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Influence of Oregano Essential Oil Nanoemulsion Incorporated Alginate Active Coatings on Quality of Pork Patties

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Abstract: Edible coatings with sodium alginate incorporated with 1.5% oregano essential oil nano emulsion were coated onto pork patties and the patties had been analyzed at standard intervals of four days under refrigeration (4±1°C). The values of pH, Thiobarbituric acid values, tyrosine values, free fatty acid values and total plate counts of the coated pork patties were on favourable facade whilst compared to uncoated patties. The lipid oxidation changed into higher in uncoated patties when compared to the coated patties. The values of the above parameters changed considerably (p□ 0. 05) in the course of storage, but the rate of change turned into at a decrease level in essential oil incorporated formulations whilst in comparison to control formulation. They were well ideal up to 32 days of refrigerated storage. The sensory quality of the patties turned into excellent due to nano emulsion incorporation and the heightened tang of essential oil was masked by means of use of nanoemulsion.

Key words: Nano Emulsions · Edible Coatings · Oregano Essential Oil · Pork Patties

INTRODUCTION

To satisfy the developing need of common packaging materials and customer requests for sheltered and great quality food, new and novel edible grade packaging materials or advancements were to be created. Expanded enthusiasm towards the utilization of edible coatings was massively expanded because of worries about constrained regular assets of the petroleum derivatives and the ecological effect brought about by the utilization of plastic-based packaging materials which are non-biodegradable. Edible coatings and coatings have for some time been known to shield perishable food items from disintegration and decrease in quality. These coatings can act as barriers against moisture, gases and volatiles mass dispersion. Edible coatings can likewise act carriers for a wide scope of nourishment added substances, including seasoning specialists, cancer prevention agents and nutrients. Alginate is a winning compound on account of its non-harmfulness, biodegradability and low cost [1]. Its utilitarian properties like thickening, emulsion-settling, suspending and gel-creating capacity have been all around contemplated [2, 3]. Sodium alginate among different sorts of alginates can shape coatings with the good properties because of being water-dissoluble, solid, reflexive, bland, unscented, adaptable, low porous to oxygen and oils [4]. Essential Oils can effectively prevent oxidation and when coupled directly in to the food items may modify the taste. To stretch out the time span of usability and to shield the item from oxidation and deterioration, Essential oils can be combined in to the edible coatings. Under these conditions the oils could be gradually discharged on to nourishment surface and can stay in adequate fixation for broadening the item timeframe of realistic usability. Nano emulsions are colloidal scatterings framed by blend of two immiscible stages balanced out by a surfactant, with oil beads of size in the scope of 10-100nms. They are optically clear and contrasted with ordinary emulsions. This component is of favourable position for nourishment applications.

Essential oils (EOs) are characteristic mixes from plants that show antimicrobial and cancer prevention properties and in this manner draw an enthusiasm as

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added substances in the nourishment applications. EOs from oregano (*Origanum vulgare L.*) and cinnamon (*Cinnamomum verum*) have potential as regular functional cell reinforcements. Numerous EOs are viewed as 'Generally Recognized as Safe' (GRAS) and endorsed by the Food and Drug Administration (FDA). The utilization and choice of EOs ought to consider the customer perceptible merit of the last item. In reality, because of their solid flavor, their immediate use is regularly constrained. To defeat this issue, EOs can be included into palatable coatings, which have been proposed as an active food packaging to improve hygiene and quality. Alginate can be cross-linked by the expansion of divalent particles, for example, Ca^{2+}, to shape solid gels and coatings. These coatings can be utilized to keep up the quality and drag out the time span of usability of foods by diminishing lipid oxidation, limiting contact with oxygen and expanding water barrier properties of the food enclosed. In veracity, there is extraordinary enthusiasm for palatable coatings because of their biocompatibility, biodegradability, wide application potential and use as transporters of practical fixings.

With this in the present test, an endeavour was made to create sodium alginate based edible coatings incorporated with essential oil nanoemulsions i.e., oregano and cinnamon and to assess their impact on the nature of pork patties. In view of the outcomes acquired, best coated product was chosen and the product was evaluated for quality and shelf life under refrigeration (4±1°C) temperature at regular intervals to evidence the impact of nanoemulsions of essential oil incorporated sodium alginate coatings on nature of pork patties and to record the viability of the coating as active packaging.

MATERIALS AND METHODS

Collection of Raw Materials: Raw materials like Essential oils i.e. Oregano Essential oil and Cinnamon Essential oil were purchased from Kamco pharmaceuticals, Hyderabad. Food grade common salt (Tata Chemicals, Mumbai) and sunflower oil (Freedom refined) required for preparation of pork patties were purchased from local market. Food grade Glycerol (vegetable Glycerine kosher usp food grade), Tween 80 (food grade) and sodium alginate (low viscosity) was procured from Loba chem. Pvt. Ltd. (Mumbai).

The examination was focussed in two sections. Oregano and cinnamon essential oils were made into nanoemulsions and they were incorporated in to sodium alginate based coatings to prepare active coatings. Their quality was assessed by coating on meat product and the best coated product was evaluated for quality. The proposed examination was carried out in the Department of Livestock Products Technology, N.T.R college of Veterinary Science, Gannavaram andhra Pradesh.

Oregano essential oil (OEO) and Cinnamon essential oil (CEO) each at 1.5 per cent were selected for using in sodium alginate based films to produce active packaging films. Coarse emulsions of above essential oils were prepared initially. Coarse emulsions were formed by slow and continuous addition of OEO and CEO each separately at 1ml V/V with distilled water. Non ionic surfactant i.e., tween 80 was added in to the above formulations at 1.5 percent level. The emulsion was subjected for stirring continuously on a magnetic stirrer (SPINOT 6030) at 3000 rpm with slow addition of essential oils and tween 80. The formed coarse emulsion was then subjected to ultrasonication (Qsonica, Q500, USA) at 20 KHZ, 200 watts 20mm diameter probe for 5minutes. The temperature of the process was controlled at less than 10°C until formation of nanoemulsions of oregano essential oil (OEON) and Cinnamon essential oil (CEON).

Coating solutions were prepared by addition of sodium alginate at 2 percent w/v level into distilled water. The solution was subjected to a temperature of 90°C for allowing gelatinization. Glycerol at 4 per cent level was added to the solution as plasticizer. After the temperature was cooled to 37°C the nano emulsions at a level of 150 micro litres each separately were added to the alginate solution to produce coating solutions with 1.5 percent essential oil concentrations. Three types of edible coatings were produced, i.e., without additives (C) and with incorporation of 1.5 % Oregano (T1) and 1.5% Cinnamon (T2). Pork patties were prepared and they were coated with coating solutions. Pork patties were dipped in the coating solutions for 1 min and then, they were drained of excess solution for 30 s followed by dipping in 2% aqueous calcium chloride solution for 30s. Coated patties were kept in hot air oven at 40°C for 30 min for the efficient casting of coating over the patties. The coated patties with SA and oregano (T1) and SA and cinnamon (T2) were subjected to sensory evaluation and the results were analysed [5]. The patties with best sensory quality were packaged along with uncoated patties as control (C) in low-density polyethylene

covers. They were labelled and stored at refrigeration temperature (4±1°C). The products were analyzed at regular intervals of 4 days for physico-chemical, sensory and microbiological qualities. pH of the preparation was estimated by following the method of Trout *et al*. [6] using a digital pH meter of (Oakton Instruments, USA). 2-thiobarbituric acid reactive substances (TBARS) were estimated through the distillation method outlined by Lawlor *et al*., [7]. Free Fatty Acid values were estimated by following the method of Koniecko *et al*. [8]. Tyrosine value was measured according to the method of Strange *et al*. [9]. For microbiological analysis, the method outlined by APHA [10] was followed. Sensory evaluation was done by subjecting the cooked patties to a six-member taste panel to evaluate colour, appearance, flavor, juiciness, tenderness and overall acceptability on a 9 point hedonic scale [5].

Statistical Analysis: The data was analyzed with SPSS version 22.0 and entire experiment was repeated six times to reduce the standard error.

RESULTS AND DISCUSSION

The results obtained in evaluating the effect of edible polymer coatings of sodium alginate with natural spice oils like oregano oil and cinnamon oil on quality of pork patties were presented below.

The pork patties coated with sodium alginate coatings incorporated with oregano oil had higher sensory quality than cinnamon oil incorporated coatings and control. These findings were in agreement with Chidanandaiah *et al*., [11].

The oregano oil incorporated sodium alginate coated patties were selected for further studies to evaluate the quality and shelf life of patties keeping in view of their high sensory quality compared to cinnamon incorporated patties. The flavor of cinnamon oil nano emulsion incorporated patties was not acceptable.

Under refrigerated storage (4±1°C) temperature, there was significant difference (P<0.05) between the values of pH, TBARS, tyrosine values and free fatty acid values of coated and uncoated pork patties. The patties coated with sodium alginate coating incorporated with nano emulsions of oregano oil (T) were significantly (P<0.05) lower in their values when compared to control. During storage, irrespective of the formulations, the pH values increased significantly (P<0.05) under refrigerated storage

Table 1: Sensory attributes of pork patties coated with different essential oils on 0th day

Sensory attributes	Oregano Essential Oil (OEE)	Cinnamon Essential Oil (CEE)
Colour	8.01±0.09	7.00±0.05
Flavour	8.00±0.08	6.92±0.09
Juiciness	7.86±0.07	6.85±0.07
Tenderness	7.90±0.08	6.18±0.09
Overall acceptability	7.76±0.03	6.23±0.04

temperature. The lower pH values in coated patties might be due the effect of phenolic compounds such as flavonoids and phenolic acids present in natural spice oils, which exhibit a wide range of antimicrobial effect [12, 13], thereby preventing the bacterial action and concomitant increase in pH. The increase in pH during storage period is due to the accumulation of metabolites by bacterial action in meat and de-amination of proteins [14]. The results were in accordance with the result of Yongling *et al*., [15] who found lower pH values in coated products.

The values of coated sample recorded significantly (P<0.05) lower TBARS values indicating the efficacy of essential oil in inhibiting the lipid oxidation. Lipid oxidation can be initiated and accelerated by various mechanisms including the production of singlet oxygen, enzymatic and non enzymatic generation of free radicals and active oxygen [16]. The antioxidant activity of oregano oil might be due to its richness in isoprenoid quinones, which acted as chain terminators of free radicals and as chelators of reactive oxygen species (ROS). In addition, the phenolic compounds existing in the commercial extracts of rosemary acted as primary antioxidants when reacting with the lipid and hydroxyl radicals to turn them into stable products [17]. These compounds could act as metal ion chelators (Fe+2 fundamentally), therefore reducing the formation ratio of the reactive species derived from oxygen [18]. Carnosic acid and carnosol might act as potent scavengers of peroxyl radicals [19]. The sodium alginate based film layers on the surface of the product might have resisted oxygen diffusion and might have retarded lipid oxidation. These results were in accordance with Wu [20], Chidanandaiah *et al.* [11], Shon *et al.* [21].

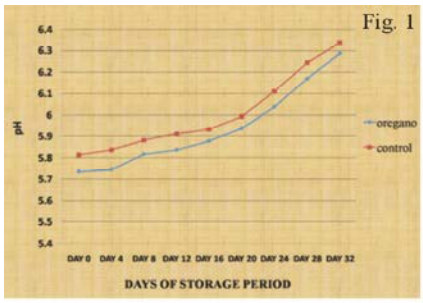
The TBARS values of treatment along with control were increased continuously during storage period. This observation was similar to the results of Yongling *et al.* [15], Lee *et al.* [22], Shon and Chin [23]. Increase in TBARS values in general during storage might be attributed to increased oxidation of unsaturated fatty acids.

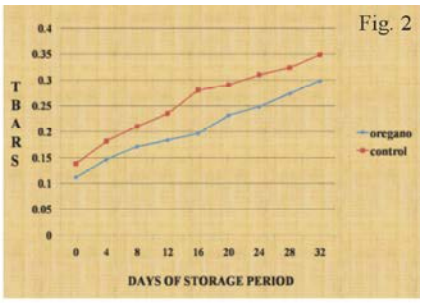
Tyrosine Value: In present study, pork patties with rosemary essential oil incorporated coating had significantly (P<0.01) lower tyrosine values (mg/100 g of product) compared to control This might be due to lower proteolysis of meat proteins and delayed spoilage due to the anti microbial activity of rosemary by the action of rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic acid, rosmanol and isorosmanol. Coating with nano emulsion of essential oil resulted in slow and sustained release. Under refrigerated storage the tyrosine values increased significantly (P<0.01). This could be attributed to hydrolytic changes in meat due to inherent tissue enzymes and bacterial proteolysis [9]. The results of the present study were collated with the observations of Biswas *et al.* [24], Naga Mallika *et al*., [25] and in pork sausages Khare *et al*., [26] in chicken fillets using chitosan and cinnamon oil edible coating under refrigeration conditions.

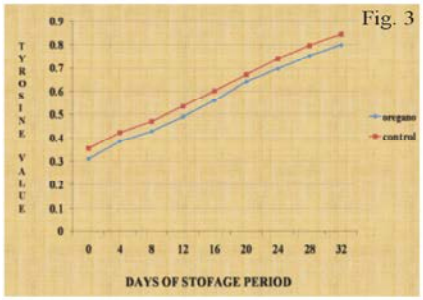
Free Fatty Acids: The Free fatty acid content of pork patties coated with nanoemulsion of rosemary essential oil incorporated sodium alginate were significantly (P<0.01) lower than control. The lower free fatty acid content in coated patties might be attributed to anti oxidant and anti microbial properties of various active principles of rosemary essential oil. The microbial lipolysis was mainly inhibited by anti microbial activity of lactone, carnosol, rosmarinic acid and hesperidin in rosemary essential oil [27]. These results were in accordance with those of Ozogul *et al*. [28] in rosemary nano emulsion added rainbow fillets, Yazgan [29] in sea bream and sea bass fillets Ozogul *et al.* [30] for wild rainbow trout and Ibrahim *et al*. [31] in Clove Essential Oil on cakes. With the progress of refrigerated storage, there was significant (P<0.01) increase in FFA values. This might be due to microbial lipolytic activities. The results were in accordance with Ucak *et al*. [32] in atlantics mackerel fish burgers added with rosemary extract and Kodal coskun *et al*. [33] in ground beef patties added with oregano essential oil.

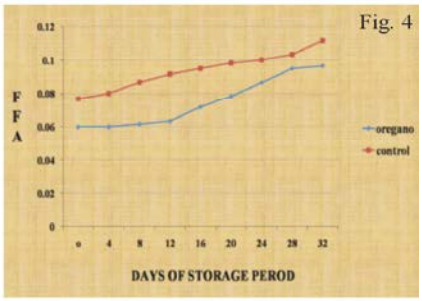
Total Plate Count: Total plate counts (TPC) were significantly (P<0.05) lower in coated samples in comparison to the control samples. This might be due to the ability of alginate to produce strong gels and due to the antimicrobial activity of oregano oil which can reduce microorganism infiltration into the coated samples. These coatings can also retard microbial growth by lowering the water activity within the coating, thereby reducing drip loss of meat products and binding water that otherwise would be available for microbial growth [34]. During storage the total plate counts increased with increasing storage period. However lower counts were noticed in treatment samples in comparison with controls. Unacceptable TPC values were found in control on day 16 under refrigerated storage temperature. However there was an increase in the shelf-life of 12 days at refrigerated storage temperature with the treatment. This improvement in microbial quality might be due to the effect of phenolic compounds that are present in essential oil which were proved to possess certain antimicrobial activity *in vitro.* The results indicated that active ingredients calvacrol and thymol exhibited strong anti microbial activity. Thus it can be stated that the usage of plant based natural spice oils and antimicrobials can be alternatives for chemicals used in food preservation. These results were in agreement with the reports of Chidanandaiah *et al.* [11], Yongling *et al*. [15] and Siripatrawan and Noipha [35], Naga mallika *et al*. [36] who found lower values in coated products and with Shaaban *et al*. [37] who found good antimicrobial activity of essential oils against gram-positive bacteria (*S. aureus and L. monocytogenes*).

Sensory Evaluation: The sensory characteristics of the coated patties were significantly higher when compared to the controls. High flavour scores might be due to specific optimal concentration of volatiles and phenolic content of rosemary essential oil [38]. The colour higher scores in treatment groups would be due to the properties of polysaccharide films in preventing dehydration, oxidative rancidity and surface browning. The overall mean tenderness and juiciness scores of coated pork patties were significantly higher than uncoated pork patties. This might be due to retention of more moisture in the coated product. In addition to this, prevention of tissue dehydration by myofibril denaturation and hydrophilic nature of corn starch molecules may also aid in increased juiciness and tenderness of the pork patties. Superior scoring in respective of colour, flavour, tenderness and juiciness had reflected in higher overall acceptability scores for the coated formulation. These sensory results were in accordance with Chidanandaiah *et al.* [11], Yongling *et al.* [15] and Siripatrawan and Noipha [35] and Sutha *et al.* [39].









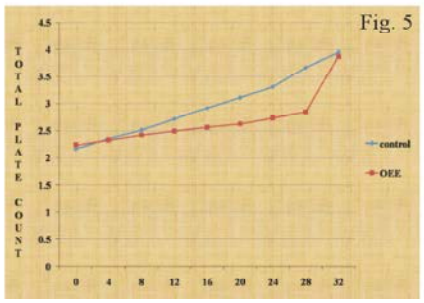


Fig.1: pH values of coated and uncoated pork patties
Fig. 2: TBARS values of coated and uncoated pork patties
Fig. 3: Tyrosine Value values of coated and uncoated pork patties
Fig. 4: Free Fatty acids values of coated and uncoated pork patties
Fig. 5: Total Plate Count Values of coated and uncoated pork patties

Table 2: Quality and Sensory attributes of coated and uncoated Pork Patties

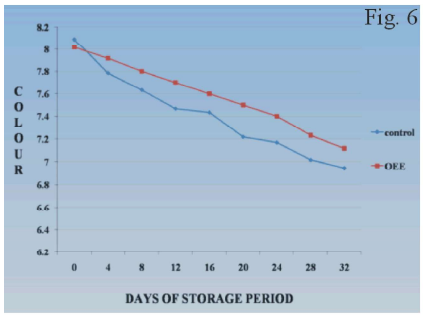
	Days of Storage								
Treatments	DAY 0	DAY 4*	DAY 8*	DAY 12*	DAY 16*	DAY20*	DAY 24*	DAY 28*	DAY 32*
pH									
CONTROL	5.81±0.007	5.83±0.01	5.88±0.01	5.91±0.01	5.93±0.008	5.99±0.01	6.11±0.009	6.24±0.008	6.33±0.009
OEE	5.78±0.009	5.74±0.008	5.81±0.008	5.83±0.009	5.87±0.01	5. 93±0.008	6.03±0.01	6.17±0.009	6.28±0.008
TBARS									
CONTROL	0.13±0.008	0.18±0.009	0.21±0.01	0.23±0.009	0.28±0.01	0.29±0.009	0.31±0.007	0.32±0.007	0.34±0.008
OEE	0.11±0.009	0.14±0.009	0.17±0.008	0.18±0.007	0.19±0.01	0.23±0.009	0.24±0.008	0.27±0.008	0.28±0.009
TYROSINE VALUE									
CONTROL	0.34±0.008	0.42±0.007	0.47±0.008	0.53±0.008	0.60±0.008	0.67±0.008	0.74±0.008	0.79±0.008	0.84±0.008
OEE	0.31±0.009	0.38±0.007	0.42±0.009	0.49±0.008	0.56±0.008	0.64±0.009	0.69±0.008	0.75±0.008	0.79±0.01
FREE FATTYACIDS									
CONTROL	0.070±0.007	0.080±0.008	0.086±0.006	0.091±0.006	0.095±0.007	0.098±0.006	0.100±0.005	0.103±0.007	0.111±0.009
OEE	0.06±0.005	0.060±0.008	0.061±0.009	0.063±0.008	0.071±0.008	0.078±0.008	0.086±0.008	0.095±0.008	0.096±0.008
TOTAL PLATE COUNT									
CONTROL	2.23±0.01	2.32±0.009	2.42±0.01	2.48±0.008	2.55±0.009	2.62±0.009	2.73±0.008	2.83±0.008	3.95±0.008
OEE	2.16±0.01	2.34±0.01	2.50±0.01	2.71±0.01	2.91±0.007	3.10±0.007	3.30±0.008	3.67±0.008	3.87±0.007

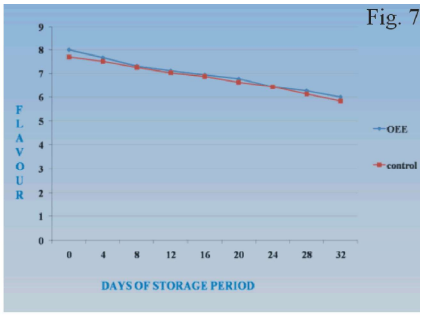
Note: (*) means significant difference between treatments (P<0.05), NS-non significant.

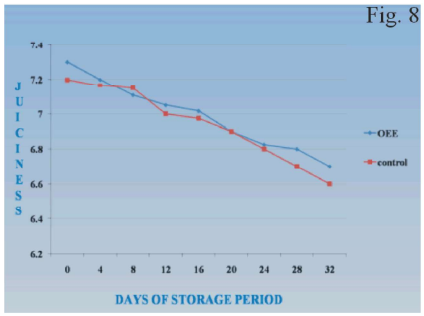
Table 3: Sensory attributes of pork patties coated with edible polymer coatings of sodium alginate with natural spice oils like oregano oil and cinnamon oil

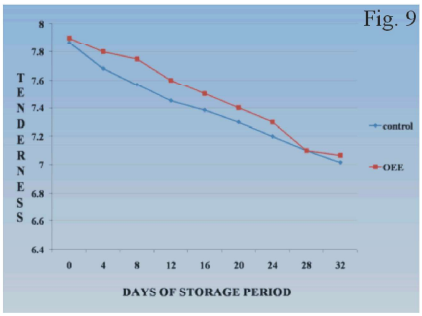
	Days of Storage								
Treatments	DAY 0	DAY 4*	DAY 8*	DAY 12*	DAY 16*	DAY20*	DAY 24*	DAY 28*	DAY 32*
COLOUR									
OEE	8.01±0.09	7.91±0.07	7.80±0.05	7.70±0.05	7.60±0.05	7.50±0.05	7.40±0.05	7.23±0.07	7.11±0.07
CONTROL	8.09±0.09	7.78±0.07	7.63±0.07	7.46±0.07	7.43±0.07	7.21±0.09	7.16±0.08	7.01±0.09	6.94±0.08
FLAVOUR									
CONTROL	7.70±0.05	7.510±0.07	7.26±0.07	7.03±0.07	6.88±0.08	6.63±0.07	6.43±0.07	6.13±0.07	5.83±0.07
OEE	8.00±0.08	7.66±0.08	7.31±0.07	7.11±0.07	6.95±0.08	6.78±0.10	6.43±0.07	6.26±0.07	6.00±0.08
TENDERNESS									
CONTROL	7.90±0.08	7.68±0.08	7.56±0.07	7.45±0.07	7.38±0.07	7.30±0.09	7.20±0.09	7.10±0.09	7.01±0.1
OEE	7.86±0.07	7.80±0.08	7.75±0.09	7.60±0.1	7.50±0.1	7.40±0.1	7.30±0.1	7.10±0.1	7.06±0.08
JUICINESS									
CONTROL	7.20±0.05	7.16±0.08	7.15±0.07	7.00±0.05	6.97±0.04	6.900±0.05	6.80±0.05	6.70±0.05	6.60±0.05
OEE	7.30±0.05	7.20±0.05	7.10±0.05	7.05±0.07	7.01±0.07	6.90±0.05	6.82±0.07	6.80±0.05	6.60±0.05
OVERALL ACCEPTIBILITY									
OEE	7.70±0.03	7.55±0.04	7.46±0.04	7.33±0.04	7.16±0.04	7.06±0.06	6.91±0.05	6.70±0.05	6.58±0.04
CONTROL	7.76±0.03	7.41±0.06	7.30±0.06	7.18±0.05	7.11±0.04	6.98±0.04	6.81±0.06	6.68±0.06	6.56±0.04

Note: (*) means significant difference between treatments (P<0.05), NS-non significant.









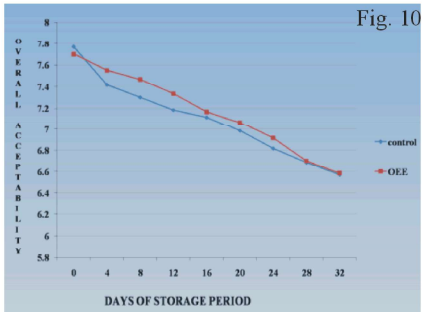


Fig. 6: Colour values of pork patties coated with oregano and cinnamon essential oils along with control
Fig. 7: Flavour values of pork patties coated with oregano and cinnamon essential oils along with control
Fig. 8: Juiciness values of pork patties coated with oregano and cinnamon essential oils along with control
Fig. 9: Tenderness values of pork patties coated with oregano and cinnamon essential oils along with control
Fig. 10: Overall acceptability values of pork patties coated with oregano and cinnamon essential oils along with control.

CONCLUSION

The current delve into showed that oregano essential oil may be successfully included into alginate coatings as nanoemulsion and could hold super antioxidant and antimicrobial activities as a part of active packaging. Outcomes of the investigation endorsed capacity of application of coatings within the form of nanoemulsions as antioxidant and antimicrobial ingredient providers to extend the shelf-life of the lined product. In this milieu it could be advised that use of nanoemulsions instead of coarse emulsions can discover new horizons in product processing and shelf lifestyles.

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