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Improving Red Color of Some Food Products using Red Beet Powder

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Abstract: ***Background**: using root beet powder pigments without extract or using organic solvents, to study the stability of pigments in model foods tomato paste, strawberry jam, juice, and burger, and to perform preliminary studies consumer acceptance of model foods containing them. **Objective**: The overall aim of this study was to use microencapsulation technique to increase the stability of red beet pigments as the natural colorant and use it to improving red color in food models. **Results**: Strawberry jam, tomato paste and burger were chosen as models to study the stability of pigments in foods, and consumer acceptance of model foods containing them. The color degradation of natural pigment from red beet powder was studied in pH, and temperature during the time was used to quantify the degradation process. The remaining absorbance at 535 nm as a function of the incubation at different values of pH for 120 hours was used to quantify the degradation process. The red beet pigment encapsulation with glycerol by freeze drying was better stabilized efficiently for pH values. unmodified pigment was reduced more than 40% with the increase of pH values. encapsulation red beet pigment was the most thermos resistant with a remaining absorbance of 95 % after 6 h at 150 °C. unmodified of red beet pigment showed remaining absorbance percentages of 64.22%, 21.38% and 8.14%, after 3 h at 150 °C respectively. In general, based on sensory evaluation data, strawberry jam could be successfully with encapsulated red beet powder without any adverse effects. Betacyanin and betaxanthin in foods with encapsulated red beet pigment were the highest content. Betacyanin content 5.84, 4.16 and 3.28 in burger, tomato paste and strawberry jam respectively. Betaxanthin content 3.13, 2.96 and 2.31 in burger, tomato paste and strawberry jam respectively. **Conclusion**: this study would be helpful in establish the betalain encapsulation techniques for increasing its stability as powdered food grade colorants.***

Keywords: Red beet powder, Betalain pigment, Betacyanin and betaxanthin, Strawberry jam, Tomato paste, burger, natural color

1. Introduction

Plants come in a huge variety of colors, and the compounds producing these colors are almost equally varied. Due to the great variation in structures and properties of these natural colorants, they offer lots of possibilities for use in different kinds of food matrices as added colorants. Although the properties of the four major plant colorant groups, chlorophylls, carotenoids, anthocyanins, and betalains are very different, there are some common properties as well. All of these colorants have antioxidative properties, which are related to many health benefits such as reduced risk of coronary heart disease and different types of cancer. Another common thing is that these compounds tend to be unstable, especially when incorporated in foods [16, 19].

Natural colorants are Generally Regarded as Safe (GRAS) substances. Therefore, they are more desirable than the synthetic ones for industrial or commercial applications as food additives. However, they are more expensive to obtain and usually they have lower stability, restricting sometimes their practical use as colorants [4, 24]. Therefore, natural pigment applications are typically limited to areas where particular reliability is required, such as food and cosmetics, where color is one of the most important attributes for product acceptance [21, 34]. Currently, the interest in using natural colorants has increased because of their non-toxicity and beneficial health effects, mainly as antioxidants [6, 30]. The number of synthetic dyes currently allowed by the FDA has been reduced from 700 to 7 [9, 5]. This is due to their negative effects on the environment and their links to allergic, toxic, carcinogenic, and harmful responses.

Beet root (Beta vulgaris) is botanically classified as an herbaceous biennial from Chenopodiaceae family and has several varieties with bulb colors ranging from yellow to red. Deep red-colored beet roots are the most popular for human consumption, both cooked and raw as salad or juice. There is growing interest in the use of natural food colors,because synthetic dyes are becoming more and more critically assessed by the consumer. But in food processing, as compared with anthocyanins and carotenoids, betalains are less commonly used, although these water-soluble pigments are stable between pH 3 and 7. To improve the red color of tomato pastes, sauces, soups, desserts, jams, jellies, ice creams, sweets and breakfast cereals, fresh beet/beet powder or extracted pigments are used [18, 23].

Betalains are N-heterocyclic water-soluble pigments found in certain plant families of the order Caryophyllales or the older name Centrospermae. Betalains and anthocyanins have never been found in the same plant and it seems that the biosynthetic routes of these two pigment groups are mutually exclusive. [16, 26, 32]. Betalains can be described as condensation products of a primary or secondary amine with betalamic acid. The general structure of betalains and betalamic acid are shown in figure 7. Betalains can be divided into two groups depending on the substitution of the amine group. In betacyanins, there is an aromatic ring in the form of a cyclodopa group and in betaxanthins, the substitution is an amine or amino acid. The aromatic ring in betacyanins extends the conjugation of the chromophore and their absorption maximum is around 540 nm (red or violet), whereas the absorption maximum of betaxanthins is about 480 nm (yellow) [16, 26].

The stability of betalain pigments is influenced by many factors including pH, temperature, oxygen, water activity, and light, similarly to other natural pigments. pH values between three and seven do not markedly affect the color, Below pH 3, the color of betanin shifts towards violet and above pH 7 the color shifts towards blue. In alkaline conditions (above pH 10), betanin degrades to yellow betalamic acid and colorlesscyclodopa-5-*O*-glucoside [15, 16, 26, 33]. This reaction is reversible and the pigment may be partially regenerated by mild heating at optimum pH of 4–5 [16, 26]. Betanin is also degraded at very acidic conditions [3, 15, 17].

Thermal degradation of betalains above temperatures of 50 °C is probably the main problem that restricts their use as food colorants. Heating of betanin causes the degradation to betalamic acid and cyclodopa-5-Oglucosidethan alkaline pH, and the reaction is similarly reversible. Other thermal degradation pathways are various decarboxylation reactions andremoval of the glycoside unit [14, 15, 16,, 26]. It has also been found out that pH affects thermal degradation rates with maximum betanin thermal stability at pH4–5 in anaerobic conditions and at pH 5–6 in aerobic conditions [33].

In food processing encapsulation is directly related to the coating of minute particles of ingredients as well as whole ingredients by micro encapsulation or macro encapsulation techniques. Encapsulation technology has been used in the food industry as a way to provide liquid and solid ingredients as an effective barrier for environmental and/or chemical interactions until release is desired. Microcapsules have the ability to preserve a substance in a finely divided state and to release it as occasion demands [20, 27, 28].

In food systems, microencapsulation prevalent objective is to protect the core material from degradation by reducing its reactivity to environmental conditions [12, 25]. There are different kinds of encapsulating agents such as polysaccharides (starches, maltodextrins corn syrups and arabic gum), lipids, proteins (gelatin, casein, soy and wheat protein) were used. The most commonly used material for microencapsulation are maltodextrins [12, 13]. Maltodextrins are starch hydrolysates that have important matrix forming properties as wall materials [7].

According to Barbosa et al. [2] typically used encapsulating materials are gum acacia, maltodextrins, hydrophobic starch, carboxy methyl cellulose and mixtures of them. Several materials such as starches, carboxy methyl cellulose, gelatin, maltodextrins, sodium alginate, sodium caseinate, pectin, gum Arabic, guar gum, chitosan etc. can be used for encapsulation. Several encapsulation techniques are used in food industries. Encapsulation of the secondary metabolities has great impact on their stability to higher temperatures such as in treatment like spray drying. Micro encapsulation is by which the secondary metabolities are coated with a thin film of protective material. They have more applications in the food industries as they increase the stability, increase the shelf life and easier to handle.

Encapsulated colors are easier to handle and offer better solubility, stability and improves flow properties and reduces dusting when the nutrient are added to dry mixtures [12]. The stabilization of pure betalain pigments may boost the use of these natural bioactive and colouring molecules in the food industry and promote their application in the pharmaceutical and cosmetic areas. Encapsulation can be performed by spray-drying, freeze drying, air suspension coating, extrusion, spray-cooling and spray- chilling, centrifugal extrusion, rotational suspension, simple and complex coacervation. The stability of betalains could be improved using spray drying [8]. Spray-drying process has been used for decades to encapsulate food ingredients such as flavors, lipids, and carotenoids, in the drying process, evaporation of solvent, thatis most often water, is rapid and the entrapment of the compound occurs quasi instantaneously [1, 11].

The aim of this thesis study was to use microencapsulation techniques with glycerol to improve the stability of natural colorant compounds of red beet powder (Beta vulgaris). Effect of glycerol as encapsulating agents on stability and color of freeze dried betalain. The results of this study would be helpful in establish the betalain encapsulation techniques for increasing its stability as powdered food grade colorants.

Other objectives were to using root beet powder pigments without extract or using organic solvents, to study the stability of pigments in model foods tomato paste, strawberry jam, juice, and burger, and to perform preliminary studies consumer acceptance of model foods containing them.

2. Materials and Methods

2.1 Plant Material Preparation

Fresh red beet root (Beta vulgaris L.), Chenopodiaceae family (Chenopodiaceae) was procured from a local market at El Arish, North Sinai, Egypt. Cleaned, washed and peeled whole beet root was crushed and the pulp was evenly spread in trays (300 X 300 X 7 mm) to form a slab. Dried by hot air dryer at (50 C°). Moisture content of beet powder was estimated and was expressed on dry basis. The initial moisture of beet root was 8.5±0.2 % and samples were dried till their moisture was reduced to around 0.04 %.

2.2 Pigment Extraction and Encapsulation

Pigment extraction was carried out in a 200-mL distilled water and 2 g beet powder, at 180 rpm at room temperature for 30 min. The supernatant waslabeled as unmodified pigment and analyzed for betalain content (betacyanin and betaxanthin). Red beet pigments extract was encapsulated with glycerol as the main carrier and encapsulating agents. Encapsulation of red beet pigments was prepared by mixing 100 ml of the unmodified pigment with 20 g of glycerol and was mixed homogeneously. The samples were frozen for 3h at −18 ° C. They were subsequently freeze dried for 2 days to ensure complete drying (freeze drier Alfa-Christ, Germany). The final product was in dry powder form and this was labeled as modified pigment.

2.3 Properties of Pigment

2.3.1 Effect of pH

A preliminary study was conducted to test the stability of unmodified pigment and modified pigment in different pH media that ranged from 2.0 to 10.0 for 120 h and then percentage of color loss was calculated [10]. Retention value of pigments were estimated according to the following equation (1) :

$$\text{Retention of pigment (\%)} = \frac{\text{Pigment. Absorbance after treatment}}{\text{Pigment.initial absorbance}} \text{X } 100$$

2.3.2 Thermal stability

The samples of unmodified pigment and modified pigment were thermostated in a water bath at the desired temperature. The degradation was followed at 150 °C for 6 h. The changes in absorbance were monitored at 535nm intervals of 0.5, 1, 2, 3, 4, 5 and 6 h. After this time, the tubes were immediately cooled in an ice-bath to stop thermal degradation. Retention value of pigments were estimated according to the following equation (1).

2.4 Model Food Applications and Color Measurement

Trials were made to utilize the encapsulated pigments in improving unmodified of some food products included strawberry jam, tomato paste and burger. The products was prepared with 0.1 % synthetic color (new coccine E124).Encapsulated red beet pigment (modified pigment) and red beet powder (unmodified pigment) were added to strawberry jam, tomato paste and burger at concentrations of 5 g/kg products. The jam was prepared according to Egyptian Standard 129, (2005), The tomato paste was prepared according to Egyptian Standard 132, (2005) and The burger was prepared according to Egyptian Standard 1688, (2005).Control sample of all products without addition of pigments was prepared under the same conditions. The samples of products were evaluated for their quality attributes at interval periods up to 6 months of storage. Strawberry jam and tomato paste storage at 25C° but burger storage at -15 C°.

2.4.1 Sensory evaluation

Sensory evaluation was carried out by ten panelists. The panelists were asked to evaluate color, taste, odor and overall acceptability for prepared strawberry jam, tomato paste and burger according to the method described by [22].

2.4.2 Determination of betalain in products

0.1 g of each prduct samples were dissolved in 10 ml of 50% ethanol, were agitated for 10 s and the homogenate was centrifuged at 6000 rpm for 10 min. The supernatant was collected as it is after centrifugation and the same was repeated for 2 more times to ensure maximum extraction of betalains. The supernatant was further used for determination of betalains. The content of betaxanthins and betacyanins in the extracts was determined spectrophotometrically at 538 nm and 480 nm with a UV–Vis spectrometer, respectively according to the methods of Stintzing, Schieber, and Carle [31]. The absorbance reading obtained was used to calculate the betalain concentration for each sample. The betalain content (BC) was calculated every tow month.

$$\text{BC (mg/g)} = \frac{(A \times DF \times MW)}{(e \times l)}$$

where A is the absorption, DF the dilution factor and l the pathlength (1 cm) of the cuvette. For quantification of betacyanins and betaxanthins, the molecular weights (MW) and molar extinction coefficients (e) (MW=550 g/mol; e=60,000 L/mol cm in H_2O) and (MW=308 g/mol; e=48,000 L/mol cmin H_2O) were applied.

2.5 Statistical analysis

All analyses were performed in triplicate and data reported as mean ± standard deviation (SD). Data were subjected to analysis of variance (ANOVA) ($P<0.05$). Data of sensory evaluation were analyzed by the analysis of variance.

3. Results and Discussion

3.1pH Stability

Relative amounts of red beet powder pigment (unmodified pigment) and encapsulated pigment (modified pigment) samples during 120h at different pH values between 2 to 10 are shown in figure (1). As the initial amounts of pigment were different in samples, pigment contents after 120 h were compared to the initial amount in the same sample easier comparison between samples. It was observed that pigments were almost stable under acidic conditions displayed their most intense red coloration at acidic pH. Even holding pigments at the acid pH 7 values for a period as long as 120 h, the red color of the pigments its high stability. With increasing pH value of up to 7, the red color greatly faded and almost appeared colorless at pH value of 10. By continuous increasing pH up to 7.0, the color of the unmodified pigment changed to violet and finally became blue at pH 8-9. Holding modified pigment at the different pH values for 120h showed the high stability.

These results may be explained based on the structural transformations of betalain pigment as a function of pH [16]. In the aqueous medium, including foods, betalain can exist in equilibrium of four possible structural forms depending on the pH: The red flavylium cation, the blue quinonoidal base, the colorless carbinol pseudo base and the colorless chalcone. At acidic pH, the flavylium structure was favoured; at pH values of 7 – 8 the colourless carbinol was found to be dominant, while at pH ≥ 7, the quinonoidal form dominated. Freeze drying encapsulation with glycerol showed a higher recovery of betalains . During increase in pH values which might have reduced the betalains content in unmodified red beet pigment when compared to modified pigment with freeze drying. The sensitive substances that are unstable in aqueous solutions may be efficiently encapsulated by this technique.In our study, the red beet pigment encapsulation with glycerol by freeze drying was better stabilized efficiently for pH values.

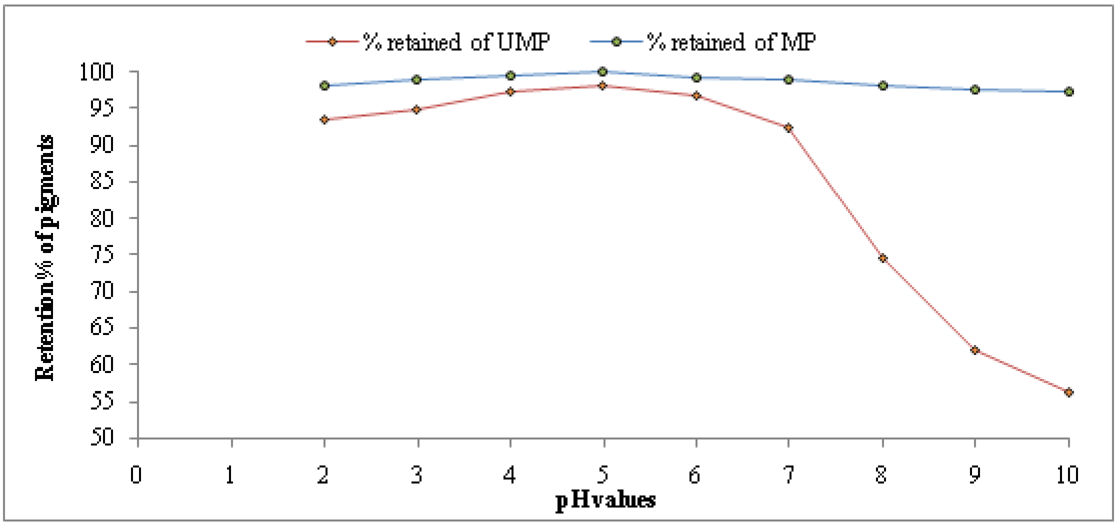

Figure 1: Retention percentage of modified and unmodified pigments from red beet as a function of pH values. Red beet powder pigment (UMP), encapsulated pigment (MP), values are expressed as a mean ± SD; n=3

3.2 Thermal stability

The instability of natural pigments to temperature results in structural and color properties. The degradation pattern depends on both the temperature and time. Most color variation patterns show a direct relationship with the intensity of the thermal treatment.

Figure (2) shows the incidence of heating at 150 °C on the absorption spectra of the red beet pigment and encapsulated red beet pigment. As can be seen, the rate of color degradation at 0, 0.5, 1, 2, 3, 4, 5 and 6 h. The most relevant result of this treatment was the pronounced instability of the red beet pigment (unmodified pigment) had lost their red color after 2 h of the test. The degradation increased as the temperature rose. The red encapsulated pigment (modified pigment) was most stable to temperature of all of them, and even after the harshest treatment (6 h at 150 °C), 85 % of absorbance at 535 nm remained. The pigment was in an encapsulation with glycerol and freeze dried was more resistant to temperature after 6 h of incubation at 150 °C, showed a remaining absorbance of 94.65 after 5 h and 87.23 % after 6 h respectively.These results directly related to the coating of minute particles of ingredients as well as whole ingredients by micro encapsulation.

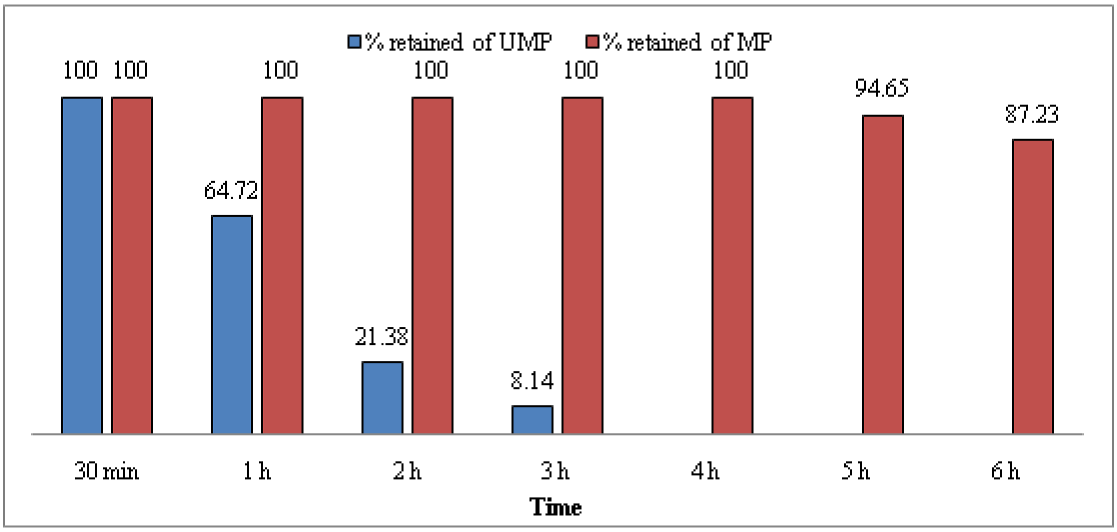

Figure 2: Retention percentage of modified and unmodified pigments from red beet as a function of time at 150°C Red beet powder pigment (UMP), encapsulated pigment (MP), values are expressed as a mean ± SD; n=3

3.3 Model Food Applications and Color Measurement

3.3.1 Sensory Evaluation

Sensory evaluation of model foods colored with red beet pigment (unmodified pigment) or encapsulated pigment (modified pigment) were done to study consumer acceptance as natural red colors. Model foods jam, tomato paste and burger colored with 0.1 % synthetic color (new coccine E124) to compared with natural colors.

The appearances all of the model foods colored with modified pigment 'strong' colors were the most liked. Mean scores with standard deviations and statistically significant differences between model foods (p<0.05) for pleasantness of appearance are shown in figure (3,4 and 5). There were no big differences between pleasantness of odor of the samples (mean scores 8 - 8.5). Similarly, the pleasantness of taste was very similar in all of the samples had the same scores 9.

Data showed in figure (3) strawberry jam sample scored higher values and was significantly preferred than control and other samples. Even the sample colored with 0.1 % synthetic color scored high value and was significantly preferred than control and unmodified sample, color of modified jam sample was still highly accepted. In general, based on sensory evaluation data, strawberry jam could be successfully with encapsulated red beet powder without any adverse effects.

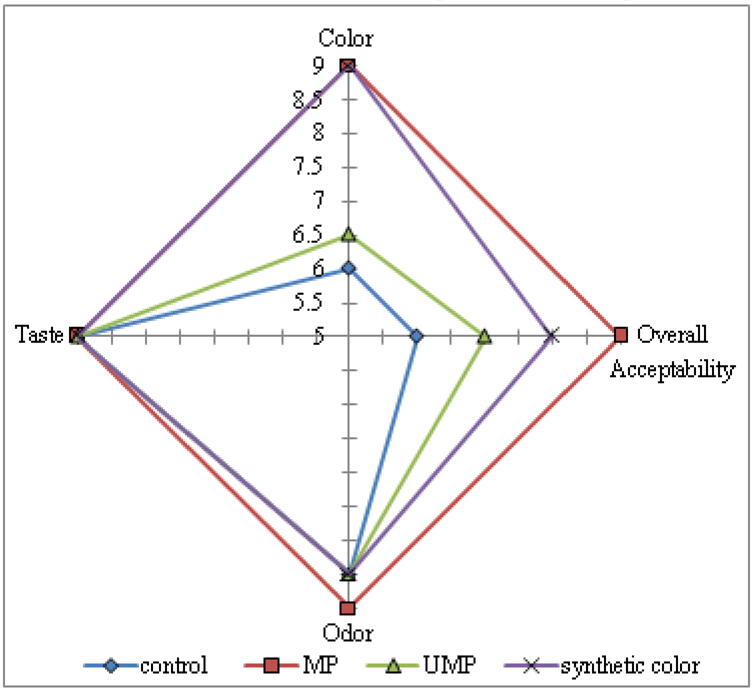

Figure 3: Sensory evaluation of Strawberry jam prepared with modified and unmodified pigments red beet Red beet pigment (UMP), encapsulated pigment (MP), synthetic color (new coccine E124) and control is sample without color.values are expressed as a mean ± SD; n=3

Tomato paste samples were sensory evaluated for their color, odor, taste, and overall acceptability. The results showed in fig (4) that color of control sample,which without color scored the lowest values of color and acceptability. No significant differences could be found between unmodified pigment sample and sample colored with synthetic dye regarding the color and overall acceptability. Samples which colored with modified pigment scored the highest values for color and overall acceptability.

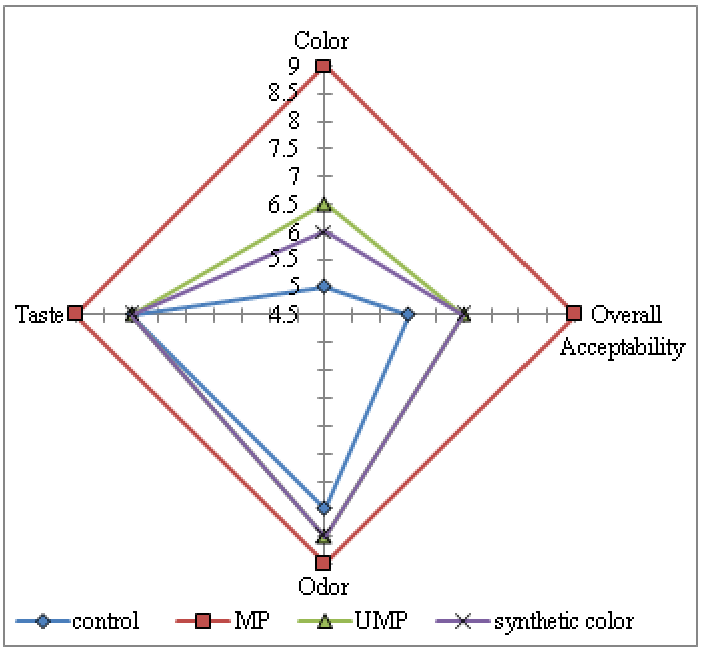

Figure 4: Sensory evaluation of Tomato paste prepared with modified and unmodified pigments from red beet

Red beet pigment (UMP), encapsulated pigment (MP), synthetic color (new coccine E124) and control is sample without color,and control is sample without color,values are expressed as a mean ± SD; n=3.

The burger prepared by adding natural color from red beet (modified and unmodified) pigments had a highest score for color and overall acceptability respectively. On the other hand, the burger prepared by adding synthetic dye had a lowest score of color and overall acceptability respectively.

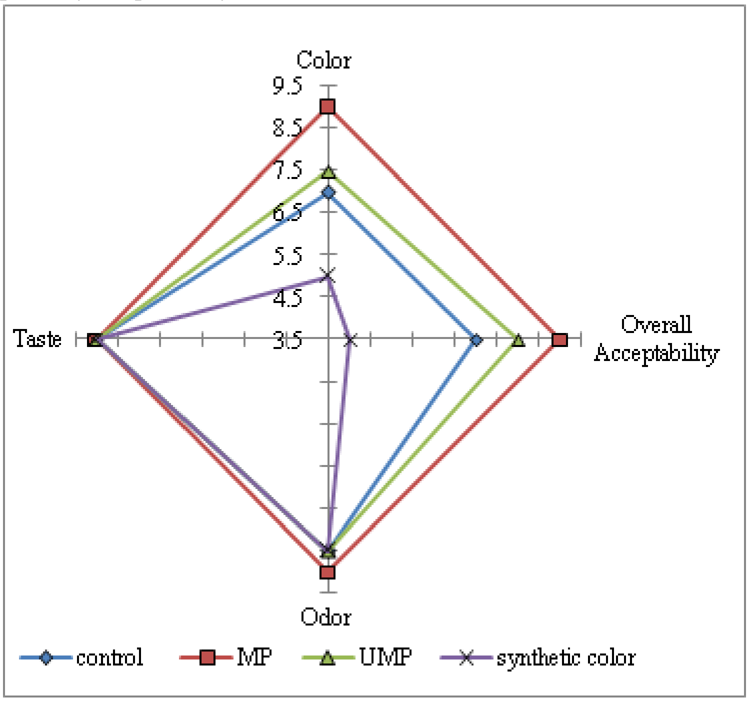

Figure 5: Sensory evaluation of burger prepared with modified and unmodified pigments from red beet
Red beet pigment (UMP), encapsulated pigment (MP), synthetic color (new coccine E124) and control is sample without color.values are expressed as a mean ± SD; n=3

3.3.2 Betalain content in products

Betacyanin and betaxanthin decreased with increase in temperature and time, they used to calculate the betalain content for each sample. the decrease in betacyanin and betaxanthin was attributed to the little availability of the pigment food samples. Results given in figure (6) showed significant differences between samples colored with modified red beet pigment and colored with unmodified pigment. This indicates that the addition of the encapsulated pigment to the food models could be successively used to improve the color without adverse effects on the quality attributes. Betacyanin and betaxanthin in foods with encapsulated red beet pigment were the highest content. Betacyanin content 5.84, 4.16 and 3.28 in burger, tomato paste and strawberry jam respectively. Betaxanthin content 3.13, 2.96 and 2.31 in burger, tomato paste and strawberry jam respectively. In food samples with unmodified pigment betacyanin and betaxanthin were affected by high temperature in strawberry jam and tomato paste so that decreased the concentrations in samples. For burger samples which colored with unmodified pigment, it can be seen that there is a tendency to a red color in all samples with a small decrease in betacyanin and betaxanthin content 4.97 and 2.76, this results directly related to the effect of low temperature in the burger producing.

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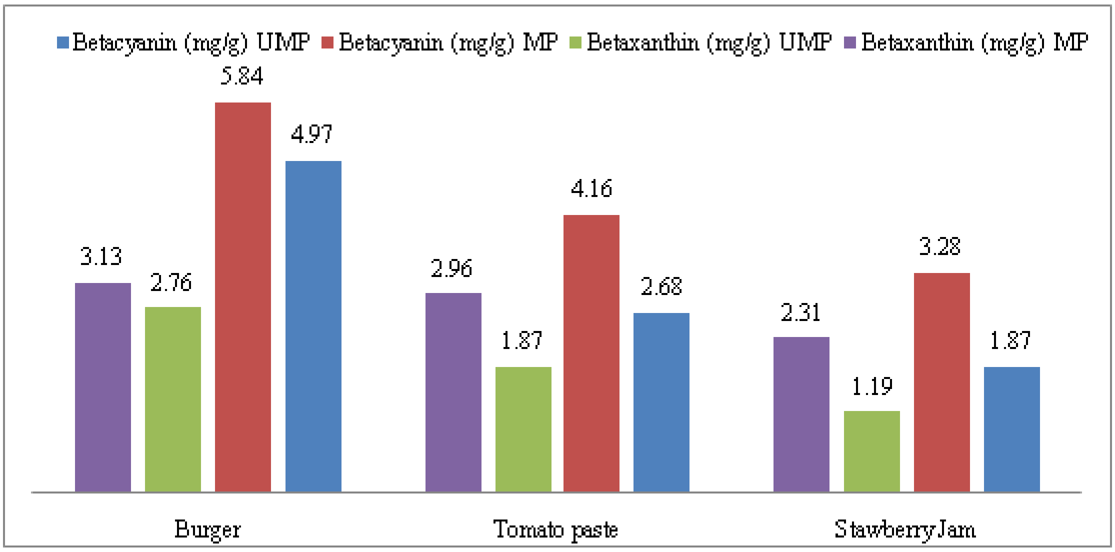

Figure 6: Betacyanin and betaxanthinas contents in food models prepared with modified and unmodified pigments from red beet

Red beet pigment (UMP), encapsulated pigment (MP).values are expressed as a mean ± SD; n=3.

4. Conclusion

Encapsulation with glycerol showed a higher recovery of red beet pigments during freeze drying techniques. This indicates that the addition of the encapsulated pigment to the food models could be successively used to improve the color without adverse effects on the quality attributes.

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