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Research Article

COMPLIANCE OF CARMINIC ACID APPLICATION WITH EUROPEAN LEGISLATION FOR FOOD SAFETY AND PUBLIC HEALTH

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ABSTRACT. The aim of this investigation is to identify suitable flours based on their mineral composition for the production of gluten-free high-protein cakes, incorporating carminic acid (E120) and ensuring compliance with food safety regulations as per European legislation. The study reveals that by combining whole grain bio spelt flour, bio sesame meal, butter, honey, carminic acid, natural colorants, bio sunflower flour, and gelatin in appropriate proportions, gluten-free cakes with favorable technological attributes can be achieved. These cakes are characterized by their high protein and omega-3 and omega-6 fatty acid content, alongside a low carbohydrate composition. Furthermore, the products are free from genetically modified organisms (GMOs) and incorporate natural colorants. They exhibit elevated levels of essential minerals such as iron (Fe), zinc (Zn), calcium (Ca), and sodium (Na), as well as a rich vitamin profile encompassing vitamins A, K, B6, B2, B12, B17, and C. With an energy value of 270 kcal per 100g, these new products cater to the dietary requirements of individuals with specific health needs, including those with type 2 diabetes, cardiovascular diseases, and gastroenterological diseases, while also appealing to the general consumer.

Keywords: *Carminic acid, natural colorants, fatty acids*

INTRODUCTION

The use of carminic acid, a prominent pigment within the anthraquinone group of animal origin, holds significant importance in the food industry due to its unique properties and diverse applications [4,14,18,20]. With a carminic acid content of approximately 50%, carmine is valued for its resistance to heat and light, contributing to its extended stability during storage, particularly in foods with a pH above 3.5. Its versatility is evident in its wide utilization across various products, including jams, gelatin desserts, baked foods, confections, toppings, dairy products, and noncarbonated drinks[1,6,8,10,13,19,26].

Carminic acid, also known as E 120, is characterized by its possession of a C-glycosidic bond, a defining feature within the anthraquinone group [3,5,7,22]. This bond imparts high resistance to acid hydrolysis and enzymatic cleavage, attributed to the glucose moiety's stability, making it less susceptible to cleavage by many enzymes involved in carbohydrate [2,9,11,23]. With a molecular formula of $C_{22}H_{20}O_{13}$ and a molecular weight of 492.4 Da, carminic acid exhibits solubility in water, alcohols, esters, acids, and alkaline solutions, while being insoluble in petroleum ether, benzene, and chloroform [20]. Notably, its color appearance is highly pH-sensitive, manifesting as pale orange at pH values below 4.5, light red and red at pH 7–7.7, and magenta-red at pH above 12.

Furthermore, carminic acid has been recognized for its antioxidant properties, with studies reporting its function as a radical scavenger in aqueous and methanolic solutions, demonstrating activity comparable to well-known antioxidants such as ascorbic acid or trolox [12,16,21,25,27].

In nature, carminic acid is primarily produced by the Dactylopius coccus (cochineal or American cochineal), which yields up to 26% carminic acid per insect dry weight, a significantly higher percentage compared to other sources such as Porphyrophora polonica L(Fig.1). (Polish cochineal) and Porphyrophora hameli Brandt (Armenian red), which yield only 0.6% and 0.8% of the pigment, respectively [17,24]. This higher yield has established the American cochineal as the primary commercial source of carminic acid, supplanting other sources since the 16th century [15].

In this study investigation is to identify suitable flours based on their mineral composition for the production of gluten-free high-protein cakes, incorporating carminic acid (E120) and ensuring compliance with food safety regulations as per European legislation. The study reveals that by combining whole grain bio spelt flour, bio sesame meal, butter, honey, carminic acid, natural colorants, bio sunflower flour, and gelatin in appropriate proportions, gluten-free cakes with favorable technological attributes can be achieved. These cakes are characterized by their high protein and omega-3 and omega-6 fatty acid content, alongside a low carbohydrate composition. Furthermore, the products are free from genetically modified organisms (GMOs) and incorporate natural colorants. They exhibit elevated levels of essential minerals such as iron (Fe), zinc (Zn), calcium (Ca), and sodium (Na), as well as a rich vitamin profile encompassing vitamins A, K, B6, B2, B12, B17, and C.

MATERIALS AND METHODS

Analytical methods used

Organoleptic assessment

The organoleptic assessment of the raw materials and bread products was conducted by established standards and methodologies to ensure a comprehensive evaluation. The BDS 15612-83 standard was employed for the organoleptic evaluation of raw materials, encompassing assessments of appearance, color, taste, and aroma. Furthermore, the organoleptic evaluation of the developed bread was conducted using the 9th Bald Hedonic Scale, as referenced in .

In addition to organoleptic assessments, the nutritional composition of the bread products was determined using standardized methods. The total protein content was determined utilizing the Kendal's method, as outlined in BDS 1671-89 , while the total fat content was analyzed using the Soxtec device, in accordance with BDS 1671-89 . Furthermore, the total ash content was determined following the guidelines of BDS ISO 2171: 1999, and the total fiber content was assessed as per the specifications of BDS ISO 5498: 1999.

Moreover, the macro and trace-element composition of the bread products was analyzed using an atomic emission photometer, specifically the AES-ICP "Varian-Liberty II", ensuring a comprehensive evaluation of the elemental composition.

These standardized methodologies and reference standards provide a robust framework for the comprehensive assessment of both the organoleptic and nutritional attributes of the bread products, ensuring accuracy, reliability, and comparability of the results.

Method for obtaining gluten-free high protein cake

A combination of whole-grain organic spelt flour, organic sesame meal, butter, honey, carminic acid, natural colorants, and sunflower flour were used to create a gluten-free high-protein cake. Along with the main flours, butter, and honey are included as additional ingredients. The flours should be brought to room temperature before use, as they are stored in a dry, cool place. It is important to measure out the required quantities of all ingredients according to the formula for the gluten-free high-protein cake. The dough should be mixed using the single-phase method.

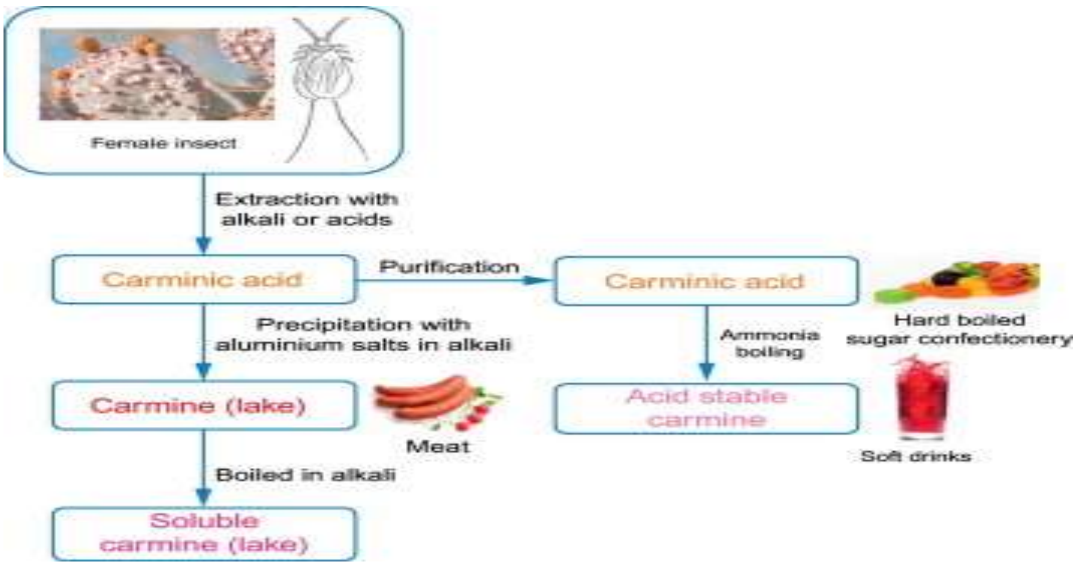

Fig. 1. *Extraction with alkali or acids to carminic acid and application carminic acid in the food meat, sugar confectionery and soft drinks.*

RESULTS AND DISCUSSION

Our team conducted research on confectionery containing carminic acid. The study aims to identify appropriate flours based on their mineral composition to produce a high-protein, gluten-free cake with the addition of carminic acid (E120), while ensuring compliance with European food safety regulations. The study revealed that by combining whole grain organic spelled flour, organic sesame meal, butter, honey, carminic acid, natural colorants, organic sunflower flour, and gelatin in suitable proportions, it is possible to produce gluten-free cakes with favorable technological characteristics.

Physicochemical analysis on the flours.

Table 1 presents the physicochemical analysis of the flours used in the production of the gluten-free high-protein cake. The analysis includes measurements of moisture, protein, carbohydrates, and fats in whole-grain bio-spelt flour and bio-sunflower flour.

In terms of moisture content, whole grain bio-spelt flour exhibits the highest value at 10.50%, while bio-sunflower flour has the lowest moisture content at 0.75%. Regarding protein content, whole grain bio-spelt flour also demonstrates the highest value at 16.12%, whereas bio-sunflower flour has the lowest protein content at 4.40%. Additionally, for carbohydrate content, whole grain bio-spelt flour again shows the highest value at 44.94%, while bio-sunflower flour has the lowest carbohydrate content at 14.80%.

Furthermore, in terms of fat content, bio-sesame flour has the highest value at 51.40%, while bio-sunflower flour has the lowest fat content at 1.20%. It is noteworthy that whole-grain bio-spelt flour is rich in both fats and proteins, while whole-grain bio-sesame flour is particularly rich in proteins.

These findings indicate that whole-grain bio-spelt flour and bio-sesame flour are suitable choices for the production of a high-protein, gluten-free cake due to their favorable nutritional profiles. The high protein content in these flours aligns with the desired nutritional characteristics for the cake, while the gluten-free nature of the flours meets the specific dietary requirements.

Therefore, based on the physicochemical analysis presented in Table 1, whole grain bio-spelled flour and bio sesame flour are appropriate and advantageous choices for the intended application in the production of the gluten-free high-protein cake.

Table 1. *Physicochemical analysis on the flours (%).(n=10)*

Type of analysis	Whole grain bio spelta flour	Bio sesame flour	Bio Sunflower flour
Moisture,%, (x±sd)	10.50±0.02	11.25±0.01	0.75±0.02
Protein,%, (x±sd)	16.12±0.01	13.05±0.02	4.40±0.02
Acidity,◦H, (x±sd)	3.12±0.01	1.70±0.01	2.80±0.01
Fat,%, (x±sd)	4.04±0.01	51.40±0.02	1.20±0.02
Carbohydrates,%,(x±sd)	44.94±0.02	24.96±0.02	14.80±0.02
Ash,%,(x±sd)	1.12±0.01	0.80±0.01	1.00±0.01
Fiber,%,(x±sd)	13.12±0.01	10.12±0.01	3.42±0.01
Energy value кcal/ 100 g product	269	267	270

$p<0,005$

Microelements in the flour

In relation to calcium, whole grain organic spelt flour has the highest amount, while organic sunflower flour has the lowest. For potassium, whole grain organic spelt flour has the highest content, and organic sunflower flour has the lowest. As for magnesium, whole grain organic spelt flour has the highest amount, and organic sunflower flour has the lowest. In terms of sodium, whole grain organic spelt flour has the highest content, and organic sunflower flour has the lowest. The technological process involves preparing the raw materials, kneading the dough, shaping and baking, analyzing the quality of the finished product, and conducting taste tests (Table 2).

Table 2. *Microelements in the flours (mg/kg)(n=10)*

Microelements, mg/kg, (x±sd)	Whole grain bio spelta flour	Bio sesame flour	Bio sunflower flour
Ca	2312.50±0.01	521.25±0.01	78.75±0.02
K	2916.12±0.01	533.05±0.02	94.40±0.02
Mg	1934.04±0.01	671.40±0.02	91.20±0.02
Na	1244.94±0.02	727.96±0.02	84.80±0.02

p<0,005

Trace-elements in the flours

Whole grain bio spelta flour has the highest iron content, while bio sunflower flour has the lowest. Similarly, whole grain bio spelta flour has the highest manganese content, and bio sunflower flour has the lowest. Additionally, whole grain bio spelta flour has the highest zinc content, while bio sesame flour has the lowest. These findings are presented in Table 3.

Table 3. *Trace-elements in the flours (mg/kg)(n=10)*

Trace-elements,mg/kg,(x±sd)	Whole grain bio spelta flour	Bio sesame flour	Bio sunflower flour
Fe	5672.50±0.02	6532.51±0.01	326.42±0.02
Mn	457.81±0.01	664.99±0.02	346.43±0.02
Zn	768.24±0.01	567.9±0.02	456.20±0.02

p<0,005

Technological preparation

The formulation of the three different mixes for the gluten-free high-protein cake involves varying proportions of whole grain bio spelt flour, bio sesame flour, bio sunflower flour, and other raw materials. Mix 1 comprises 70% whole grain bio spelt flour, 5% bio sesame flour, 5% bio sunflower flour, and other raw materials. Mix 2 consists of 60% whole grain bio spelt flour, 10% bio sesame flour, 10% bio sunflower flour, and other raw materials. Mix 3 includes 50% whole grain bio spelt flour, 20% bio sesame flour, 10% bio sunflower flour, and other raw materials. The preliminary preparation involves ensuring that the butter reaches a temperature of 45°C before being incorporated into the dough, which is kneaded using the one-phase mixing method.

The cakes are baked in cake pans at 200°C with a fan for 35 minutes. This baking technology is characterized by its innovative approach, which utilizes a fan to reduce the baking time from the traditional 55 minutes to 35 minutes, thereby saving energy consumption. The cakes are designed to cater to the dietary needs of individuals with specific health conditions such as type 2 diabetes, cardiovascular diseases, and gastroenterological diseases, as well as for the general consumer.

The composition of the cake is distinguished by the use of whole-grain high-protein flours, supplemented with sunflower flour to enhance the energy value based on the protein content, at the expense of the carbohydrate component.

Microelements in the cake

Concerning calcium, mix 1 exhibits the highest concentration, while mix 3 demonstrates the lowest concentration. Similarly, for potassium, mix 1 displays the highest content, and mix 3 shows the lowest content. In terms of magnesium, mix 1 contains the highest amount, and mix 3 contains the lowest. For sodium, mix 1 has the highest content, and mix 2 has the lowest (Table 4). The technological process involves the preparation of raw materials, dough kneading, shaping, baking, analysis and quality assessment of the final product, and sensory evaluation.

Table 4. *Microelements in the cake (mg/kg)(n=10)*

Microelements,mg/kg,(x±sd)	Cake,Mix 1	Cake,Mix 2	Cake,Mix 3
Ca	641.60±0.02	632.25±0.01	101.85±0.02
K	3046.12±0.01	1673.05±0.02	1184.42±0.02
Mg	2134.04±0.01	1181.40±0.02	1131.20±0.02
Na	1234.94±0.02	934.96±0.02	967.80±0.02

$p<0,005$

Trace elements in the cake

With the presence of iron (Fe), mix 2 exhibits the highest concentration, while mix 3 demonstrates the lowest concentration. Similarly, in terms of manganese (Mn), mix 2 displays the highest content, and mix 3 exhibits the lowest content. Furthermore, concerning zinc (Zn), mix 1 showcases the highest concentration, while mix 3 presents the lowest concentration, as indicated in Table 5.

Table 5. *Trace-elements in the cake (mg/kg)(n=10)*

Trace-elements, mg/kg,(x±sd)	Cake Mix 1	Cake Mix 2	Cake Mix 3
Fe	5670.50±0.02	6542.51±0.01	577.5±0.02
Mn	456.81±0.01	654.99±0.02	336.40±0.02
Zn	768.04±0.01	467.9±0.02	455.20±0.02

$p<0,005$

Fatty acids in the cake

The fatty acid composition of gluten-free cakes made from different mixes of flour is presented in Tables 6 and 7. The cakes predominantly contain unsaturated fatty acids, with variations in the content of monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and saturated fatty acids (SFA) across the different mixes.

In terms of MUFA content, mix 1 exhibits the highest concentration at 30.13 g/100g fat, while mix 3 has the lowest at 23.51 g/100g fat. Conversely, for PUFA, mix 3 demonstrates the highest content at 56.19 g/100g fat, whereas mix 1 has the lowest at 54.20 g/100g fat. The SFA content ranges from 15.34 g/100g fat in mix 1 to 16.87 g/100g fat in mix 2. Notably, palmitic acid (C16:0) constitutes a significant proportion of the SFA, with mix 2 exhibiting the highest concentration at 11.13 g/100g fat and mix 1 the lowest at 10.19 g/100g fat. The variation in stearic acid content across the mixes is minimal, ranging from 3.34 to 3.84 g/100g fat. Oleic acid content is highest in mix 1 at 27.47 g/100g fat and lowest in mix 3 at 24.63 g/100g fat.

Among the PUFA, linoleic acid (C18:2) predominates, with mix 3 showing the highest content at 54.87 g/100g fat, followed by mix 2 at 53.95 g/100g fat and mix 1 at 53.55 g/100g fat. The content of α-linolenic acid (C18:3n3) ranges from 0.66 to 1.16 g/100g fat across the different mixes. The total content of omega-3 fatty acids is low, varying from 0.63 g/100g fat in mix 1 to 1.26 g/100g fat in mix 2, while the omega-6 fatty acid content ranges from 54.20 to 56.19 g/100g fat. Consequently, the ratio of omega-6 to omega-3 fatty acids varies from 43.02 to 84.94 g/100g fat across the different mixes.

In conclusion, gluten-free cakes are found to be rich in oleic and linoleic fatty acids, with variations in the composition of MUFA, PUFA, and SFA across the different mixes. These findings provide valuable insights into the fatty acid profiles of gluten-free cakes and their potential implications for dietary considerations.

Table 6. *Fatty acids in the cake (g/100g fat)(n=10)*

	Cake Mix 1	**Cake Mix 2**	**Cake Mix 3**
		SFA	
C-10:0	0,00	0,17	0,16
C-12:0	0,02	0,04	0,04
C-14:0	0,29	0,57	0,33
C-15:0	0,03	0,05	0,16
C-16:0	**10,19**	**11,13**	**10,46**
C-17:0	0,00	0,01	0,17
C-18:0	**3,84**	**3,34**	**3,56**
C-20:0	0,28	0,41	0,26
C-21:0	0,03	0,00	0,06
C-22:0	0,42	0,55	0,29
		MUFA	
C-16:1n7	0,07	0,13	0,11
C-17:1n7	1,17	0,01	0,02
C-18:1t4	0,01	0,04	0,06
C-18:1t9	0,14	0,02	0,11
C-18:1t10	0,03	0,00	0,15
C-18:1t11	0,08	0,01	0,07
C-18:1c9	**27,47**	**26,25**	**24,63**
C-18:1c11	0,75	0,88	0,59
		PUFA	
C-18:2c9,12	**53,55**	**53,95**	**54,87**
αC-18:3n3	**0,66**	**1,16**	**0,85**
C-20:2n6	0,01	0,00	0,17
C-22:2n6	0,00	0,02	0,00
C-22:5n3	0,00	0,07	0,15

$p<0,005$

Table 7. *Fatty acids composition in the cake (g/100g fat)(n=10)*

FA	Cake Mix 1	Cake Mix 2	Cake Mix 3
SFA	**15,34**	**16,87**	**16,20**
MUFA	**30,13**	**27,51**	**23,51**
PUFA	**54,20**	**55,28**	**56,19**
trans- FA	0,02	0,09	0,48
cis- FA	28,22	27,16	25,64
Σ n-3	0,63	1,26	1,01
Σ n-6	53,58	54,02	55,32
Σ n-6/Σn-3	**84,94**	**43,02**	**54,90**

$p<0,005$

Characteristic and sensory evaluation of cakes

The evaluation of the three different cake mixes reveals distinct characteristics in terms of appearance, texture, taste, and aroma. Mix 1 yields a cake with a regular shape and a cracked upper crust, exhibiting a golden to reddish hue with normal thickness. The crumb is colorful, displaying small pores with occasional larger air pockets. The taste is sweet, and the flavor is typical of this type of cake (Fig.2).

In contrast, mix 2 results in a cake roll with a regular shape and a torn top surface. The top crust is golden to reddish, with normal thickness and no signs of burning. The crumb is colorful and well-baked, displaying thick-walled pores with large air pockets, more developed than those in mix 1 and 3. The taste is sweet, and the aroma is typical and characteristic of the cake's composition.

Mix 3 produces a cake with the correct shape and a golden top crust of normal thickness, without any signs of burning. The crumb is colorful, but underdeveloped, making it difficult to restore its original volume under pressure. The porosity is thick and compact, resulting in a chewy texture. The taste is sweet, lacking distinct flavor, and the aroma is pleasant and characteristic of the cake's composition(Fig.3).

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These sensory evaluations provide valuable insights into the visual, textural, gustatory, and olfactory attributes of the cakes produced from the different mixes, contributing to a comprehensive understanding of their overall quality and consumer appeal.

Fig. 2. *High protein gluten-free cake*

Fig. 3. *Slice of high protein gluten-free cake*

CONCLUSIONS

The utilization of whole grain bio spelt flour, bio sesame flour, bio sunflower flour, and other raw materials in the production of gluten-free high-protein cakes is highly advantageous due to their favorable technological parameters. These cakes are characterized by their high protein and omega-3 and omega-6 fatty acid content, coupled with low carbohydrate composition. Furthermore, the incorporation of natural colorants ensures compliance with food safety regulations, aligning with European legislation such as Regulation EU 1924/2006 on nutrition and health claims made on foods. Notably, the new products are free from genetically modified organisms (GMOs) and boast high levels of essential minerals including iron (Fe), zinc (Zn), calcium (Ca), and sodium (Na), as well as a rich vitamin profile encompassing vitamins A, K, B6, B2, B12, B17, and C. With an energy value of 270 kcal per 100g, these cakes cater to the dietary requirements of individuals with specific health needs, such as those with type 2 diabetes, cardiovascular diseases, and gastroenterological diseases, while also appealing to the general consumer. Moreover, the competitive pricing of these products presents an economic advantage over other offerings in the market, making them an attractive option for consumers seeking a balance between quality and affordability.

To effectively position these products in the market, strategic investments in marketing efforts and educational campaigns are crucial. Building consumer awareness regarding the health benefits of organic flours for addressing specific health concerns will be instrumental in driving market share growth and fostering consumer acceptance.

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