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EFFECT OF SOME TYPES OF CARRAGEENAN ON CHEMICAL AND BIOLOGICAL CHARACTERISTIC IN THE STRAWBERRY JAM

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Abstract

The aim of the research is to introduce Carrageenan (Kappa and Iota) into Strawberry jam instead of Pectin and know its effect on chemical and biological characteristics, using three weights (0.1, 0.3, 0.5g) for each model and at three replicates and compare the results with the control treatment (strawberry jam containing pectin). And chemical and biological tests and know the effect of added weight on these products. The data were analyzed in a statistical program and the mean differences between the averages were compared with (L.S.D) test the lowest significant difference ($P \leq 0.05$). The result of the study found significant differences in chemical tests in the proportion of reducing and total sugars and the amount of total Phenols and Anthocyanin. No significant differences were found in the percentage of restraint of free radicals using the DPPH method. As for biological examination, the study showed that there is no growth of microorganisms in all sample.

Key words: strawberry jam, (kappa, iota) carrageenan, pectin.

Introduction

Marine plants are more primitive and richer than wild plants. Seaweeds are important marine resources that play a major role in supporting the rich marine biodiversity. Seaweed is a large algae, which is found naturally in marine habitats. The main commercial values of seaweed are increasing with their chemical components such as alginate, carrageenan and agar which are multiple sugars, and these sugars are widely used in food and industrial applications (Venkatesan, *et al.*, 2017). Carrageenan is an anionic Hydrocolloid extracted from the red seaweed of the Hypnea, Eucheuma, Gigartina, Chondrus and Iridaea species. It is a polysaccharide contain of sulfate group that is widely used in the food industry to give density and as a stabilizing agent and to be a gelatinous and cohesive material in toothpaste.

Recently, Carrageenan has been used on a variety of other fields such as pharmaceuticals, tissue engineering and biological sensor applications (Thakur, *et al.*, 2018, Venkatesan, *et al.*, 2017). Carrageenans are high molecular weight mucopolysaccharides that contains in its composition a sulfate half-ester which makes it have an ionic strength that the gel materials will be formed in the presence of positive ions such as calcium, sodium and potassium (Visakh, *et al.*, 2019). Carrageenan's structure contains many groups of hydroxyl and sulfate groups with negative charge and therefore makes it hydrophilic and multi-negative charge (Perez and Claudio, 2020) the molecular weight of carrageenan is high (between 100 and 1,000 kDa) (Ramawat and Me´rillon, 2015). Carrageenan is generally formed by attaching 3.6-anhydrogalactose units to galactose sulfate units. These products can be used as additives in making jelly sweets, pizza, barbecue sauces, and canned pet food.

In the European system, carrageenan is symbolized as E407 and has a galactan structure (Fleurence and Levine, 2016) Carrageenan is divided into six forms based on the number and locations of SO_3 groups: kappa(k), lambda (λ), iota (ι), mu (μ), nu (ν), and theta (θ) Among of these Six species, three from those species are commercially important (κ, λ and ι). The difference in the basic structure is the result of substitution with hydroxyl groups (OH) in sugar units and the absence of 3, 6-ether bonds. Different types of carrageenan are created depending on the number and locations of sulfate groups. These groups in the carrageenan have a strong negative charge and are responsible for the chemical reaction (Ahmed and Soundararajan, 2019). Kappa-carrageenan (K car) is a naturaly acidic multi-sugar (Ghanam, 2010). It consists of a repeated units of bonding 3 linked β-D-galactose 4-sulphate and bonding 4 linked 6-anhydro-α galactopyranose with one sulfate group per repetition of disaccharide. κ-carrageenan generally forms strong and solid gels with potassium salts but the gel becomes brittle with calcium salts (Ahmed and Soundararajan, 2019). In the presence of potassium ions, aggregation of K car as helix together in larger units where the potassium ion forms electrostatic reactions with sulfate groups and Anhydrous Oxygen (Ramawat and Me´rillon, 2015). As for the type Iota carrageenan (I car), it has two groups of sulfates in the repeat unit of disaccharide and unlike kappa, the resulting gel is flexible with calcium salts (Ahmed and Soundararajan, 2019). This type differs from other basic forms due to the presence of a sulfate group on C_4 for the unit of galactose and another group of sulfate on C_2 for a 3, 6-anhydro-galactose unit which is among the three synthetically important carrageenan. When this type forms a three-dimensional network of spirals, it is observed that the sulfate groups are directed towards the outer part that is necessary for the properties of gel formation (Perez and Claudio, 2020). The Joint Committee of Experts of the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization on Food Additives (WHO) issued a technical report that the use of carrageenan is not a source of concern and can be added to infant formula as food and for medical purposes at concentrations of up to 1000 mg/l (WHO, 2015).

China is one of the countries topping the list in marine algae cultivation for use in nutrition, medicine, biofuel manufacture and many other areas. The Arab region can enter this sector if it is developed and employed according to peaceful principles. In view of the lack of cultivation of marine algae on a commercial scale in Iraq and consequently the lack of production of carrageenan, bearing in mind that their use in food manufacturing has a significant economic return compared to other food additives and the lack of comprehensive and extensive studies for this product, this study aimed to:-

1- The use of different concentrations of carrageenan in the manufacture of strawberry jam.
2- Study the chemical properties of strawberry jam, choose the most suitable form of carrageenan, and use ratios.

Material and method

Sources of experience materials

Carrageenan (kappa and Iota) was obtained from Xian Wonderful Herb Biotechnology/China, frozen strawberries and pectin were from the local markets in Baghdad.

Preparation of jam:-

Strawberry fruits are taken at a rate of (202,97)g, cut and added water at a rate of (49.5)g, sugar (247,52)g and pectin at a rate of (0.73)g as control, after which the mixture is cooked and before the completion of cooking, and adding (1.76)ml of citric acid (Mehdi and Al-Hakim, 1985).

The work of jam with different concentrations of carrageenan of the two types (Kappa and iota)

Preparing jam, as in the above method, but instead of adding pectin, the carrageenan kappa and iota are added in different concentrations and in two experiments separately. K car type is added in three different concentrations (0.1, 0.3, 0.5)g with Potassium chloride (kcl) is at a concentration of (5) mM and then. I car it uses the same method of preparation and work, but instead of adding potassium chloride (kcl), calcium chloride ($CaCl_2$) is added at a rate of (2.4) mM. This experiment was conducted with three replicates of the control model and three replicates using three concentrations of carrageenan (Kappa and iota).

Chemical analysis

Sugar analysis

Reduced and total sugars were estimated using the Lane-Eynon method, (Ruck,1963).

Determination of phenols and anthocyanin

Extraction of sample

The method proposed by Vukoja *et al.*, (2019): 1 g of the sample is taken with 10 ml of acidified methanol ratio as (methanol: hydrochloric acid 98 : 2) and left for 12 hours at 4^0c after which the mixture is filtered and the filtrate is taken.

Quantification of phenolic compounds

By following the method proposed by Rodrigues *et al.*, (2017) using the Folin-Ciocalteau (FC) detector. As 0.5 ml of each sample extract is added to tubes containing 2.5 ml of Reagent Solution (FC) at 10%. Then add 2 ml of 4% sodium carbonate solution, moving the tubes and leaving it for two hours in darkness at room temperature. The blue color resulting from the detector (FC) reduction is measured by phenolic compounds by measuring the spectrum of the wavelength of 750 nm in spectrophotometer, the phenol content was calculated from the line equation that was Obtained from the standard curve of Gallic acid The results are expressed in mg of gallic acid equivalent per 100 g sample (mg GAE/100g). The standard curve is prepared using a solution of Gallic acid by dissolving 10 mg of the acid in 10 ml of ethanol and then diluting it to 100 ml of water The final concentration becomes 0.1 mg/ml, after which different concentrations are taken and diluted to 100 ml of water and the concentration is µg/ml (Waterhouse, 2002) as shown in table 1.

Table 1: Preparation of standard curve.

Tube number	Volume of added gallic acid solution (ml)	Distilled water(ml)	Concentration of Gallic acid
1	0.1	0.9	20
2	0.2	0.8	40
3	0.3	0.7	60
4	0.4	0.6	80
5	0.5	0.5	100

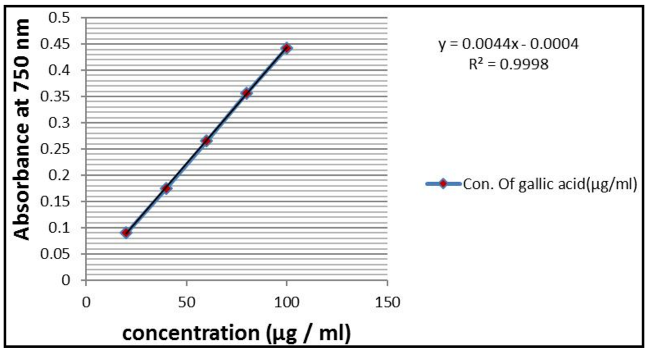

Fig. 1: Standard curve of Gallic acid.

Anthocyananin analysis

Using differential pH method proposed by Giusti and Wrolstad (2001), the total anthocyanin content was calculated as milligrams (Cyanidin-3-Glucoside) equivalents per gram sample (mg cy-3-glu/g). Calculate the sample absorption (A) as follows:

$$A = (A\lambda \text{ vis-max} - A700) \text{ pH } 1.0 - (A\lambda \text{ vis-max} - A700) \text{ pH } 4.5$$

Calculate the concentration of anthocyanin the original sample using the following formula:

$$\text{Anthocyanin dye concentration (mg/L)} = (A \times MW \times DF \times 1000) / (\varepsilon \times 1)$$

Whereas:

MW = molecular weight (449.2 g/mol).

DF = Dilution Factor.

ε = molar absorptivity (26.900 l / mol. cm).

Estimating the antioxidant activity using a DPPH method

Extraction of sample

The method proposed by Rodrigues *et al.*, (2017), followed that 2 g of the sample is taken and 20 mL of methanol alcohol 50% concentration is added then homogeneity of the sample and left for one hour at room temperature, then the mixture is centrifugation for 15 minutes.

Determining the antioxidant activity by DPPH

The method proposed by Rufino *et al.*, (2007) followed, 0.1 ml of the extract is taken and 3.9 ml of DPPH solution is added. The control sample was by adding 0.1 mL methanol to the DPPH instead of the sample. And then measured after 2 hours on a 515 nm wavelength in spectrophotometer.

Results are expressed as a percentage of free-radical inhibition activity Sequestration (FRS%) according to the following formula:

$$FRS\ \% = (Ac - Am) \times 100\ Ac^{-1}$$

whereas:

Ac = absorbance of the control

Am = absorbance of the sample

Microbiological tests

The total account of microorganisms in strawberry jam was estimated using the Nutrient Agar medium (Jay, 1996). Yeasts and fungi were estimated using Potato Dextrose Agar (PDA) medium (A.P.H.A, 1984).

Result and discussion

Chemical Analysis

Sugar analysis

Table 2 shows that there are significant differences ($p \leq 0.05$) in the values of reducing sugars and total sugars of strawberry jam samples with added carrageenan (Kappa and iota) in comparison with the control, and the reason for that is due to the difference in the chemical composition between the two carrageenan types, as the

Table 2: Shows the percentage of reducing sugars and total sugars of strawberry jam models.

	Treatment	Glucose%	Fructose%	Total Sugars%
1	Pectin	29.43abc	31.28abc	60.71_b
2	K car 0.1	29.43abc	31.28abc	60.71_b
3	K car 0.3	28.98bc	30.81bc	59.79_b
4	K car 0.5	28.12c	29.83c	57.95_c
5	I car 0.1	30.88a	32.75a	63.63_a
6	I car 0.3	30.38ab	32.27ab	62.65_a
7	I car 0.5	29.43abc	31.28abc	60.71_b
	L.S.D	*1.7309	*1.7309	*1.7309

- (*) ($P \leq 0.05$).
- The numbers represent the average of three iterations of the sample.
- The various small letters indicate significant differences ($p \leq 0.05$).

Iota contains on sulfate groups more than the Kappa type and this leads to a smaller number of reactive sites (OH groups) to linked the Iota type with sugars by hydrogen bonds and thus the amount sugars association with Iota is less than the Kappa type, and it is noted from the results also that the increased concentration of carrageenan. The percentage of total and reduced sugars decreased due to the increase in associations with sugars, and this is consistent with (Yang, *et al.*, 2020; Raman, *et al.*, 2019; Miele, *et al.*, 2016).

The highest value for reducing sugars % (glucose, fructose) and total sugars % in strawberry jam for the models added to it is I car (0.1 and 0.3)g. The glucose sugar reached 30.88% and 30.38%, fructose sugar 32.75% and 32.27% and total sugars 63.63% and 62.65%, respectively and the lowest value was in the jam added to Kaba Carrageenan (0.3 and 0.5) g. The glucose sugar reached 28.98% and 28.12%, fructose sugar 30.81% and 29.83% and total sugars 59.79% and 57.95%, respectively, although Jam added to K car 0.1 g and I car 0.5 g, which has a sugar level of glucose 29.43% fructose sugar 31.28% and total sugars 60.71% which is equal to the control treatment as shown in Fig. 2.

The percentage of total sugars in strawberry jam with added pectin (control treatment) is consistent (Berni, *et al.*, 2017). Stenner *et al.*, (2016) explained that sugars enhance the conversion of carrageenan to gel through hydroxyl OH groups for sugar where hydrogen bonds are formed with carrageenan filaments, thus facilitating the formation of the Junction Zone and thus gel formation. Perumpuli *et al.*, (2018) indicated that proper sugar content is a critical factor in gel formation and works to preserve jam. Okut and others (2018) indicated that sugar is one of the main components in the formation of streamlined behavior of jam and helps to extend expiry and enhance taste and the texture of jam and must be the proportion of reduced sugar (30-35)% of the total sugar in the jam to avoid the problem of crystallization.

Determination of total phenols, anthocyanin and free-radical inhibition activity ratio by DPPH

Table 3 shows that there were significant differences in the amount of total phenols and anthocyanin, while there were no significant differences in the percentage of free-radical inhibition activity in the DPPH method for strawberry jam models with two carrageenan types (Kappa and Iota) compared to the control model.

The reason for the variation in the total amount of phenols between the samples is due to the difference in the chemical composition between the types of carrageenan (Kappa and Iota) as the phenols have the ability to interact in the medium with the carrageenan because they contain hydroxyl groups (OH) and they decrease by increasing the concentration of carrageenan and its quantity in the type Kappa is less of an Iota type for increased correlations and this is consistent with what it came to (Nassar, *et al.*, 2016). The values of

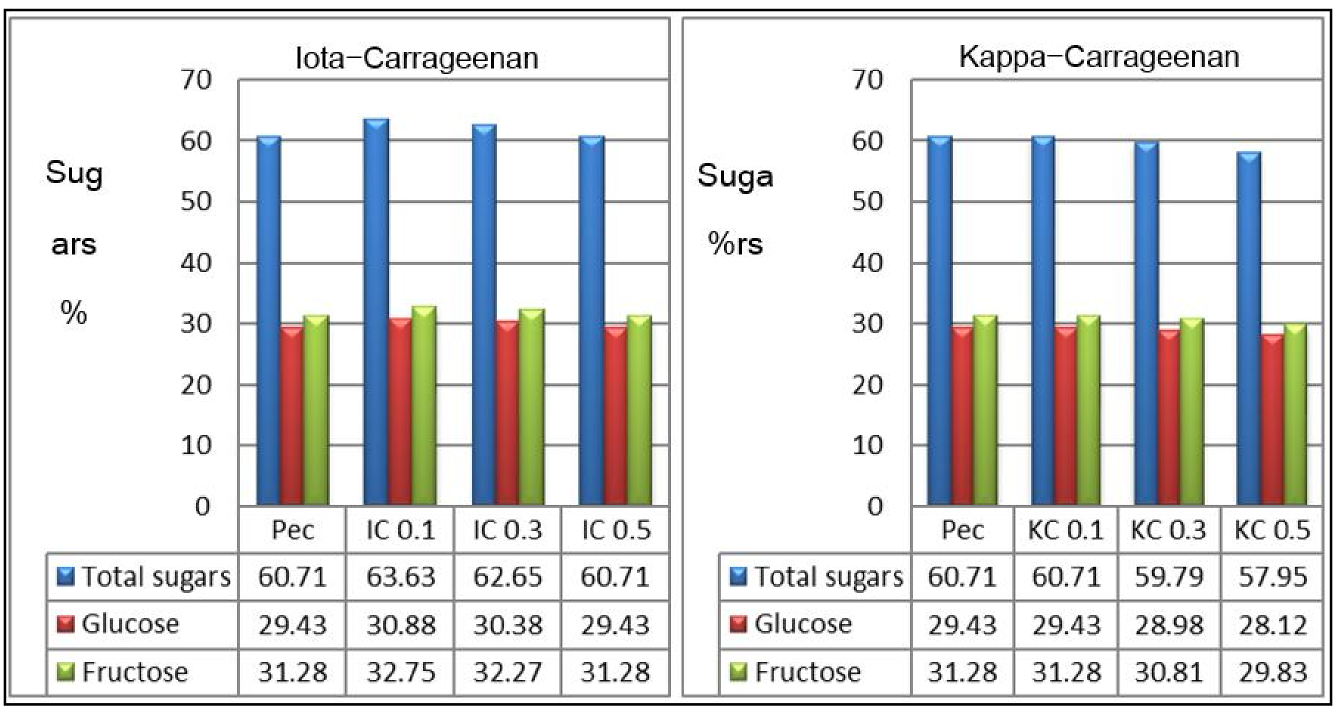

Fig. 2: Shows the difference in the values of reducing and total sugars between the strawberry jam models for which two carrageenan (Kappa and Iota) are added to the control model.

Table 3: Shows the amount of total phenols, anthocyanin, and ratio of DPPH for strawberry jam.

	Treatment	Total phenol (mg GAE/100g)	Anthocyanin (mg cy-3-glu/g)	DPPH%
1	Pectin	265.52_c	154.79_d	58.96_a
2	K car 0.1	263.35_d	219.13_a	58.52_a
3	K car 0.3	261.41_e	172.69_b	59.03_a
4	K car 0.5	259.64_f	132.93_f	59.13_a
5	I car 0.1	278.58_a	168.75_c	59.06_a
6	I car 0.3	274.82_b	139.91_e	59.81_a
7	I car 0.5	261.94_{de}	$_g128.93$	60.15_a
L.S.D		*1.7309	*1.7309	N.S 1.7309

- (*)($P \leq 0.05$).
- (N.S) Not Significant.
- The numbers represent the average of three iterations of the sample.
- The various small letters indicate significant differences ($p \leq 0.05$).

anthocyanin change in models, and it is noted that the greater the concentration of carrageenan, its content in the jam decreases due to the electrostatic connections between the positive charge of anthocyanin and the negative charge of carrageenan and this is consistent with what it says (Chesnokova, *et al.,* 2015), where he shows that the more concentration of K car leads To the saturation of the heterocyclic anthocyanin compound, thus lower dye release. The use of multiple sugars, whatever their charge, in extracting anthocyanin results in a change in the pH of the system. The pH of the anthocyanin dye solution depends on the concentration and type of sugars. Multiple negatively charged polysaccharides react electrostatically with the heterocyclic oxygen of anthocyanin which creates a stable compound (Xie, *et al.,* 2019 Navikaite, *et al.,* 2016; Chesnokova, *et al.,* 2015; Klimaviciute, *et al.,* 2015).

Fig. 3 shows that the highest value of total phenols was in strawberry jam with I car 0.1 g which amounted to 278.58 mg/100 g and the lowest value was in the K car 0.5 g with amount of 259.64 mg/100 g compared to the treatment jam Added control for pectin which amounted to 265.52 mg/100 g. The total amount of phenols for the control treatment is consistent with (Martinsen, *et al.,* 2020) and is not consistent with what it says (Rodrigues, *et al.,* 2017; Pineli, *et al.,* 2015).

Brandão *et al.,* (2018) said that thermal energy is not the only factor that converts biologically active agents in foods but treatment with citrus-related acids can enhance the appearance of other phenolic compounds such as the decomposition of anthocyanin into phenol acids. This decrease in phenolic compounds is due to the numerous oxidation reactions that occur during thermal treatment, and this in turn affects the sensory properties of a particular product because these compounds affect changes in the flavor, color, and smell of the product. Waterhouse (2002) showed that phenols are affected by sugar level as they increase when sugar levels are low.

Fig. 4 shows that the highest value of anthocyanin in strawberry jam was k car 0.1 g which amounted to 219.13 mg/g and the lowest anthocyanin content for the I car 0.5 g which amounted to 128.93 mg/g compared to the control treatment jam which has a value of 154.79 mg/g. The amount of anthocyanin for the control treatment is consistent with what it says (Mahdi, *et al.,* 2019).

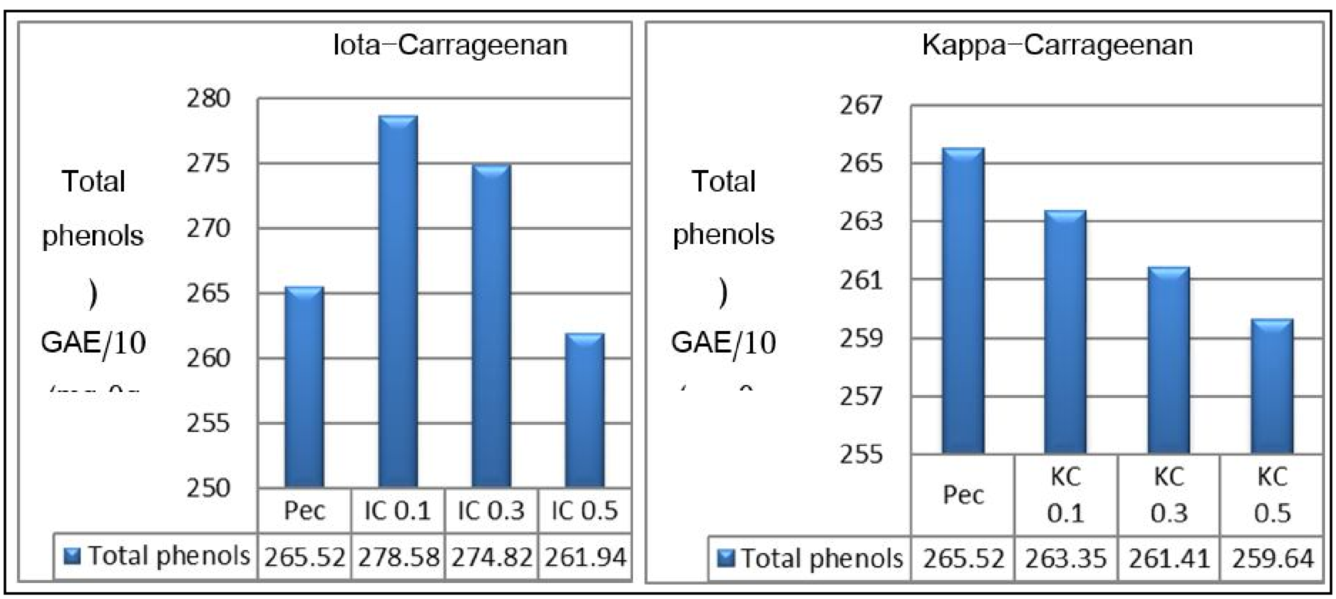

Fig. 3: Shows the difference in the values of total phenols between the strawberry jam models for which two carrageenan (Kappa and Iota) are added to the control model.

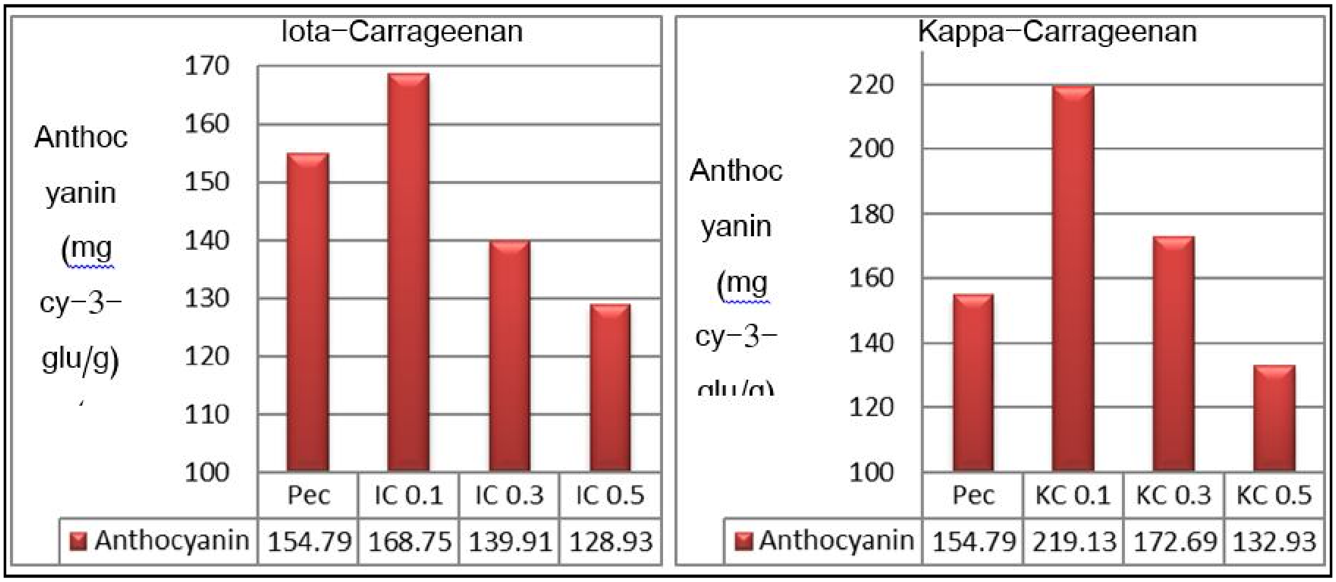

Fig. 4: Shows the difference in the values of anthocyanin between strawberry jam models for which two carrageenan (Kappa and Iota) are added to the control.

The major changes in chemical composition and quality that occur in the product are not only due to thermal treatment during manufacture, but after this process, due to the insufficient inhibition of the enzyme Polyphenol Oxidase (PPO), which has a role in the decomposition of anthocyanin and their content in the product is reduced (Martinsen, *et al.*, 2020; Aaby, *et al.*, 2018). Kukin (2018) stated that the color intensity (absorption) of different anthocyanin solutions decreases in the presence of amylose, amylopectin, alpha and beta-cyclodextrin while the presence of glucose, maltose and sucrose causes the color intensity to increase at pH 4 and these changes are noticeable in decreasing the pH to 2. And the effect of pectin the change in color intensity is minimal. Fig. 5 shows that the highest percentage of free radical restraint in the DPPH method in strawberry jam was for I car 0.5 g witch amounted to 60.15% and the lowest amount in the jam of K car 0.1 g which 58.52% compared to jam The control treatment witch amounted to 58.96%. The proportion of free radical inhibition of the DPPH method for the control treatment is consistent with its findings (Rodrigues, *et al.,* 2017).

Martinsen *et al.*, (2020) stated that the natural antioxidant activity in strawberry jam has not seen a

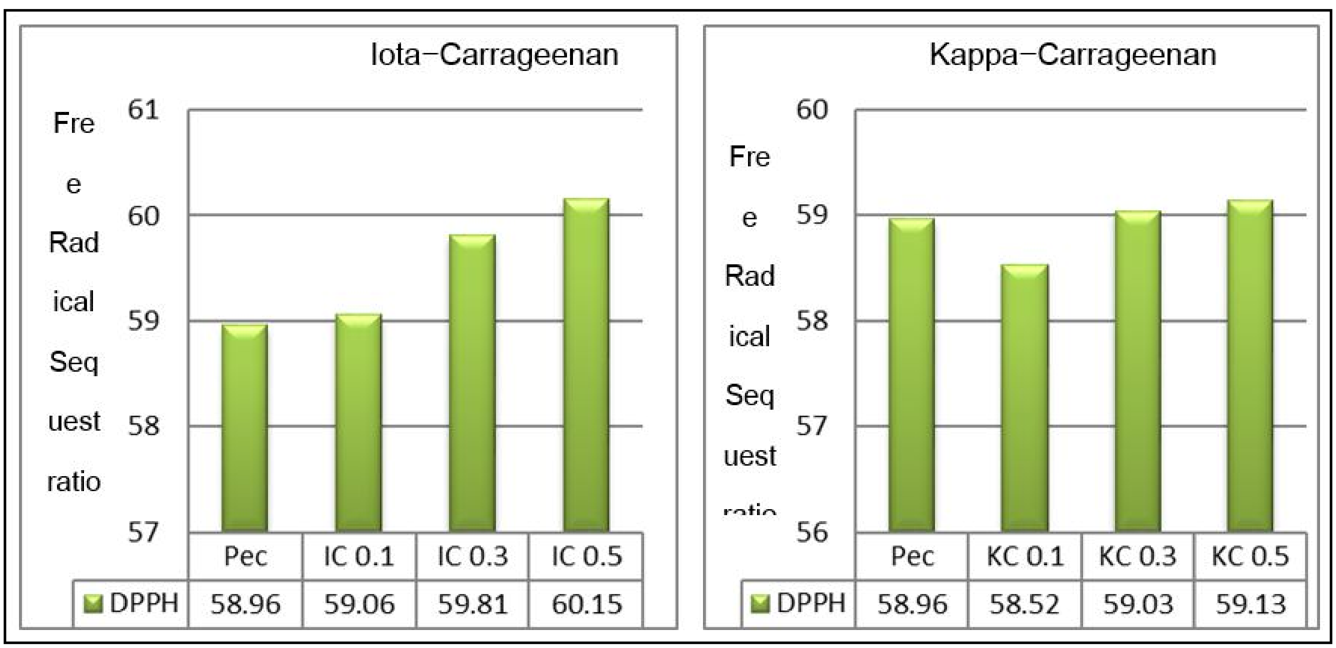

Fig. 5: Shows the percentage of free radical inhibition of DPPH method between strawberry jam models with two car types (K and I) for control model.

significant decrease due to the formation of post-industrial decomposition products that have a higher antioxidant capacity than the original phenolic compounds. Lafarga *et al.*, (2018) stated that the natural antioxidant capacity is closely related to total phenol and anthocyanin content. The fresh DPPH solution has a dark purple color that turns colorless when an antioxidant such as carrageenan is present in the medium by providing hydrogen atoms or by electronic donation (Suganya, *et al.,* 2016; Rafiquzzaman, *et al.,* 2015).

Microbiological Test

No viable numbers (bacteria, fungi, yeasts) were recorded in all strawberry jam models. This is due to the high sugar concentration that prevents the growth of microorganisms, and also indicates that the manufacturing process was good and this corresponds to what was described in it (Isah, 2017). Wang *et al.,* (2012) has shown that K car has a clear effect on yeasts, molds, and fungi that can lead to some problems in the product. In addition, K car also showed a clear inhibition of bacteria, I car has shown antibacterial activity as carrageenan is effective in producing antibacterial defense (Ramawat and Me´rillon, 2015).

Conclusions

The results obtained in this study showed the chemical structure of Carrageenan types and their concentration in the product have an effect on reducing and total sugars, phenols, anthocyanin and the ratio of free radical inhibition, the high concentration of sugar and low pH value in the product demonstrated the effective ability of carrageenan in producing antimicrobial defenses when added to food products within the permissible limits.

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