MSc. Eng. Bogumiła URBAŃSKA
PhD, DrSc Jolanta KOWALSKA prof. WULS
Department of Technology and Food Evaluation, Institute of Food Sciences
Warsaw University of Life Sciences (WULS), Poland
Katedra Technologii i Oceny Żywności, Instytut Nauk o Żywności
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie (SGGW), Polska

ANALYSIS OF 5-HYDROXYMETHYLFURFURAL (HMF) CONTENT IN CONCHED CHOCOLATE MILK MASSES AND SELECTED RAW MATERIALS USED FOR THEIR PRODUCTION®

Analiza zawartości 5-hydroksymetylofurfuralu (HMF) w konszowanych czekoladowych masach mlecznych i wybranych surowcach użytych do ich wyprodukowania®

The aim of the work presented in the article is to compare the HMF content in chocolate milk mass after the conching process and in selected raw materials used to obtain them. The conching process was carried out under changing conditions of time and temperature. A statistically significant effect of roasting and conching time and temperature on the tested parameter was demonstrated in cocoa beans and chocolate milk masses, respectively.

Key words: 5-hydroxy-methylfurfural, powdered milk, cocoa beans, chocolate mass, chocolate.

Celem pracy przedstawionym w artykule była analiza porównawcza zawartości HMF w czekoladowych masach mlecznych po procesie konszowania oraz w wybranych surowcach wykorzystanych do ich otrzymania. Proces konszowania prowadzono w zmiennych warunkach czasu i temperatury. Wykazano statystycznie istotny wpływ prażenia oraz czasu i temperatury konszowania na badany parametr odpowiednio w ziarnach kakaowych i czekoladowych masach mlecznych.

Słowa kluczowe: 5-hydroksymetylofurfural, mleko w proszku, ziarna kakaowe, masa czekoladowa, czekolada.

INTRODUCTION

The use of raw materials with the right characteristics in chocolate production is very important [7], but it must be combined with proper knowledge of the processes and their influence on the quality of the final product. By modifying the processing conditions of cocoa beans or semi-finished products that will be made from them, one can control the properties of the final product such as gloss, hardness, taste or smoothness, as well as the content of bioactive components.

In addition to sensory impressions or technological aspects, product quality also includes product safety. Contaminants can be introduced with the raw material come from the environment, but they can also arise in the technological process, e.g. 5-hydroxymethylfurfural. Legislation defines acceptable levels of contaminants in chocolate products, which are monitored in this regard.

HMF is a widespread heat-induced food contaminant. 5-Hydroxymethylfurfural (HMF), which is considered carcinogenic in high concentrations. HMF is widely used in food analysis as an indicator compound that provides evidence of the degree of heat treatment of products [13]. It has been attributed genotoxic and carcinogenic effects, so monitoring its presence in raw materials and products is very important. Studies indicate that the average daily human intake of HMF ranges from 30 to 150 mg. Acceptable limits for HMF in food have not yet been established, but the European Food Safety Authority (EFSA) has set a theoretical maximum daily intake of 16 mg/day [3, 14].

The aim of this study was to analyze the comparative content of 5-hydroxymethylfurfural (HMF) in chocolate milk masses after conching process and in the raw materials used to obtain them.

RESEARCH METHODOLOGY

Research material

Analyzed were: 5 milk powders (3 milks produced by spray drying and 2 milks produced by roller drying), roasted and unroasted cocoa beans, 5 chocolate liquor from 3 different manufacturers, obtained from roasted and unroasted cocoa beans [Tab.1].

Corresponding author – Adres do korespondencji: Jolanta Kowalska, Department of Technology and Food Evaluation, Institute of Food Sciences, Warsaw University of Life Sciences (WULS), Nowoursynowska Str. 159c, 02-776 Warsaw, e-mail: jolanta_kowalska@sggw.edu.pl

Table 1. The types of raw materials used for the tests and the marking of samples

Tabela 1. Rodzaje surowców użytych do badań oraz oznaczenie próbek

Research material	Manufacturer	Sample Code
Roasted cocoa beans	Z	Z1
Unroasted cocoa beans	Z	Z2
Cocoa liquor/roasted beans/Ghana	X	MZG1
Cocoa liquor/roasted beans/Ghana	X	MZG2
Cocoa liquor/roasted beans/Ghana	X	MZG3
Cocoa liquor/roasted beans/Ivory Coast	Y	MZG4
Cocoa liquor/unroasted beans/Peru	Z	MZG5
Milk preparation (cylindrical drying technique)	X	MLK1
Milk preparation (cylindrical drying technique)	W	MLK2
Whole-milk powder (drying technique)	X	MLK3
Whole-milk powder (drying technique)	X	MLK4
Whole-milk powder (drying technique)	Y	MLK5
Milk chocolate	S	CZ1

Source: The own study

Źródła: Badania własne

Preparation of chocolate masses

Chocolate masses were prepared in Thermomix device according to the developed recipe. On the basis of the results of analyses of physico-chemical properties obtained for the raw materials for the preparation of masses, 5 out of 19 analysed milks were selected (Table 1) [15], cocoa mass based on unroasted beans, deodorized cocoa butter, powdered whey, powdered sugar, lecithin, ethyl vanillin..

Table 2. Parameters of the chocolate mass preparation process and sample marking

Tabela 2. Parametry procesu przygotowywania mas czekoladowych oraz oznaczenie próbek

Process time	Process temperature			
	50°C	60°C	70°C	80°C
	MC1	MC2	MC3	MC4
35 minutes	MC11	MC21	MC31	MC41
45 minutes	MC12	MC22	MC32	MC42
55 minutes	MC13	MC23	MC33	MC43
65 minutes	MC14	MC24	MC34	MC44
75 minutes	MC15	MC25	MC35	MC45
85 minutes	MC16	MC26	MC36	MC46
95 minutes	MC17	MC27	MC37	MC47
105 minutes	MC18	MC28	MC38	MC48
120 minutes	MC19	MC29	MC39	MC49

Source: The own study

Źródła: Badania własne

The first step in making the chocolate mass was to liquefy the fat and chocolate liquor. The fat was dissolved at 60°C, while the liquor was dissolved at 50°C for about 5 minutes. After obtaining a liquid consistency, the liquor, 10% fat, sugar, milk powder and whey were combined together. The ingredients were mixed for 10 minutes at medium speed of the mixer at 40°C. Then the remaining fat, lecithin and ethylowanilin were added. Thus combined ingredients were conched (mixed) at four temperature ranges (50, 60, 70 and 80°C) for 35 to 120 minutes, taking a sample of the mass for analysis every 10 minutes (Table 2). Conching parameters were determined based on information obtained from chocolate manufacturers.

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Analytical methods

Determination of dry matter content [11].

Determination of the dry matter content was carried out using a standardized method based on PN-84/A-88027. It consisted in evaporation of the water present in the sample during drying at 105°C for 3 hours (with reduction to a constant weight), and then weighing the sample after the process was completed. The dry matter content was calculated from the difference in weight of the sample before and after drying. The final result was obtained by calculating the arithmetic mean of three parallel replicates that did not differ by more than 0.2%.

The dry matter content was calculated according to the formula:

$$s.s.=100-[(mps)-mw)/(mps)\ ]x\ 100\% \quad (1)$$

where: mw – mass of test material after drying [g],
mps – mass of the test material before drying [g].

Determination of 5 - hydroxymethylfurfural [8, 12]

HMF determination in the studied raw materials was carried out by spectrophotometric method using thiobarbituric acid (TBA).

The preparation of samples for analysis consisted in measuring with a pipette 10 ml of regenerated milk into a conical flask, adding 5 ml of 0.3N oxalic acid solution and thorough mixing of the flask contents. The flasks were covered with foil and transferred to a boiling water bath for about 1 hour, after which the samples were cooled to 20°C. 5 ml of 40% trichloroacetic acid (TCA) solution was added to the flask, mixed and filtered through tissue paper. Then 4 ml of the resulting filtrate was transferred to a test tube and 1 ml of 0.05 mol TBA solution was added to it. The samples were placed in a water bath for 30–40 minutes at 40°C. After the samples were removed from the bath and cooled to room temperature, their absorbance was measured in a spectrophotometer at λ = 443 nm against distilled water.

The beans, cocoa liquor,and chocolate masses were weighed into conical flasks in an amount of about 5 g and poured with 30 ml of water. The resulting mixture was fluidized and homogenized, and then 10 ml of solution was taken. In further steps of the determination, the procedure was analogous to that for milk powder.

The hydroxymethylfurfural content (μmol/l) was calculated according to the formula

$$HMFGeneral = (A - AH_20) * 72.6 - 0.55 \quad (2)$$

where: A – measured absorbance value for individual samales.

Calculation methods

Statistical analysis was performed to investigate the significance of the effect of selected parameters of the chocolate mass production process on specific parameters characterizing their quality. For this purpose, the Excel 2010 program was used, in which the standard deviation was calculated, which is a measure of the deviation of the tested value from its arithmetic mean, and the Statistica 13.1 program, was used ANOVA analysis of variance and industrial DoE statistic at the significance level α=0.05.

RESULTS AND DISCUSSION

The content of dry matter in chocolate milk masses

The conching stage in the manufacturing process of chocolate has many functions, among others it is aimed at a thorough mixing of all ingredients, allows to obtain the right consistency, as well as to reduce the content of water present in the chocolate mass and to get rid of the residue of undesirable acetic acid [10].

The dry matter content of the chocolate paste can vary due to the use of ingredients with different water content and also due to different process parameters. Gutierrez [5] and Hinneh et al. [6] state that the dry matter content of chocolate masses should be a minimum of 99%.

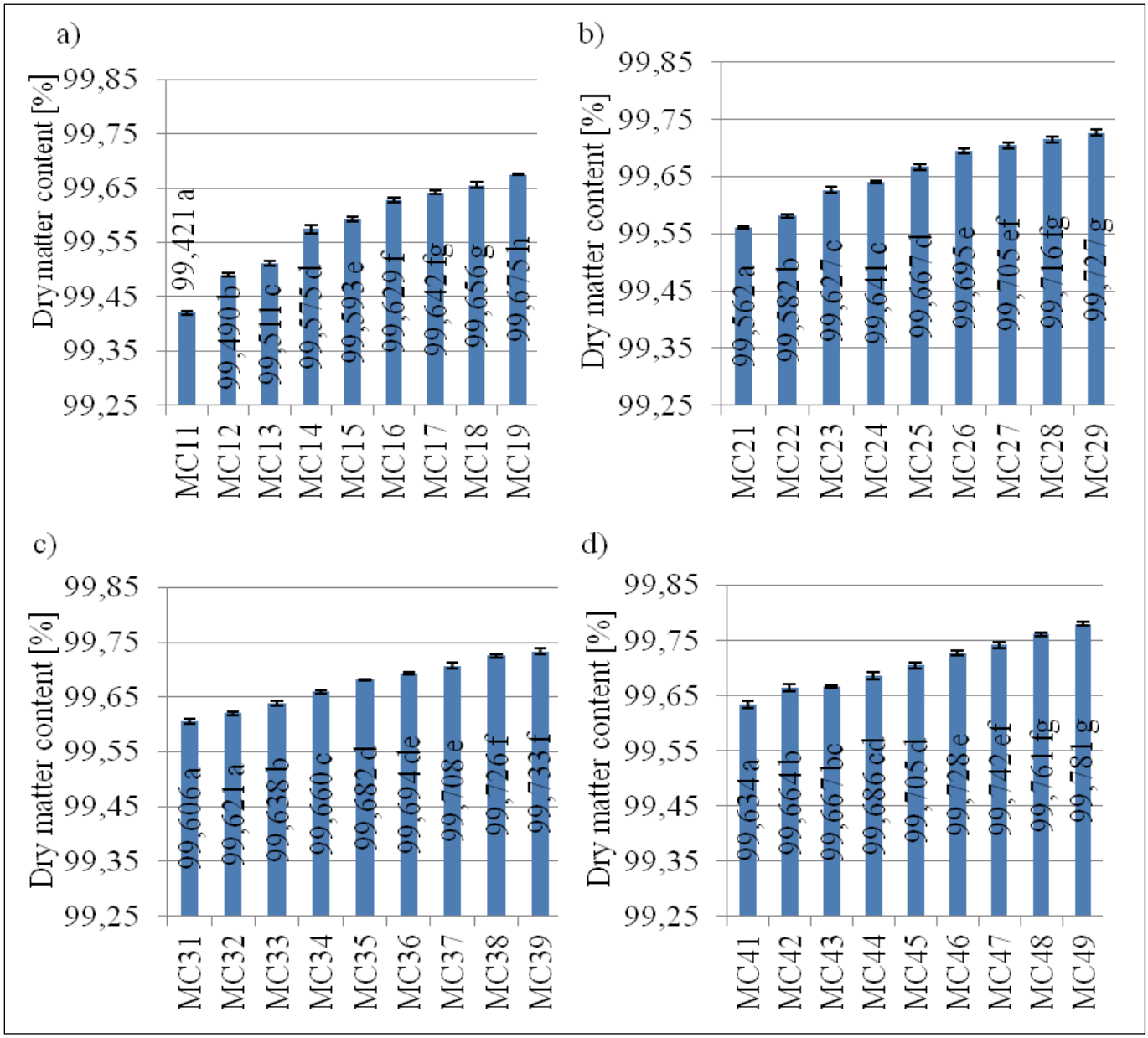

Fig. 1. The average dry matter content of a) MC1 chocolate liquor, b) MC2 chocolate liquor, c) MC3 chocolate liquor, d) MC4 chocolate liquor (sample designations – Tab. 2).

Rys. 1. Średnia zawartość suchej substancji w a) w masie czekoladowej MC1, b) w masie czekoladowej MC2, c) w masie czekoladowej MC3, d) w masie czekoladowej MC4 (oznaczenia próbek – Tab. 2).

Source: The own study

Źródła: Badania własne

In all the masses analyzed in this study, dry matter content above 99.4% was determined, which is confirmed by the studies of the authors quoted.

HMF content in the investigated milk powders

Among the analyzed milks, MLK2 rolled milk enriched with sugar addition had the highest HMF content (Figure 1). Statistical analysis showed a significant difference between milk obtained by the same technology MLK1, whose average level of 5-hydroxymethylfurfural was lower by as much as over 70 µmola/litre. One of the reasons for such a large discrepancy in the results may be the analytical method, whose main drawback is the lack of specificity of the reaction with thiobarbituric acid. This may result in the reaction of this acid with other carbonyl groups contained in the milk (e.g. from added sucrose), which in turn may cause unexpected overestimation of the results [4]. Additionally, hexoses, which include the glucose and fructose that make up sucrose, are precursors to 5-hydroxymethylfurfural. Ferrer et al. [4] comparing the HMF content of whole milk powder and milk powder enriched with 21.6% maltodextrin addition showed more than three times higher HMF content in the enriched milk, which confirms the results obtained in this study presented in Fig. 2.

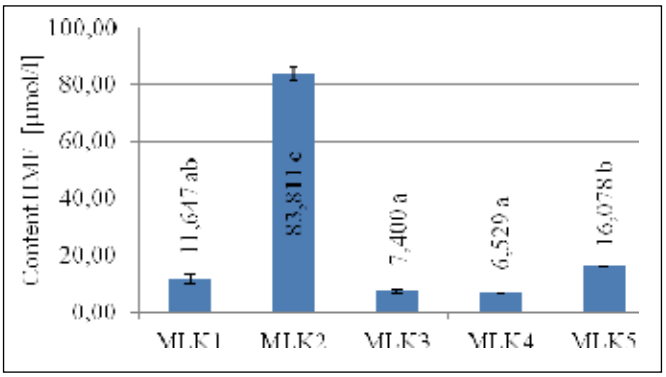

Fig. 2. The average content of HMF in the tested milk powders (the same letters mean no significant differences between the compared means for the assumed significance level a-0.05).

Rys. 2. Średnia zawartość HMF w badanych proszkach mlecznych (jednakowe oznaczenia literowe oznaczają brak istotnych różnic pomiędzy porównywanymi średnimi dla założonego poziomu istotności a-0,05).

Source: The own study

Źródła: Badania własne

MLK3 and MLK4 milks from the same producer (spray-dried) did not differ significantly in HMF content. Similar levels were recorded by Chudy et al. [2] determining the amount of HMF depending on the storage time of milk powder (1, 6, 12 months, respectively) at 7.25 µmola/litre, 9.90 µmola/litre and 15.90 µmola/litre. Statistical inference revealed a statistically significant difference between MLK1 and the analyzed spray-dried milk powders (MLK3, MLK4, MLK5), while comparison of HMF content in the spray-dried milks classified them into two homogeneous groups, indicating small but significant differences in the content of the analyzed compound.

HMF content in roasted and unroasted cocoa beans

The formation of 5-hydroxymethylfurfural in food can take place in two ways. One of them is long term heating of the product rich in sugars at temperature above 150°C in acidic environment (cocoa beans before roasting have pH about 5.7). This leads to caramelization of sugars, which is most easily done by glucose, fructose, xylose or ribose [9].

Due to the high roasting temperature of cocoa beans of about 150°C, we decided to investigate the differences in HMF content between roasted and unroasted beans (Fig. 3).

The roasted Z1 beans had significantly higher 5-hydroxymethylfurfural content in comparison with the unroasted Z2 beans. This indicates a significant effect of high temperature on HMF content in the final product.

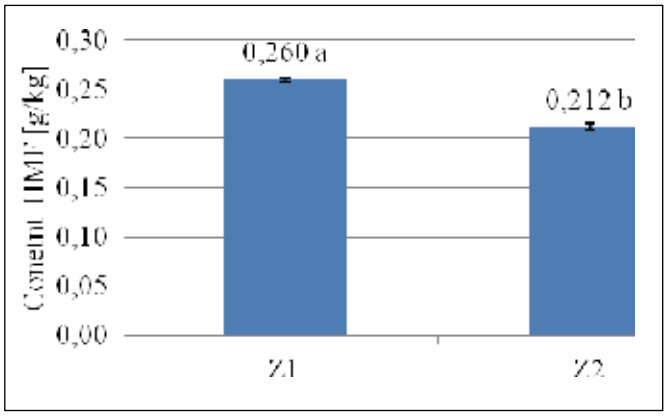

Fig. 3. Average HMF content in roasted beans Z1 and unroasted Z2.

Rys. 3. Średnia zawartość HMF w ziarnie prażonym Z1 i nieprażonym Z2.

Source: The own study

Źródła: Badania własne

Studies conducted by Sacchetti et al. [13] show a higher content of 5 - hy-droxymethylfurfural in roasted beans than in beans not subjected to this process. In addition, the analysis carried out on the effect of increasing time and temperature of liquor conching showed that higher process parameters favor the formation of higher amounts of this genotoxic compound.

HMF content in cocoa masses

The obtaining of cocoa bean liquor is not accompanied by any high-temperature process that could significantly affect the content of 5-hydroxymethylfurfural in its composition. However, HMF is not only an indicator of the degree of temperature cycling, but also an indicator of "aging" of the product. As a result of non-enzymatic browning reactions taking place in the product, the 5-hydroxymethylfurfural content can increase Quiroz – Reyes and Fogliano [16].

Five chocolate liquor from different producers and obtained from cocoa beans harvested in different seasons were analyzed (Figure 4). The statistical inference performed to separate homogeneous groups did not show significant differences between the HMF content of the analyzed liquor. The lowest HMF content was characterized by liquor MZG1 (producer X) obtained from beans harvested in April 2018. The liquor MZG2 and MZG3 from the same producer were distinguished by a higher value, but not exceeding 0.02 g/kg.

These liquor were obtained from beans harvested in July 2017, so they were a year older. It can be presumed that it is the "age" of the liquor that may indicate a higher HMF content in their composition, but due to the lack of a statistically significant difference shown in this study, no such conclusion can be made. A more extensive study on a larger amount of study material would have to be conducted to confirm this thesis.

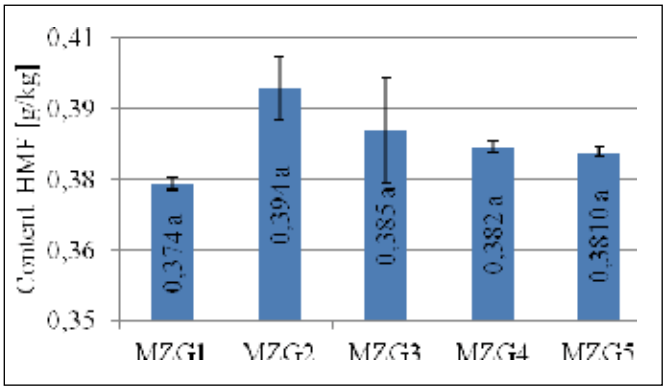

Fig. 4. Average HMF content in cocoa liquors.
Rys. 4. Średnia zawartość HMF w miazgach kakaowych.
Source: The own study
Źródła: Badania własne

The MZG5 liquor (producer Z, no data available for the date of bean harvest) made from unroasted cocoa beans was characterized by similar HMF content compared to MZG4 liquor (producer Y, no data available for the date of bean harvest). In this case, there is no significant difference between the HMF content of the products obtained from roasted and unroasted beans as shown in the beans analysis (Figure 4).

This difference can be attributed to a number of factors. The most important one is the different origin of the roasted beans. MZG5 liquor and Z2 unroasted beans were obtained from producer Z. Whereas MZG4 liquor and Z1 roasted beans were obtained from producers Y and Z, respectively. Producer Y did not provide information on the date of harvest of beans from which the liquor was produced nor on the parameters of the beans processing, which can significantly differentiate the HMF content in the final product.

HMF content in conched chocolate mash under varying time and temperature parameters.

Conching is the last stage in chocolate production where elevated temperature is used. Tempering, which follows conching, is carried out at temperatures not exceeding 50°C, so it can be assumed that it does not significantly affect the formation of Maillard reaction by-products. Due to the thermal induction of the 5-hydroxymethylfurfural formation reaction, it was considered reasonable to trace the effect of varying temperature parameters and conching time on the HMF content of chocolate [1].

Figure 5 shows the average HMF content in conching chocolate masses at varying temperatures (50°C, 60°C, 70°C, 80°C, respectively).

The MC1 mass conched at 50°C was characterized by the lowest HMF content. Its result was on average 0.16 g/kg lower than the other masses, in which the HMF content slightly increased in direct proportion to the increase of conching temperature. Nevertheless, statistical analysis showed no significant differences between the samples, classifying all of them in one homogeneous group.

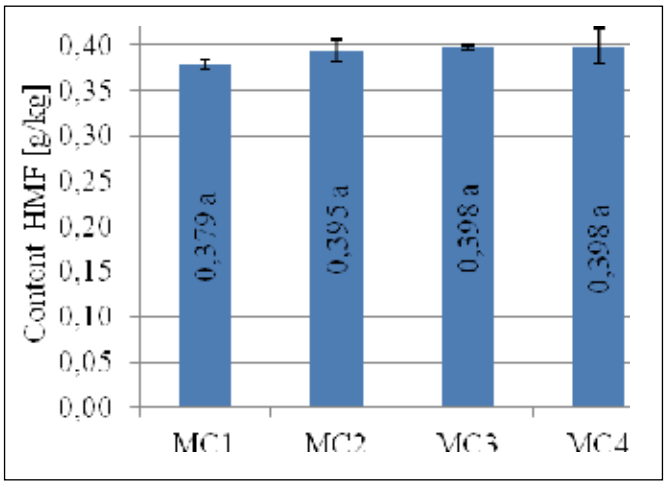

Fig. 5. Average HMF content in conched chocolate masses at various temperatures: MC1 (50°C), MC2 (60°C), MC3 (70°C), MC4 (80°C) (sample markings – tab. 2).
Rys. 5. Średnia zawartość HMF w masach czekoladowych konszowanych w różnych temperaturach: MC1 (50°C), MC2 (60°C), MC3 (70°C), MC4 (80°C) (oznaczenia próbek – tab. 2).
Source: The own study
Źródła: Badania własne

The distribution of 5-hydroxymethylfurfural content in individual chocolate masses was also analyzed taking into account the initial and final conching times (45 minutes and 120 minutes). Statistical analysis of MC1, MC2 and MC4 masses showed significant differences between 45 and 120 minutes of conching. However, in the masses conched at 70°C this difference was not shown.

There is a lack of literature data relating to the analysis of HMF content in cocoa masses, but a study by Sacchetti et al. [13] addresses the issue of the effect of roasting time and temperature on 5-hydroxymethylfurfural content in cocoa beans. This study showed a positive correlation between HMF content and increasing roasting time and temperature.

Similar correlations were obtained in the present study (Fig. 7), but even so, the correlation test based on them did not show a relationship between increasing time and conching temperature. Even for MC3 mass, in which there was no significant difference between the initial and final conching time, there was a non-significant increase in the content of one of the intermediate products of the Maillard reaction.

CONCLUSION

1) The content of HMF in milk powders was on a similar level, with the exception of milk preparation MLK2. This product had more than seven times higher content of 5 - hydroxymethylfurfural due to the presence of sugar in its composition, which is a precursor of this compound.
2) Unroasted beans contained significantly less HMF in its composition in comparison with roasted beans. However,

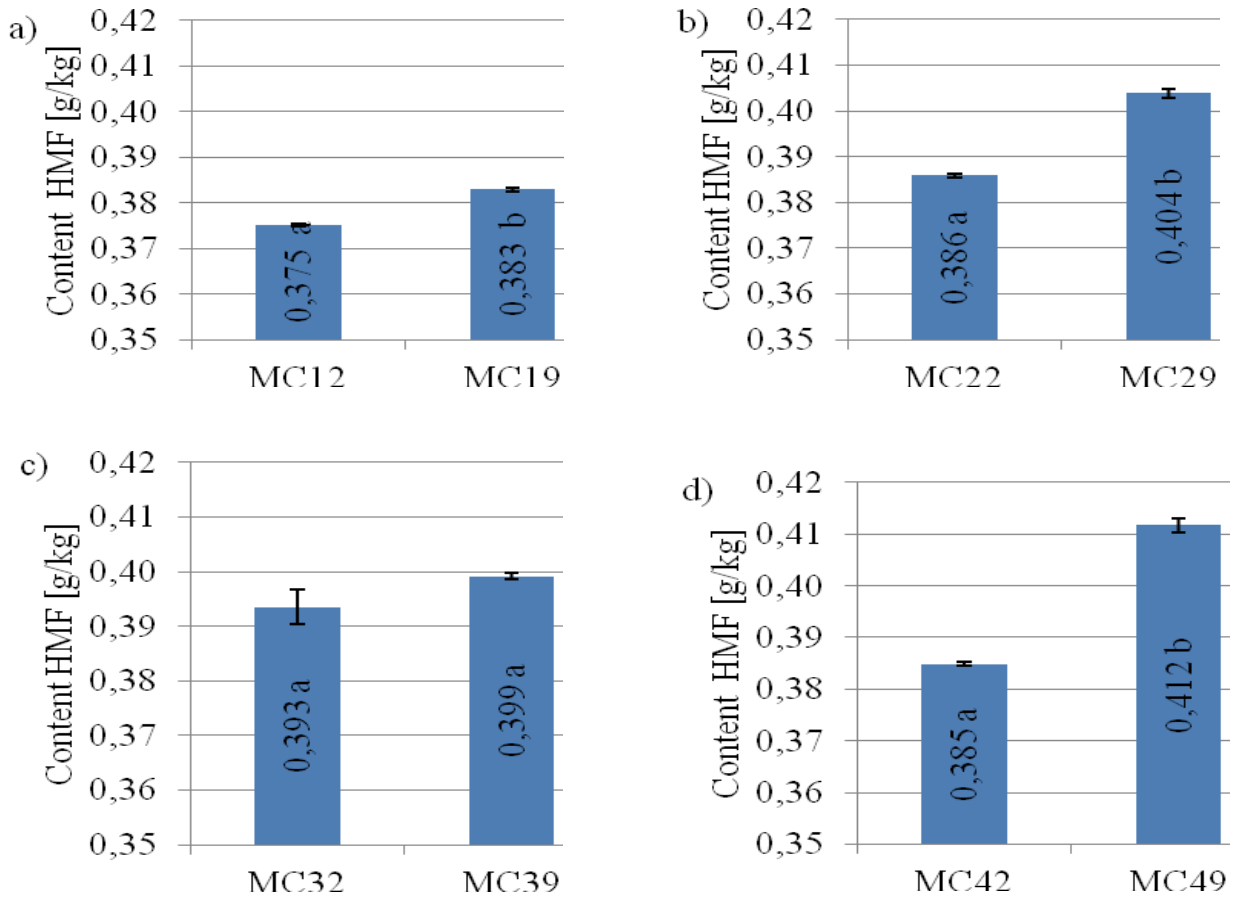

Fig. 6. Average HMF content in conched chocolate masses for 45 and 105 minutes: a) at 50°C, b) at 60°C, c) at 70°C, d) at 80°C (sample markings – tab. 2).

Rys. 6. Średnia zawartość HMF w masach czekoladowych konszowanych przez 45 i 105 minut: a) w temperaturze 50°C, b) w temperaturze 60°C, c) w temperaturze 70°C, d) w temperaturze 80°C (oznaczenia próbek – tab. 2).

Source: The own study

Źródła: Badania własne

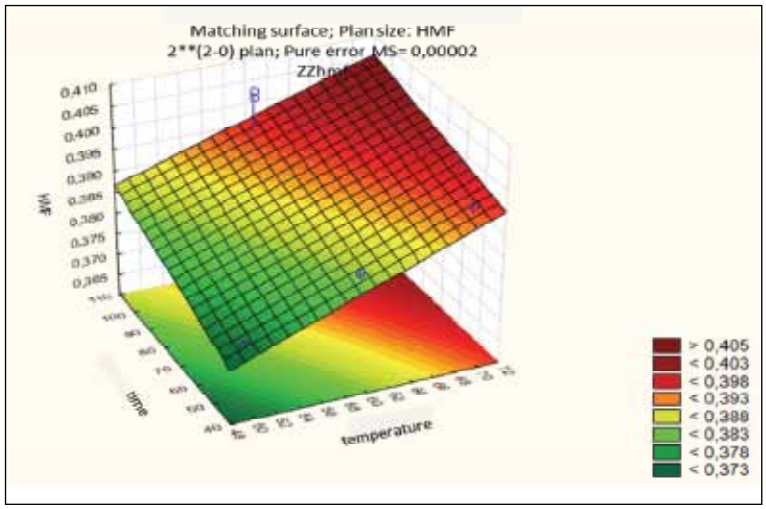

Fig. 7. Average HMF values for all conched chocolate mixes in different time and temperature parameters.

Rys.7. Średnie wartości HMF dla wszystkich mieszanek czekoladowych konszowanych w różnych parametrach czasu i temperatury.

Source: The own study

Źródła: Badania własne

there was no statistically significant difference in HMF content in the mash obtained from roasted and unroasted beans.

3) Chocolate masses conched at higher temperatures and for longer time cha-racterized significantly higher share of 5 - hydroxymethylfurfural in their composition.

4) The thermal processes applied have an influence on the content of HMF, which are formed under the influence of high temperature in products containing sugars.

PODSUMOWANIE

1) Zawartość HMF w mleku w proszku była na podobnym poziomie, z wyjątkiem preparatu mlecznego MLK2. Produkt ten posiadał ponad siedmiokrotnie wyższą zawartość 5-hydroksymetylofurfuralu ze względu na obecność w swoim składzie cukru, który jest prekursorem tego związku.

2) Ziarna nieprażone zawierały w swoim składzie znacznie mniej HMF w porównaniu z ziarnami

prażonymi. Nie stwierdzono jednak statystycznie istotnej różnicy w zawartości HMF w miazgach otrzymanych z ziaren prażonych i nieprażonych.

3) Masy czekoladowe konszowane w wyższych temperaturach i przez dłuższy czas charakteryzowały się istotnie większym udziałem 5 - hydroksymetylofurfuralu w ich składzie.

4) Stosowane procesy termiczne mają wpływ na zawartość HMF, które powstają pod wpływem wysokiej temperatury w produktach zawierających cukry.

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