the chemical compositions of sorghum and millet with other grains.

# 1 Introduction

Sorghum and millet have an important role in the semi-arid tropics of Asia and Africa. These crops are the main sources of energy, protein, vitamins, and minerals for millions of the poorest people in these regions (FAO, 1995). Due to their antioxidant activity, they have a great benefit for the human organism. They have a positive effect on gut microbiota and work as inhibitors of chronic diseases (obesity and cancer). Moreover, sorghum contains carotenoids and has a high content of vitamin E (*Cardoso et al.*, 2015).

Keywords and phrases: gluten, sorghum, millet, chemical compositions

### 1.1 Alternative grains

Sorghum and millet are alternative grains, which are cultivated in small areas. Their uses are mostly traditional (*Berényi*, 2013). There are major emerging uses for gluten-free food and beverage products (*Taylor & Duodu*, 2010).

### Sorghum

Sorghum bicolor (L.) is the fifth most important cereal in the world after rice, wheat, maize, and barley (Food Security Department, 1999). It can grow under excessive heat or in infertile soils (*Mokrane et al.*, 2010). The sorghum's area is 42 million ha. The biggest part is situated in Africa (24,5 million ha) and in Asia (10,6 million ha). In India, this number is 9,1 million ha, in USA is 6,6 million ha, and in Australia is 0,7 million ha (*Kiran*, 2014). The crop is suited to hot and dry agroecologies. Sorghum production includes two groups:

- group I countries (mainly in Asia and Africa) use sorghum for food;
- group II countries (developed and some developing countries) produce sorghum for animal feed. The crop yields an average of 3–5 t/ha (FAO, 1996).

Sorghum has the following species:

- Sorghum vulgare Pers var. technicum
- Sorghum vulgare Pers var. saccharatum
- Sorghum vulgare Pers var. frumentaceum
- Sorghum vulgare Pers var. sudanense (Németh, 2009).

#### Millet

Millet (*Panicum miliaceum* L.) is a small-grained cereal. The most important strains of millets are:

- pearl millet (*Pennisetum glaucum*);
- finger millet (*Eleusine coracona*);
- proso millet (*Panicum miliaceum*);
- foxtail millet (*Stalia italica*).

The genus *Pennisetum* includes about 140 species, which include domesticated and wild-growing species. Millet areas are distributed in most of the Asian and African countries and also parts of Europe (*Kajunas*, 2001). Developing countries account for 94% of global output and an estimated 28 million tons: pearl millet -15 million tons, foxtail millet -5 million tons, proso millet -4 million tons, finger millet -3 million tons (FAO, 1996).

#### 1.2 Chemical compositions of sorghum and millet

Sorghum provides several benefits such as essential fatty acids, proteins, carbohydrates, energy, minerals, vitamins, phytic acid, carotenoids, alcohols, flavonoids, phenolic acids, and bioactives (*Abugri et al.*, 2013). Due to lysine, sulphur-containing amino acids, threenine and tryptophan, millet has a good amino acid balance (*Ajiboye et al.*, 2014).

Cereals	Starch	Protein	Ash	Raw fat
Hard wheat	77.4	13.5	0.56	0.98
Soft wheat	77.9	11.0	0.71	0.86
Barley	53.6	19.4	2.88	2.31
Rye	58.0	13.3	1.96	2.53
Sorghum	67.7	12.1	1.87	3.32
Millet	67.4	8.8	1.82	4.22

Table 1: Chemical parameters of cereals (% dry matter)

According to Bagli (2008)

The protein content in sorghum is 12.1%, the protein content in millet is 8.8% (*Table 1*). The protein content in sorghum is quite variable. In most literatures, this value ranges from 6 to 16% (*Mokrane et al.*, 2010). The starch content in these grains is approximately equal (67%). The raw fat content in millet is 4.22%; therefore, the energy content is very high in this grain (*Table 1*).

Both grains have a large content of leucine: in sorghum: 832 mg/kg, in millet: 598 mg/kg. However, the phenylalanine and isoleucine content are also high in these cereals. The amount of valine and threonine is high as well; consequently, the essential amino acid composition in sorghum and millet is great (*Table 2*).

The mineral content in millet is very high compared with other cereals; it is especially rich in P, K, Mg, Ca, and Fe. Sorghum has a lot of P, K, and Mg (*Table 3*).

	Sorghum	Pearl millet	
Isoleucine	245	256	
Leucine	832	598	
Lysine	126	214	
Methionine	87	154	
Cystine	94	148	
Phenylalanine	306	301	
Tyrosine	167	203	
Threonine	189	241	
Tryptophan	63	122	
Valine	313	345	
Chemical score	37	63	

Table 2: Essential amino acid composition (mg/kg) and chemical score of sorghum and millet proteins

According to Bagli (2008)

Table 3: Mineral content of cereals (mg/kg)

	Hard wheat	Soft wheat	Barley	Sorghum	Rye	Millet
Р	3498	977.6	4570	349.9	3620	2879
Κ	826.2	1225	4572	239.9	3570	2798
Mg	301.2	306.5	1971	187.7	1328	1488
Ca	159.5	202.2	736.2	27.3	348.7	508.6
Na	46	38.4	238.4	4.6	67.2	60.89
Zn	30.8	7.6	74.2	3.1	30.6	65.9
Fe	13.2	13.9	128.4	10.6	44	199.8
Mn	5.2	8.1	9.2	1.2	24.4	8.1
Cu	1.4	1.6	5.7	0.2	2.9	3.4
$\operatorname{Cr}$	0.1	0.001	0.9	0.8	0.7	7.7

According to Bagli (2008)

Sorghum has the highest phenol content among cereals and the most intensive antioxidant activity. The phenolic compounds are 4128  $\mu$ g/g, while in wheat this amount is only between 501 and 562  $\mu$ g/g. The antioxidant capacity in sorghum is 195.8  $\mu$ mole/g, while in wheat it is only 4.17–4.33  $\mu$ mole/g (*Table 4*).

	Phenol	"DPPH"	"ABTS"
	$\operatorname{compounds}$	antioxidant capacity	antioxidant
Cereals	(gallic acid	after 10 minutes	capacity after 3
	equivalents)	$(\mu mole/g),$	minutes
	$(\mu g/g)$	"ABTS"	$(\mu mole/g)$
Hard wheat	562	4.33	8.8
Soft wheat	501	4.17	8.3
Barley	879	21.00	14.9
Sorghum	4128	195.8	51.7
Rye	1026	12.17	13.0
Millet	1387	23.83	21.4

Table 4: Antioxidant activity and phenol compounds of cereals

According to Bagli (2008)

Table 5: Dietary fibre in cereals (% dry matter	Table 5:	Dietary	fibre in	cereals	(% dry)	matter
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Cereals	Soluble dietary fibre	Resistant starch	Insoluble dietary fibre	Total dietary fibre content
Hard wheat	1.61	0.20	2.98	4.59
Soft wheat	1.78	0.55	1.87	3.65
Barley	2.56	0.23	22.07	24.63
Sorghum	1.42	1.77	19.59	21.01
Rye	3.70	0.20	14.07	17.77
Millet	1.45	1.96	13.50	14.95

According to Bagli (2008)

Sorghum contains 21% of total dietary fibre content. The insoluble dietary fibre is also high, 19.59%. The total dietary fibre content in millet is lower, 14.95%; otherwise, the soluble dietary fibre in sorghum (1.42%) and in millet (1.45%) is approximately equal (*Table 5*). Waxy starch includes about 100% amylopectin, while non-waxy sorghum starch has 75% amylopectin and 25% amylose (*Wong et al.*, 2009).

Finger millet contain phytates (0.48%), polyphenols, tannins (0.61%), trypsin inhibitors, and fibres, which have antinutrient effects due to chelating and inhibitor activities (*Palanisamy et al.*, 2014). Finger millet, from cereals, is a rich source of Ca, containing 300 to 350 mg/100g of it (*Kiran*, 2014).

#### 1.3 Utilization as food

During the past few years, sorghum and millet production has increased. Sorghum is used as steamed, leavened, and fat-fried product (FAO, 1995). Furthermore, sorghum use is similar to that of corn: starch, glucose, syrup, and oil can be produced. Moreover, it can be used in preparing whole-grain products, bread, pancake, dumpling, mush, cake, pasta, and beer. Broom and forage can also be prepared from them. Millet is used as traditional food and beverage products, with malting and lactic acid fermentation technologies (*Taylor & Kruger*, 2016).

## 2 Conclusions

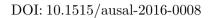
Sorghum and millet have a great essential amino acid composition. The mineral content in millet is very high in comparison with other cereals; it is especially rich in P, K, Mg, Ca, and Fe. Sorghum has the highest phenolic compounds among cereals and the most intensive antioxidant activity.

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## Impact of DDGS-supplemented diet with or without vitamin E and selenium supplementation on the fatty acid profile of beef

I. Holló<sup>1</sup> e-mail: hollo.istvan@ke.hu

DE GRUYTER OPEN

J. Csapó<sup>2,3</sup>

e-mail: csapojanos@sapientia.siculorum.ro csapo.janos@gmail.hu

## G. Holló<sup>1</sup>

e-mail: hollo.gabriella@sic.ke.hu

<sup>1</sup>Kaposvár University, Faculty of Agricultural and Enviromental Sciences, H-7400, Kaposvár, Guba Sándor St 40., Hungary <sup>2</sup>Sapientia Hungarian University of Transylvania, Faculty of Miercurea Ciuc, Department of Food Science, RO-4100 Miercurea Ciuc, Piata Libertății 1., Romania <sup>3</sup>University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Food Technology, HU-4032 Debrecen, Böszörményi út 138., Hungary

**Abstract.** The impact of supplementation of vitamin E or organic selenium in DDGS (dried distillers grains with solubles) diet on fatty acid composition in two meat cuts of finishing Holstein bulls was investigated. Twenty-four Holstein bulls were allotted to treatments in three groups of eight bulls per group for a 100-day trial. The treatments were adequate Se and vitamin E supplementation in control group (C), supranutritional vitamin E supplementation in vitamin Group E (E), supranutritional Se

Keywords and phrases: vitamin E, Selenium, DDGS, cattle, fatty acid profile

supplementation in selenium group (Se). At similar age, slaughtering Group C had higher slaughter/carcass weight and EUROP fat score than Se counterparts. The killing out percentage and proximate composition of muscles differed among treatments. Inclusion of the vitamin E or Se supplement led to expected increases (P < 0.05) in vitamin E and Se contents of the brisket and loin. Higher vitamin E concentration caused significant lower SFA and greater PUFA. Higher Se level influenced significant SFA in brisket and PUFA in both muscles. Vitamin E or Se dietary treatments in DDGS-supplemented diet resulted in beef meat cuts considerably beneficial PUFA/SFA but markedly higher n-6/n-3 PUFA ratio and even higher health index in both meat samples opposite to Group C.

## 1 Introduction

Vitamin E and Se are important components of the antioxidant defence system of living animals. After slaughter, they delay the onset of oxidation reactions in meat products. Supplementing cattle diet with vitamin E for 100 days in the last finishing period has been recognized as an effective tool for improving the appearance of beef cuts and results less lipid oxidation during retail display, while vitamin E supplementation had no significant effect on feedlot performance and carcass quality (*Kobayashi & Takasaki*, 1985, *Arnold et al.*, 1992).

Increased selenium (Se) intakes are associated with reduced risks of certain cancers in humans and an adequate intake is needed to decrease the risk of several diseases and other selenium deficiency syndromes. The Se concentration of meat is primarily determined by its geographical origin. Hungary belongs to the selenium-deficient regions in Europe; therefore, the improvement of Se supply of the population through increasing the Se content of animal products is desired. Se increments in muscle strongly depend on dietary level and the chemical form of Se (*Lee et al.*, 2006, *Cozzi et al.*, 2011). Otherwise, beef is a major natural source of dietary selenium for humans and is considered as a "highly bioavailable" compound (*Shi & Spallholz*, 1994). Due to the above mentioned beneficial effects, supplementation of animal diets with vitamin E and Se has been strongly proposed as a production strategy in cattle production.

The use of dried distiller's grains with solubles (DDGS) in cattle diets has increased in recent years. Feeding diets containing 0-30% DDGS in the finishing period either do not change (*Leupp et al.*, 2009) or change (*Mapiye et al.*, 2012) performance traits and yield of beef carcasses. Corn-based DDGS have some positive effects on meat quality compared to barley control diet (Aldai et al., 2010). Previous reports showed that DDGS appears to alter fatty acid profile and resulted a higher PUFA/SFA ratio in beef (*Depenbusch et al.*, 2008).

On the other hand, it should be considered that a higher proportion of PUFA (pro-oxidant) has negative influence on the oxidative stability of beef. This later depends on the balance between pro- and antioxidant components. The balance between the pro- and antioxidant compounds in beef can be provided by antioxidant supplementation, with the dietary inclusion of antioxidants such as vitamin E and Se. Knowing the exact effect of supplementations on carcass value is essential upon the introduction of a novel feeding system.

As a consequence of this, the main aim of the present work was to determine the influence of DDGS-supplemented diet with or without vitamin E and selenium on the fatty acid profile of beef in order to evaluate meat quality.

## 2 Materials and methods

#### 2.1 Materials

#### Animals and diet

Experiments were conducted on a private commercial cattle-fattening farm (Bull Farms Ltd., Csengele). Twenty-four Holstein young bulls were included in the experiment. Animals were allotted randomly to one of three dietary treatments, with 8 animals per treatments. All of the animals were fattened under semi-intensive conditions, ad libitum maize silage, grass hay, and moderate concentrate. The diet was supplemented with 20% DDGS (*Table 1*).

On DM basis, $\%$	Group E	Group Se	Control
Maize silage	40	40	40
Grass hay	10	10	10
Concentrate	30	30	30
DDGS	20	20	20
Premix with selenium	-	+	-
Drinking water with vitamin E	+	-	-

Table 1: Ingredients of the dietary treatments

Treatments included vitamin E supplementation (Lutavit E50, BASF) for vitamin Group E (E). Lutavit E 50 S water dispersible powder was administered directly via the drinking water. Animals in Group E received drinking water containing 2 mg of Lutavit E/day/animal.

Selenium supplementation was carried out by adding selenium-enriched yeast (Selplex-2300 Saccharomyces cerevisiae CNCM, I-3060 Alltech) for the Selenium (Se) group. We prepared a premix using ground corn grain in a way that 10 g of the premix contained 2 mg of selenium in order to ease the administration. This premix was added to the daily feed of the cattle in Group Se.

The bulls in Group C received sufficient vitamin E and Se as the requirement for growing cattle. The length of the experimental period was 100 days. The average final weight and age of bulls were  $499\pm67$  kg and  $502\pm87$  day, respectively.

#### 2.2 Methods

#### Slaughter procedure, meat sampling

The animals were slaughtered at similar live weight at the commercial abattoir according to the Hungarian Standard. The carcasses were assessed for conformation (an 18-point scale: scale 1 (poorest) to 18 (best)) and fatness (a 15 point-scale: scale 1 (leanest) to 15 (fattest)) according to the EU beef carcass classification scheme with the use of subclasses. One hour after the slaughter, the dressed carcasses were weighed (hot carcass weight). After 24 hours, chilling samples were taken from the right half carcass from two commercial meat cuts (brisket, loin) from muscles (*superficial pectoral* (SP), and *longissimus dorsi* (LD) muscles) to determine the vitamin E and selenium content as well as the fatty acid profile of beef. The *superficial pectoral* muscle was trimmed from boneless brisket. Longissimus dorsi muscle was separated from thoracic vertebrae between the  $12^{\text{th}}$  and  $13^{\text{th}}$  ribs.

# Chemical analysis, determination of vitamin E and selenium content, and the fatty acid profile of muscles

Laboratory examinations were carried out in the Analytical Laboratory of Kaposvár University, Faculty of Agricultural and Environmental Sciences. The chemical composition, selenium content, and fatty acid profile were determined as previously described (*Holló et al.*, 2008). The Se-content determination is based on the method written in the Hungarian Food Codex (HFC, 1990). Total vitamin E concentration in muscles was measured according to the method by *Csapó & Csapó-Kiss* (2003).

Proportions of fatty acids were expressed as a percentage of total fatty acid methyl esters. Besides individual fatty acids, 7 groups of fatty acids were calculated. Health Index (HI) was calculated as previously described by *Zhang* 

et al. (2008). HI = (Total MUFA + Total PUFA) /  $(4 \times C14: 0 + C16: 0)$ . Statistical analysis

For the statistical evaluation, the IBM SPSS 20.0 software (2011) was used. In addition to basic statistical results (mean, SD), the effect of diet was evaluated with multivariate analysis of variance, general linear model (GLM) III. The differences between the groups were evaluated with LSD test – the level of significance was set at P < 0.05. In Table 3, significance level at P < 0.1 was shown, too.

## 3 Results and discussion

#### Carcass characteristics

At similar slaughtering age, Group C had the highest slaughter weight regarding hot carcass weight (*Table 2*).

Table 2: Effect of dietary supplement on the carcass value of beef from bulls fed DDGS diets

Compo	- Slaughter	Hot carcass	Killing out,	EUROP meat	EUROP fat
nents	weight, kg	weight, kg	%	score, point	score, point
Е	$496.13 \pm 44.16^{ab}$	$256.95 \pm 30.54^{\mathrm{ab}}$	$51.70 \pm 2.11^{\rm a}$	$6.75 {\pm} 1.98$	$3.38 \pm 0.74^{\rm ab}$
$\mathbf{Se}$	$464.00 \pm 56.27^{\rm a}$	$242.68 \pm 34.64^{\rm a}$	$52.22 \pm 2.31^{\rm a}$	$7.13 \pm 1.36$	$3.00\pm0.00^{\rm a}$
С	$536.13 \pm 81.07^{b}$	$294.78 \pm 48.87^{\rm b}$	$54.88 \pm 2.60^{b}$	$8.13 \pm 1.81$	$4.38 \pm 1.41^{\rm b}$
Mean	$498.75 {\pm} 66.83$	$264.80{\pm}43.36$	$52.94{\pm}2.66$	$7.33{\pm}1.76$	$3.58{\pm}1.06$

<sup>a,b</sup> means significant differences among groups

Group Se showed significantly lower slaughter and carcass weight than the other two groups. Carcass dressing percentage was 53% and the majority of carcasses were classified into conformation class R – and to fat classes 2 – (Group C) and 1+ (Group E and Se). Dietary vitamin E did not affect hot carcass weight and EUROP grading, but dressing percentage was affected, in line with previous findings (*Nassu et al.*, 2011). Similarly to Group E, the dietary inclusion of Se resulted lower killing out percentage in Group Se, and, what is more – mainly due to lower carcass weight –, a significantly lower EUROP fat grade than in Group C. In literature, it is generally reported that Se and vitamin E contents of diets did not affect carcass characteristics, but the effects of supplementation depend on its level and type of source, previous nutritional history, and handling of animals (*Cozzi et al.*, 2011, *Nassu et al.*, 2011).

#### Proximate composition

The protein, fat, and ash content on dry matter basis showed significant differences in both muscles between control and supplemented groups. The intramuscular fat contents of loin in supplemented groups are considerably lower than the content of 2 to 2.5% reported to be optimal for beef-eating quality. Intramuscular fat content in longissimus muscle of Charolais young bulls showed a similar trend (*Cozzi et al.*, 2011); however, there were no significant differences among Se treatments. The dietary treatment effect on protein content of muscles was significant; the highest protein level was measured in Group E. A significant difference was observed for ash content between muscles; in brisket, it was measured a higher level than in loin. Higher ash contents were recorded from Se- or vitamin-E-supplemented groups in both muscles.

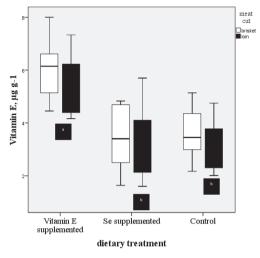
#### Muscle vitamin E and Se content

The vitamin E supplementation resulted in higher vitamin E concentrations in meat cuts (*Figure 1*). In agreement with the previous findings, vitamin E concentration in Group E was more than 1.5-fold greater than that measured in Group C (brisket: 6.1 vs. 3.6 µg g<sup>-1</sup>; loin: 5.2 vs. 3.1 µg g<sup>-1</sup>). It was found that vitamin E level in muscle is significantly higher in beef fed WDGS diet supplemented daily with vitamin E than that of the non-supplemented group (*Driskell et al.*, 2011). In line with the results of *O'Grady et al.* (2001), muscle vitamin E concentrations were not significantly affected by dietary Se supplementation (brisket: 3.5 vs. 3.6 µg g<sup>-1</sup>; loin: 3.3 vs. 3.1 µg g<sup>-1</sup>). The oxidative stability can be influenced effectively if vitamin E content in beef reached 3–3.5 µg g<sup>-1</sup> (*Arnold et al.*, 1993). Both muscles in Group E contained a vitamin E level above the mentioned threshold value.

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Table 3: Effect of	fed DDGS diets
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Compt	- Dry mat	ry matter, $\%$	Proteir	Protein, DM%	Fat, ]	Fat, DM%	$\operatorname{Ash}$	Ash, $DM\%$
nents	brisket	loin	brisket	loin	brisket	loin	brisket	loin
E	$24.76\pm 2.09^{ab}$	$24.40\pm 1.36$	$24.40\pm1.36$ $88.68\pm6.27^{a}$	$92.54{\pm}1.92^{a}$	$6.54{\pm}4.00^{ m b}$	$3.74{\pm}2.02^{ m b}$	$4.19 \pm 0.43^{a}$	$4.19\pm0.43^{a}$ $3.72\pm0.34^{a}$
$\mathbf{Se}$	$24.03{\pm}0.89^{ m b}$	$23.91{\pm}1.44$	$87.72\pm3.9^{\mathrm{ab}}$	$92.13{\pm}1.92^{a}$	$7.20{\pm}3.96^{ m b}$	$3.99{\pm}1.91^{ m b}$	$4.27 \pm 0.20^{a}$	$3.87{\pm}0.33^{a}$
U	$26.67{\pm}2.63^{a}$	$23.58{\pm}2.72$	$81.29 \pm 9.27^{ m b}$	$88.02 \pm 3.94^{ m b}$	$14.93 \pm 9.76^{a}$	$8.67 \pm 4.11^{a}$	$3.60{\pm}0.67^{ m b}$	$3.31{\pm}0.31^{ m b}$
Mean	$25.15{\pm}2.23$	$23.97{\pm}1.89$	$85.90{\pm}7.35^*$	$90.90 \pm 3.37^{**}$	$9.56{\pm}5.91^{*}$	$5.47{\pm}3.58^{**}$	$4.02{\pm}0.54^{*}$	$3.63{\pm}0.40^{**}$

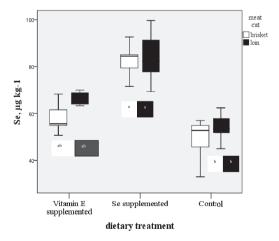
 $^{\rm a,D}$  means significant differences among groups \*,\*\* means significant differences between muscles P < 0.05



 $^{\rm a,b}$  means significant differences among groups  ${\rm P} < 0.05$ 

Figure 1: Effect of dietary supplement on Vitamin E content of beef from bulls fed DDGS diet

As expected, Group Se had significantly higher concentrations of Se in muscles (Se: 83.42  $\mu$ g kg<sup>-1</sup>) than those not receiving dietary supplementation (E: 61.72  $\mu$ g kg<sup>-1</sup> and C: 51.73  $\mu$  kg<sup>-1</sup>) (*Figure 2*).



 $^{\rm a,b}$  means significant differences among groups  $\rm P < 0.05$ 

Figure 2: Effect of dietary supplement on Se content of beef from bulls fed DDGS diet

There were significantly higher concentrations of Se in muscles of Group Se  $(82.58\pm7.79 \ \mu \ kg^{-1} \ and \ 83.94\pm9.81 \ \mu \ kg^{-1})$  compared to Group C  $(49.14\pm8.49 \ \mu \ kg^{-1} \ 54.32\pm5.95 \ \mu \ kg^{-1})$ , whereas intermediate Se concentration (58.20\pm6.84 \ \mu \ kg^{-1}, \ 65.82+2.83 \ \mu \ kg^{-1}) was found in muscles of Group E. Higher Se concentration was observed in loin (longissimus muscle:  $68.89\pm14.63 \ \mu \ kg^{-1}$ ) than in brisket (pectoralis muscle:  $61.23\pm15.50 \ \mu \ kg^{-1}$ ), although the difference was not significant.

#### Fatty acid profile of muscles

The results of total fatty acid composition are presented in Table 4. Treatment differences in fatty acid composition were mainly for supplemented versus non-supplemented groups in both muscles. In vitamin-E- and Se-supplemented groups, decreased proportions of SFA and higher levels of PUFA can be observed. Briskets from Group C tended to have the highest SFA content, too, due to higher intramuscular fat content. These differences can be largely attributed to increased levels of myristic and palmitic acids as well as to a higher percentage of C 12:0, C 15:0, and C 17:0 in the brisket of control group. From human nutritional point of view, the lauric (C12:0), myristic (C14:0), and palmitic (C16:0) acids deserve attention. These are the primary fatty acids associated with increasing plasma low-density lipoprotein and cholesterol concentrations in humans. Palmitic acid (C 16:0) is the main end-product of de novo fatty-acid synthesis; this can be elongated to stearic (C 18:0) acid. The C 18:0 is the main end-product of linoleic (C 18:2 n-6) and linolenic acid (C 18:3 n-3) biohydrogenation process, too. In our study, a lower (P < 0.1) C 18:0 was measured in the brisket of control bulls than in Group Se. In line with stearic acid content, the long-chain saturated fatty acids (C 20:0, C 21:0, C 22:0) were found in a significantly higher proportion in both muscles of Se group compared to control group. It seems that biohydrogenation in Group Se was higher than in the other two groups. The average SFA content of loin in Group C significantly differed from the SFA detected in vitamin Group E. The same individual saturated fatty acid differences were detected between dietary treatments for loin as well as for brisket, except for C 17:0 and C 18:0. The MUFA content of muscles varied between 39 and 43%; significant differences were detected for C 14:1, C16:1, and C 20:1 fatty acids. In this study, C 18:1 trans isomers were found in a significantly higher proportion in supplemented groups than that of the muscles in control group. Contrary to trans isomers, in the loin of control group, a higher level of C 18:1 *cis* fatty acid was observed than in Group Se. Cis-9, trans-11 CLA (conjugated linoleic acid) are considered highly beneficial to human health.

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	Drisket	10111	Drisket	IOIII	DFISKet	IOIII	Drisket	IOII
C10:0	$0.03 \pm 0.0$	$0.02 \pm 0.0$	$0.02 \pm 0.0$	$0.02 \pm 0.0$	$0.03 \pm 0.0$	$0.03 \pm 0.0$	$0.02 \pm 0.0$	$0.02 \pm 0.0$
C12:0	$0.04\pm0.0^{\rm b}$	$0.03\pm0.0^{b}$	$0.03\pm0.0^{\rm b}$	$0.03\pm0.0^{\rm b}$	$0.05\pm0.0^{a}$	$0.05\pm0.0^{a}$	$0.04\pm0.0$	$0.04\pm0.0$
C13:0	$0.01 \pm 0.0$	$0.01 \pm 0.0$	$0.01 \pm 0.0$	$0.01 \pm 0.0$	$0.02 \pm 0.0$	$0.01 \pm 0.0$	$0.01 \pm 0.0$	$0.01 \pm 0.0$
C14:0	$1.98{\pm}0.6^{ m b}$	$1.61 \pm 0.5^{\rm b}$	$1.65\pm0.5^{ m b}$	$1.58{\pm}0.2^{ m b}$	$3.01 \pm 0.5^{a}$	$2.65 {\pm} 0.6^{ m a}$	$2.21 {\pm} 0.8$	$1.90 \pm 0.7$
C14:1	$0.28{\pm}0.2^{ m b}$	$0.24{\pm}0.1^{ m ab}$	$0.22{\pm}0.1^{ m b}$	$0.19{\pm}0.1^{ m b}$	$0.56 \pm 0.2^{a}$	$0.40{\pm}0.2^{a}$	$0.35 \pm 0.2$	$0.27 {\pm} 0.2$
C15:0	$0.36\pm0.1^{ m b}$	$0.41\pm0.1^{ m b}$	$0.43\pm0.1^{ m b}$	$0.51 \pm 0.1^{ab}$	$0.57\pm0.1^{a}$	$0.58{\pm}0.1^{\rm a}$	$0.45 \pm 0.1$	$0.49 \pm 0.1$
C16:0	$24.14 \pm 4.0^{b}$	$22.02 \pm 3.1^{ m b}$	$22.22\pm2.3^{ m b}$	$21.91{\pm}1.2^{ m b}$	$28.50 \pm 1.8^{a}$	$27.18\pm 2.5^{a}$	$24.95 \pm 3.8$	$23.46 \pm 3.3$
C16:1	$2.39\pm0.5^{\mathrm{ab}}$	$2.32\pm0.5^{\mathrm{ab}}$	$1.99{\pm}0.4^{ m b}$	$1.84{\pm}0.4^{ m b}$	$2.88 \pm 0.7^{a}$	$2.39\pm0.4^{a}$	$2.42 {\pm} 0.6$	$2.18 {\pm} 0.5$
C17:0	$1.08\pm0.1^{ m b}$	$1.17 \pm 0.2$	$1.23\pm0.2^{\mathrm{ab}}$	$1.32 \pm 0.2$	$1.26 \pm 0.2^{a}$	$1.29 \pm 0.2$	$1.19 \pm 0.2$	$1.25 \pm 0.2$
C17:1	$0.57 \pm 0.1$	$0.68 \pm 0.1$	$0.59 \pm 0.1$	$0.60 \pm 0.1$	$0.59 \pm 0.1$	$0.59 \pm 0.1$	$0.59 \pm 0.1$	$0.63 \pm 0.1$
C18:0	$20.49\pm0.9^{AB}$	$20.07 \pm 1.0$	$22.35\pm3.3^{A}$	$23.75 \pm 4.0$	$19.43 \pm 4.0^{B}$	$19.95 \pm 3.3$	$20.76 \pm 3.1$	$21.26 \pm 3.3$
C18:1 t-1	$2.32\pm0.5^{\mathrm{ab}}$	$2.94{\pm}1.1$	$2.68\pm0.9^{a}$	$3.04{\pm}0.9$	$1.81\pm0.3^{ m b}$	$1.89 \pm 0.3$	$2.27 {\pm} 0.7$	$2.67{\pm}1.0$
C18:1 t-2	$1.67{\pm}0.2^{\mathrm{a}}$	$1.79 \pm 0.2^{a}$	$1.55\pm0.2^{\mathrm{ab}}$	$1.55{\pm}0.2^{ m b}$	$1.41{\pm}0.1^{ m b}$	$1.49 \pm 0.1^{ m b}$	$1.54 {\pm} 0.2$	$1.62 \pm 0.2$
C18:1n-9c	$33.30 \pm 2.8$	$33.66 \pm 2.7^{ m ab}$	$34.16 \pm 2.2$	$31.60{\pm}2.0^{ m b}$	$35.70 \pm 2.8$	$35.67 \pm 3.3^{a}$	$34.39\pm 2.7$	$33.55 \pm 3.0$
C18:2n-6	$5.88{\pm}2.8^{ m a}$	$7.14{\pm}2.3^{a}$	$5.90{\pm}1.7^{\mathrm{a}}$	$6.81{\pm}1.6^{a}$	$2.45\pm0.5^{ m b}$	$3.39{\pm}0.8^{ m b}$	$4.75 \pm 2.5^{*}$	$5.95\pm 2.4^{**}$
C20:0	$0.21\pm0.1^{ m b}$	$0.20{\pm}0.0^{ m ab}$	$0.29 \pm 0.1^{a}$	$0.27\pm0.1^{a}$	$0.18\pm0.1^{b}$	$0.18 \pm 0.1^{ m b}$	$0.23 \pm 0.1$	$0.22 \pm 0.1$
C18:3n-6	$0.03 \pm 0.0$	$0.04 \pm 0.0$	$0.03 \pm 0.0$	$0.04{\pm}0.0$	$0.03 \pm 0.0$	$0.03 \pm 0.0$	$0.03 \pm 0.0^{*}$	$0.04\pm0.0^{**}$
C20:1	$0.22 \pm 0.2^{a}$	$0.27\pm0.2^{a}$	$0.19 \pm 0.1^{a}$	$0.21 \pm 0.1^{a}$	$0.03\pm0.0^{b}$	$0.07\pm0.1^{\rm b}$	$0.15 \pm 0.2$	$0.19 \pm 0.1$
C18:3n-3	$0.39\pm0.1^{B}$	$0.44{\pm}0.1^{ m b}$	$0.46\pm0.3^{AB}$	$0.46\pm0.1^{ m b}$	$0.56{\pm}0.1^{ m A}$	$0.70 \pm 0.1^{a}$	$0.47 {\pm} 0.2$	$0.52 \pm 0.2$
c-9,t-11 KLS	$0.29 {\pm} 0.1$	$0.34{\pm}0.1^{\rm C}$	$0.40 \pm 0.1$	$0.41{\pm}0.1^{ m A}$	$0.35 \pm 0.1$	$0.35\pm0.1^{B}$	$0.35 \pm 0.1$	$0.36 {\pm} 0.1$
C21:0	$0.03\pm0.0^{ab}$	$0.03 \pm 0.0^{ab}$	$0.04{\pm}0.0^{a}$	$0.04\pm0.0^{a}$	$0.02\pm0.0^{b}$	$0.02\pm0.0^{\rm b}$	$0.03 \pm 0.0$	$0.03 \pm 0.0$
C22:0	$0.05\pm0.0^{ab}$	$0.04 \pm 0.0^{ab}$	$0.06\pm0.0^{a}$	$0.05\pm0.0^{a}$	$0.03\pm0.0^{\rm b}$	$0.03\pm0.0^{\rm b}$	$0.05 \pm 0.0$	$0.04 \pm 0.0$
C20:4n-6	$2.81\pm 2.5^{a}$	$3.04{\pm}1.5^{a}$	$2.18{\pm}1.2^{ m a}$	$2.50{\pm}1.2^{a}$	$0.21 \pm 0.3^{b}$	$0.56 \pm 0.7^{\rm b}$	$1.74 {\pm} 1.9$	$2.15{\pm}1.6$
C23:0	$0.05\pm0.0^{ab}$	$0.06\pm0.0^{a}$	$0.06\pm0.0^{a}$	$0.04{\pm}0.0^{ m ab}$	$0.02 \pm 0.0^{b}$	$0.02\pm0.0^{ m b}$	$0.04 \pm 0.0$	$0.04 \pm 0.0$
C22:5n-3	$0.60\pm0.4^{a}$	$0.61 \pm 0.3^{a}$	$0.53 \pm 0.3^{a}$	$0.50\pm0.2^{ab}$	$0.09\pm0.1^{ m b}$	$0.20{\pm}0.1^{\rm b}$	$0.41 {\pm} 0.4$	$0.46 \pm 0.3$
C22:6n-3	$0.04{\pm}0.0^{a}$	$0.05\pm0.0^{a}$	$0.03 \pm 0.0^{ab}$	$0.04{\pm}0.0^{ m ab}$	$0.01 \pm 0.0^{b}$	$0.02\pm0.0^{ m b}$	$0.03 \pm 0.0$	$0.03 \pm 0.0$
SFA	$48.36 \pm 4.6^{\rm b}$	$45.66 \pm 3.5^{ m b}$	$48.28 \pm 4.5^{ m b}$	$49.54 \pm 4.7^{\mathrm{ab}}$	$53.00 \pm 3.1^{a}$	$51.98\pm 2.8^{a}$	$49.88 \pm 4.5$	$48.76 \pm 4.5$
MUFA	$40.67 \pm 3.2$	$41.77 \pm 2.0$	$41.34 \pm 3.0$	$38.94 \pm 2.6$	$43.07 \pm 3.4$	$42.54 \pm 3.4$	$41.70 \pm 3.2$	$41.05 \pm 3.0$
PUFA	$10.86 \pm 6.4^{a}$	$12.57{\pm}4.4^{\mathrm{a}}$	$10.27 \pm 3.4^{a}$	$11.52 \pm 3.4^{a}$	$3.83{\pm}0.8^{ m b}$	$5.49{\pm}1.8^{ m b}$	$8.32 \pm 5.2$	$10.20 \pm 4.5$
n-6	$9.31 \pm 5.8^{a}$	$10.85 \pm 4.0^{a}$	$8.61 \pm 3.1^{a}$	$9.88{\pm}2.9^{ m a}$	$2.77 \pm 0.8^{b}$	$4.13{\pm}1.6^{ m b}$	$6.90 \pm 4.7$	$8.61 \pm 4.2$
n-3	$1.25{\pm}0.7^{ m A}$	$1.36 {\pm} 0.5$	$1.22\pm0.7^{ m AB}$	$1.20 {\pm} 0.4$	$0.69 \pm 0.1^{B}$	$0.99 \pm 0.3$	$1.05 \pm 0.6$	$1.20 \pm 0.4$
PUFA/SFA	$0.24{\pm}0.2^{a}$	$0.28 \pm 0.1^{a}$	$0.22 \pm 0.1^{a}$	$0.24{\pm}0.1^{ m a}$	$0.07\pm0.0^{\rm b}$	$0.11\pm0.0^{\rm b}$	$0.18 \pm 0.1$	$0.22 \pm 0.1$
n-6/n-3	$7.52{\pm}2.0^{a}$	$8.08{\pm}2.2^{a}$	$7.97{\pm}2.6^{a}$	$8.44{\pm}1.2^{a}$	$4.03\pm0.9^{b}$	$4.11{\pm}0.6^{ m b}$	$6.51 {\pm} 2.6$	$7.06 \pm 2.4$
	$1.70{\pm}0.6^{a}$	$1.98\pm0.5^{a}$	$1.84{\pm}0.4^{ m a}$	$1.80{\pm}0.3^{a}$	$1.16 \pm 0.1^{b}$	$1.29\pm0.2^{\rm b}$	$1.57 \pm 0.5$	$1.72 {\pm} 0.4$

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This fatty acid differed (P < 0.1) among treatments in the case of loin; the highest was in the Se-group, followed by Group C, and the lowest was in Group E. Based on our data, Se supplementation might improve the proportion of CLA level in loin.

The linoleic acid proportion was more than 2-fold higher in supplemented groups. Significant muscle effect on fatty acid composition was detected only for C 18:2 *n*-6 and C 18:3 *n*-6 fatty acids. The linolenic acid (C 18:3 *n*-3) content was higher in the brisket and the loin of Group C compared to the same values of Group E and the value of loin detected in Group Se. The long-chain fatty acids belonging to *n*-3 and *n*-6 fatty acid families were generally higher in muscles of Group E and Se than in control. However, in loin, no significant differences were observed between control and Se group for C 22:5 *n*-3 and C 22:6 *n*-3 and in brisket for C 22:5 *n*-3. The lack of difference might occur due to biohydrogenation of docosahexaenoic acid (C 22:6 *n*-3) converting into behenic acid (C 22:0) in Group Se. At the same time, it seems that including high levels of vitamin E in the diet resulted in higher levels of *n*-3 fatty acids, somehow modifying the long-chain fatty acid synthesis. Besides this, higher levels (P < 0.05) of *n*-6 fatty acids were found in vitamin-E- and Se-supplemented groups than in the control group in both muscles.

A previous report (*Depenbusch et al.*, 2008) showed that feeding DDGS appears to alter fatty acid profiles in beef. In our study, the PUFA/SFA ratio was considerably higher in group E and Group Se than that of control group, however, less than the lowest limit recommended for improving human health (0.45). It can be difficult to recommend a ratio when individual fatty acids within groups/families can have decisively different biological effects, namely the n-6/n-3 ratio did not change favourably in the supplemented group due to greater content of n-6 fatty acids. From this point of view, the control group showed a desirable value.

Health index (HI) is a ratio which was calculated directly from the sum of MUFA and PUFA in numerator and C14:0 and C16:0 in denominator; consequently, this greater HI value is beneficial. According to the data, Group E and Group Se had markedly higher health-promoting effects than Group C.

## 4 Conclusions

Adding a supplement containing vitamin E or Se during the finishing period of Holstein bulls successfully produced greater contents of these desirable components in beef. Vitamin-E or Se-enriched beef made some changes in the fatty acid composition of beef. It is concluded that PUFA/SFA ratio and Health Index generally enhanced supplementing the diet with vitamin E or selenium in DDGS diet, but higher PUFA/SFA ratio resulted in a less desirable n-6 to n-3 ratio in beef.

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## Antioxidant activity as indicator of UV radiation and other abiotic stress factors on *Agaricus bisporus* (Lange/Imbach) and *Sedum hybridum* (L.)

A. Szabó

DE GRUYTER OPEN

A. Geösel

e-mail: anna.szabo@uni-corvinus.hu Corvinus University of Budapest, Faculty of Food Science, Department of Vegetable and Mushroom Growing, H-1118 Budapest, Villányi St 29–43., Hungary

## Z. Kókai

e-mail: zoltan.kokai@uni-corvinus.hu Corvinus University of Budapest, Faculty of Food Science, Department of Postharvest Science and Sensory Evaluation, H-1118 Budapest, Villányi St 29–43., Hungary

Cs. Orbán e-mail: orban.csaba@se-etk.hu K. Töreki e-mail: toreki.kristof@gmail.com

Semmelweis University, Faculty of Health Sciences, Department of Dietetics and Nutriton Sciences, H-1088 Budapest, Vas St 17., Hungary

## A. Szőke

e-mail: szoke.andrea83@gmail.com

Corvinus University of Budapest, Faculty of Horticultural Sciences, Department of Floriculture and Dendrology, H-1118 Budapest, Villányi St 29–43., Hungary

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**Abstract.** Investigation of stress level might be facilitated also in plant and horticultural sciences, but currently mainly morphological parameters are in use. Antioxidant activity routinely measured in food-oriented researches and several studies indirectly indicated that stress factors can influence this parameter. Our aim was to assess the potential direct indicator role of antioxidant activity in stress conditions. We measured the effects of UVB and soil-delivered stress on *Agaricus bisporus* and *Sedum hybridum*. Our results indicate that UVB slightly decreases, while the inadequate soil conditions increase antioxidant activity; hence these measurements are suitable for determining the level of stress in different living samples.

## 1 Introduction

Antioxidant activity is a parameter that describes the capacity of a sample to neutralize the free radicals. It has got a crucial role in human health, as many diseases (e.g. malignant tumours, non-alcoholic fat liver, autoimmune diseases etc.) (*Felton et al.*, 1994; *Geösel et al.*, 2011; *Ozzard et al.*, 2008) can be developed by the damages in DNA and the structural and functional proteins evoked by these reactive substances (*Bagchi et al.*, 2000). Because of this, many studies investigated how efficient the different foods are (e.g. fruits and vegetables) in delivering protective antioxidants to the human body.

In plants, the role of the antioxidant type of characteristic substances is the same as in the human body: protect the structure and function of plant cell organelles from free-radical-caused damages, and thus maintain homeostasis.

In mammalian, plant, and fungal cells, the antioxidant capacity mainly comes from two systems: the first one is the non-enzymatic, which utilizes organic substances (e.g. ascorbic acid, lycopene, a-tocopherol, glutathione) to quench the Reactive Oxygen Species (ROS), while the enzymatic antioxidant system contains enzyme proteins, such as Glutathion-S-transferase, Ascorbateperoxidase, to facilitate electron or hydrogen atom transfer (*Edwards et al.*, 2000; *Venisse*, 2003).

Most of the non-enzymatic participants are represented in a relatively small amount in the different samples. Increasing of their levels can be influenced by many factors, e.g. the concentration of lycopene levels depends on temperature (*Helyes et al.*, 2007). Polyphenols are expanded by the photo-oxidative UVstress in many fruits, e.g. *Sambucus sp.*, *Fragaria sp.* (*Asami et al.*, 2003; *Murugesan et al.*, 2012). The joint feature of these induction factors is that they both mean stress to the plants. Many horticultural products have been tested for this phenomenon with different methods (*Csambalik et al.*, 2014; *Geösel et al.*, 2011; *Sipos et al.*, 2013). Most of the studies investigate the effect of environmental factors on the health-promoting molecule contents. Some studies suggested that stress factors indicate the elevated synthesis of carotenoids (*Kim et al.*, 2012). Others show that polyphenol content increases during UV-stress to protect the plant tissues from damages (*Ozzard et al.*, 2008). Similar results were obtained from measurements performed on fruits exposed to insect invasions (*Felton et al.*, 1994; *Hemingway & Laks*, 1992). Other abiotic stress factors, such as water shortage or chilling injury, also alter the bioactive compound levels (*Esteban et al.*, 2001). The availability of mineral elements determine the redox status as well (*Tewari et al.*, 2006). Competition with weed also means relevant stress to the plants.

The antioxidant-producing ability of the different species describes the capacity of the plants to adapt to these stress factors and hence contribute to the viability characteristics. As measurements of antioxidant activity can be performed by rapid and economical methods, another paradigm also arises (*Huang et al.*, 2005). It is hard to deceive the effect of stress factors to the investigated species. Most of the agricultural and food engineers assess the optimal condition by the quality parameters e.g. size, yield, growing rate and dynamics, ornamental value (*VanWoert et al.*, 2005). These are indirect parameters.

As antioxidant capacity measures the total ROS elimination capacity of the processed tissue, and these radicals formed during the exposition to the stress factors, it is possible that the antioxidant capacity indicates the intensity of stress itself.

Because of the aforementioned points, we came up with a novel approach to determine the connection of antioxidant activity with the different stress factors in mushrooms, as a relevant part of human nutrition, and in *Sedum-s* as an ornamentally interesting species.

UVB-treated white button mushrooms were chosen for the purpose of this study since nowadays they have become one of the novelty mushroom products with their increased vitamin D content, representing a natural source of this vitamin of high importance for the human nutrition (*Ozzard et al.*, 2008). The other object is *Sedum hybridum*, a commonly applied species on green roofs, especially extensive green roofs for its exceptional tolerance for climatic conditions and high decorative value (*Szőke et al.*, 2012).

## 2 Materials and methods

#### 2.1 Investigated samples

Ultraviolet-B (UVB)-treated Agaricus bisporus samples were provided by the Department of Vegetable and Mushroom Growing. The biologically active, still developing fruitbodies were treated by a 15W output 290–315 nm wavelength VL-115 M lamp. 0, 5, 15, 20, 25, and 30 minutes of UVB radiation were applied on three consecutive days (a total of: 0, 15, 30, 45, 60, 75, 90 minutes). Samples were taken on 15<sup>th</sup> January 2015.

Sedum hybridum plants were propagated vegetatively. The samples were grown on a 3-year extensive green roof in four different substrate mixtures (S1: soil-zeolite-sand-based substrate, S2: riolite-based substrate, S3: brick-fracture- and ytong-based substrate and S4: soil) and in a 10-cm layer, respectively, as potential sources of substrate-associated stress factors. The control plants were grown on the ground in the original soil of the plot under the same ambient conditions. Samples were collected at the same phonological phase on the  $10^{\text{th}}$  of April 2015.

#### 2.2 Sample preparation

Samples were homogenized with distilled water in a 1:1 ratio at 24000 min<sup>-1</sup> RPM by a teflon homogenizer. This homogenate was kept in ultrasonic water bath for 15 min, and then spanned at 2000 g for 15 min. The supernatant was used for all of the measurements.

### 2.3 Antioxidant activity measurements

Ferric reduction antioxidant power (FRAP) was determined according to *Benzie & Strain* (1996). 100 µl of sample was added to pH = 3.6, 300 mM acetate buffer – 10 mmol/litre TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl – 20 mmol/litre FeCl<sub>3</sub> · 6H<sub>2</sub>O, and then, after 5 minutes, absorbance was read at  $\lambda = 593$  nm. Results were generated in ascorbic acid equivalence (*Benzie & Strain*, 1996).

DPPH (2,2-diphenyl-1-picrylhydrazyl) elimination assay was performed as described by *Brand-Williams et al.* (1995). 100 µl of sample was added to 3.9 ml of  $6 \cdot 10^{-5}$  M methanolic DPPH solution, and then incubated for 20 minutes in dark. Absorbance readings were performed at  $\lambda = 517$  nm, and inhibition % was calculated (*Brand-Williams et al.*, 1995).

CUPRAC assay was measured by the method of Apak et al. (2008). 1 ml

 $10^{-2}$  M CuCl<sub>2</sub>, 1 ml  $7.5 \cdot 10^{-3}$  M neocuproine solution, 1 ml pH = 7.4, 1 M NH<sub>4</sub>Ac buffer, 100 µl sample, and 1 ml distilled water was mixed and incubated for 30 minutes in dark at room temperature. After the absorbance reading at  $\lambda = 450$  nm, values were calculated to trolox equivalents (*Apak et al.*, 2008).

ABTS-assay was measured as described by *Huang et al.* (2005). Reaction mixture contained 10 µl of sample; 20 µl of 3.50 mg/ml myoglobin in 50 mM, pH = 7.4, 9% NaCl and 1% glucose containing potassium-phosphate buffer; 150 µl of 1 mg ABTS and 25 µl 3% H<sub>2</sub>O<sub>2</sub> in 0.1 M pH5 citrate buffer. This mixture was shaken for 5 minutes at 37 °C, then alkaline stop solution was added and measured at  $\lambda = 405$  nm against trolox calibration curve (*Huang et al.*, 2005).

Total Phenolic Compound (TPC) was recorded as described by *Singleton* and *Rossi* (1965). 1250 µl of 10-fold diluted Folin-Cioalteau reagent, 240 µl methanol, 10 µl of sample, and 1 ml of 0.7 M NaCO<sub>3</sub> was mixed, and then kept at 50 °C for 5 minutes, then measured at  $\lambda = 765$  nm to calibration curve set-up with gallic acid (*Singleton & Rossi*, 1965). All of the measurements were carried out in five replicates.

#### 2.4 Statistical analysis

Raw data were processed during statistical analysis. Since the resulting dataset is considered to be low from statistical point of view (n < 13), the conditions for parametric tests were not met. That is why the Kruskal-Wallis nonparametric test (with the exact p-value calculation) was chosen (equivalent to ANOVA parametric test), which is not sensitive either to the normality of our data distribution or to the heterogeneity of its deviation (*Conover*, 1980).

Multiple pairwise comparisons using Dunn's procedure – as a suitable method for detecting significant differences with Bonferroni correction – was applied. The analyses were implemented by the XLStat-Sensory solution software, version 2013.1.01 (Addinsoft, 28 West 27<sup>th</sup> Street, Suite 503, New York, NY 10001, USA).

To visualize and compare data in graphs, a 0 to 100 scaling method was applied to normalize the data.

### 3 Results and discussions

Our results (Table 1) indicate that in the case of UVB exposition the antioxidant activity does not alter. None of the applied methods show either increasing or decreasing patterns via photo-oxidative stress.

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Species	Stressor	DPPH	CUPRAC	ABTS	FRAP	TPC
		$mean\pm SD$				
	UVB-0	$61.76\pm 5.34^{a}$	$100.00\pm0.80^{\rm b}$	$100.00\pm 5.30^{c}$	$99.53{\pm}2.86^{ m b}$	$100.00\pm 1.54^{\rm b}$
	UVB-15	$96.48{\pm}2.61^{ m b}$	$81.66{\pm}1.76^{ m b}$	$48.09\pm0.38^{\mathrm{ab}}$	$68.56 \pm 4.16^{ m ab}$	$85.88{\pm}1.34^{\rm ab}$
A	UVB-30	$79.50{\pm}0.00^{ m ab}$	$66.50{\pm}0.69^{ m ab}$	$47.96{\pm}0.29^{\mathrm{ab}}$	$66.06\pm0.00^{ m ab}$	$74.92{\pm}0.00^{a}$
Agaricus	UVB-45	$97.98{\pm}2.33^{ m b}$	$77.45{\pm}1.59^{ m ab}$	$47.99{\pm}0.67^{a}$	$75.13\pm0.25^{\rm ab}$	$81.47\pm1.56^{\mathrm{ab}}$
surodsia	UVB-60	$82.19{\pm}1.38^{\rm ab}$	$50.19{\pm}0.00^{\mathrm{ab}}$	$53.85{\pm}0.03^{ m abc}$	$100.00 \pm 4.79^{\rm b}$	$84.81{\pm}4.58^{\mathrm{ab}}$
	UVB-75	$98.67{\pm}2.70^{ m b}$	$65.55 \pm 1.11^{ m ab}$	$54.17\pm0.17^{ m abc}$	$95.36{\pm}0.11^{ m b}$	$95.66{\pm}5.87^{ m b}$
	UVB-90	$100.00\pm 6.86^{ m b}$	$50.94{\pm}0.61^{a}$	$54.59{\pm}0.00^{ m bc}$	$18.90{\pm}3.29^{a}$	$71.09{\pm}2.46^{a}$
	Range	38.24	49.81	52.04	81.10	28.91
	Control	$9.29{\pm}0.00^{a}$	$69.65{\pm}0.26^{ m ab}$	$100.00\pm0.00^{\rm ab}$	$61.20{\pm}4.72^{\mathrm{ab}}$	$29.34\pm0.88^{\mathrm{ab}}$
	$\mathbf{S1}$	$85.76{\pm}5.90^{\mathrm{ab}}$	$69.70{\pm}1.51^{ m ab}$	$55.15{\pm}13.86a$	$61.48\pm0.00^{a}$	$27.65{\pm}1.33^{ m a}$
Sedium	S2	$85.91 \pm 0.43^{\rm ab}$	$68.61{\pm}0.00^{a}$	$97.53{\pm}11.69^{ m b}$	$65.62{\pm}0.88^{\mathrm{ab}}$	$28.45\pm0.86^{a}$
hybridum	1 S3	$79.50{\pm}0.78^{ m a}$	$100.00{\pm}0.13^{ m b}$	$52.28{\pm}23.39^{ m a}$	$100.00\pm 3.75^{\rm b}$	$100.00{\pm}22.27^{ m b}$
	$\mathbf{S4}$	$100.00 \pm 0.92^{ m b}$	$80.82{\pm}1.71^{ m ab}$	$68.03{\pm}21.32^{\rm ab}$	$72.01{\pm}7.00^{ m ab}$	$32.87{\pm}0.00^{\mathrm{ab}}$
	Range	90.71	31.39	47.72	38.80	72.35

ab and abc indicate heterogeneous groups. Homogeneity and heterogeneity are indicated by methods and species, respectively.

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Although the size of changes is not meaningful, there was a slight loss of radical-eliminating molecules during the treatments. The highest values were observed in the control samples. The total phenolic compound changes also demonstrate the decreasing pattern observed in the case of antioxidant activity (*Figure 1*).

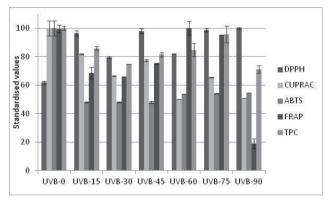


Figure 1: Antioxidant activity and phenolic compound characteristics of UVB-treated white button mushroom samples (mean, SD, n=5)

In the case of soil-derived stress, we observed the organic-poor substratederived stress, as the highest increase in the antioxidant activity was manifested in the S3 growth sample. Values of high-soil-content S1 and S4 samples show similar patterns in the case of each of the methods (*Figure 2*).

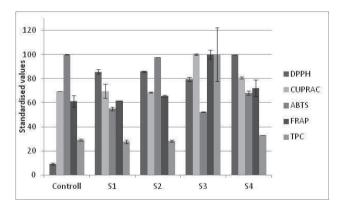


Figure 2: Antioxidant activity and phenolic compound characteristics of Sedum hybridum samples (mean, SD, n=5)

Range values show that the sensitivity of the applied 5 methods is different. In the case of *Agaricus*, the increasing order of ranking is TPC<DPPH<CUPRAC<ABTS<FRAP. In the case of Sedum, the following ranking applies: CUPRAC<FRAP<ABTS<TPC<DPPH.

## 4 Conclusion

In this study, we assessed the potential role of antioxidant-activity assays in the monitoring of photo-oxidative UVB- and substrate-associated stress factors on a nutritionally relevant edible mushroom and on an ornamentally valuable plant. Phenolic compound levels were also determined. During the measurements, we utilized the most common methods that can be easily adapted by any laboratory without the need of expensive instrumentation (*Huang et al.*, 2005).

Our results indicated that UVB stress did not cause an increasing pattern in mushroom samples, which is similar to what was observed in shiitake mushrooms by *Jiang et al.* (2010). It is maybe due to the fact that mushrooms do not possess a photosynthetic apparatus. There is evidence that the thylakoid membrane-bound enzymes of the photosynthetic system are the most sensitive proteins in the plants to the ROS damages. Avoiding the function loss can be achieved by the enhanced antioxidant capacity (enzymatic and non-enzymatic as well). The difference between *Sedum* and *Agaricus sp*-s may come from the lack of chloroplast, whence the necessity of fast-reacting antioxidant systems (*Halliwell*, 1989). The absence of phenolic content changes is due to the fact that mushrooms are not considered as a good source for these molecules (*Wong et al.*, 2013).

The results also indicated that alteration in antioxidant activity is a hallmark of the response to the related stress, as in the case of *Sedum hybridum* species the soils with the hypothesized different adequacy levels. The highest values measured in S3 substrates verified this assumption. This result is in line with others, who also highlighted the relevance of adequate salinity or other soil-delivered necessary compounds (*Esteban & Villanueva*, 2001; *Sairam et al.*, 2005).

Alteration in phenolic compound levels was also significant, which is in agreement with the results obtained from test performed on berries (Asami et al., 2003; Murugesan et al., 2012).

Our different range/rank/order results between the *Sedum* and *Agaricus sp*s are also in line with others' conclusion that the simultaneous utilization of different antioxidant activity methods is necessary as their sensitivity differs due to their different chemical background (*Huang et al.*, 2005).

On the basis of our results, we can conclude that antioxidant activity is a suitable derived parameter for the assessment of stress level.

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