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Application of simplex lattice mixture design for optimization of active modified atmosphere for pomegranate arils (cv. Wonderful) based on microbial criteria

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ABSTRACT

Despite the advantages of low O_2 and enriched CO_2 for extending the storage life of pomegranate arils, limited studies on systematic selection and optimization of gasses to reduce the microbial growth during storage have been reported. Therefore, this study was undertaken to optimize the gas composition for cold storage (10 °C) of pomegranate arils (cv. Wonderful) based on microbiological criteria. Selecting experimental points and optimizing gas concentration were carried out according to the simplex lattice mixture design with three factors (O_2, CO_2 and N_2). Seven gas combinations including low O_2 (2–18 kPa), enriched and high CO_2 (2–18 kPa) and 80–96 kPa N_2 were used in varying concentrations and the bacterial, yeast and mould growth on arils were analysed. Aerobic mesophilic bacteria growth varies between 3.95–5.89 log CFU g^{-1} and yeast growth was between 3.84–5.91 log CFU g^{-1}, whereas, mould growth was between 2.15–3.63 log CFU g^{-1} across the modified atmospheres. Data from these analyses were used to fit linear and cubical polynomial models. Pareto analysis and ternary contour plots showed that the main effects (CO_2, N_2 and O_2) as well as their interaction had significant effects on microbial growth. For all microbial criteria's, the lowest growth was tend to move to the highest CO_2 in the modified atmosphere system. On the contrary, the presence of higher O_2 concentration stimulated the growth. This was evident that, the gas mixture containing 12.67–18 kPa CO_2, 2–4.67 kPa O_2 and 80 − 82.67 kPa N_2 significantly reduced microbial count on pomegranate arils. Validation of the model showed that the cubical model predicted the microbial counts effectively with high correlation coefficients of $R^2 > 94\%$ for mould and > 99% for aerobic mesophilic bacteria and yeast, whereas, the linear model overestimated bacteria and yeast counts and underestimated mould count.

1. Introduction

Minimally processed ready-to-eat pomegranate arils packed in modified atmosphere packaging have become very popular due to its convenience, good sensory attributes and health benefits (Martínez-Romero et al., 2013). Since minimally processed arils easily loose quality together with an increase in microbial spoilage and decay, maintaining the quality has become a major challenge (O'Grady, Sigge, Caleb, & Opara, 2014). According to López-Rubira, Conesa, Allende, and Artés (2005) and Maghoumi et al. (2013), microbial growth is one of the main problems affecting the overall quality of minimally processed pomegranate arils, which commonly determines acceptability and shelf life. In order to overcome this limitation, the objective of some modified atmosphere systems can be to create an unsuitable atmosphere for microbial growth by generating sufficiently low O_2 and enriched CO_2. An atmosphere of 3–6 kPa of O_2 and 2–10 kPa CO_2 was shown to achieve microbial control and extend the shelf life of a variety of fresh cut products (Oliveira et al., 2015). The effect of active modified atmosphere on the microbial safety of pomegranate arils has not been extensively studied. In the few reported studies, similar experimental approaches have been used to determine the inhibitory effect of active modified atmospheres (; López-Rubira et al., 2005; Martínez-

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Romero et al., 2013). The reports from these studies showed that the effect of modified atmosphere in inhibiting microbial growth was influenced by establishing optimum gas composition.

Furthermore, several studies have associated the potential microbial inhibitory effect of modified atmosphere (MA) with either the impact of low O_2 or critically high CO_2. Allende, Luo, McEvoy, Artés, and Wang (2004) reported that higher concentration of CO_2 played a major role in inhibiting the presence of microorganisms in minimally processed baby spinach leaves under super atmospheric and MA conditions. Belay, Caleb, and Opara (2017) found out a significant reduction of aerobic mesophilic bacteria, yeast and mould using super atmospheric O_2 condition. Similarly, Oliveira et al. (2015) described CO_2 as the only gas under MA that has a direct and significant antimicrobial effect. However, the inhibitory effect of CO_2 was suggested to be dependent on the microbial flora present and produce characteristics (Caleb, Opara, & Witthuhn, 2012). On the other hand, the response of microbial growth to O_2 levels varies considerably in literature. For instance, Ayhan and Eştürk (2009) reported that O_2 concentration affected on the growth of aerobic and anaerobic bacteria. Similarly, a reduction in aerobic psychrotrophic growth in chicory endives was attributed to high O_2 concentration (Jacxsens, Devlieghere, Van der Steen, & Debevere, 2001).

In recent studies the use of simplex lattice design approach to select a components and to optimize gas concentration on mixture experiments has been reported (Mahajan, Luca, & Edelenbos, 2014; Hron and Macak, 2015; Wyrwisz et al., 2016). This approach enables better understanding of how the individual components or their interaction affect a response variable by using a simple interactive model coefficients and ternary graphs (Azevedo, Cunha, Mahajan, & Fonseca, 2011; Karaman, Yilmaz, & Kayacier, 2011; Kayacier, Yüksel, & Karaman, 2014; Yilmaz et al., 2015). However, no study has been reported for pomegranate arils on selection or optimization of gasses using simplex lattice design approach. Therefore, the objective of this study was to determine the optimal gas combination for storage of pomegranate arils based on the microbial criteria (aerobic mesophilic bacteria, yeast and mould). This objective was achieved by designing experimental points using three gas components (O_2, CO_2 and N_2), followed by the optimization of gas proportion based on simplex lattice mixture design approach.

2. Materials and methods

2.1. Sample preparation and experimental design

Pomegranate fruit were obtained at commercially ripened stage with characteristic deep-red skin and arils with mature kernel (Crisosto, Mitcham, & Kader, 2001), from Sonlia pack house, Wellington, Western Cape (33°38′23″S, 19°00′40″E), South Africa. Fruit were air freighted and delivered in well-ventilated boxes at cool temperature to Department of Horticultural Engineering, Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Germany. On arrival, the fruit were stored in a cold storage chamber (5 °C) until processing to extract the arils. Damaged fruit was removed and the outer skin of selected healthy fruit was surface disinfected using 70% ethanol (Belay et al., 2017). Arils were extracted manually by carefully removing the husk. Extracted arils were collected in a pre-sterilized tray (using 70% ethanol) and mixed to assure uniformity.

Simplex lattice design was used to determine the number of experimental runs and the proportion of the gasses in each experiment (Table 1). In this study, three gas components (CO_2 (2–18 kPa), O_2 (2–18 kPa) and N_2 (80–96 kPa)) were used by changing their concentrations simultaneously and keeping the total concentration constant (100 kPa). Simplex lattice design for a three component system is represented by an equilateral triangle in two dimensional spaces. For this study a {3, 3} simplex lattice design resulted in 7 experimental design points, of 3 runs with a single component at the vertex, 3 runs with 3 components at the augmented points and 1 run with 3 components at the centre point were used. All modified atmospheres selected by using simplex lattice design were evaluated for total aerobic mesophilic bacteria, yeast and mould counts. Closed system was used to create a modified atmosphere. Arils (350 g) were transferred to 4000 mL air-tight glass jars which were designed to achieve a completely hermetic seal and stored at 10 °C. This storage temperature was selected to simulate the average supermarket shelf or retail conditions. Each jar lid had three valves (inlet, out late and a gas sampling port). A rubber ring was fixed between the bottle and the lid seal to prevent air leakage. A plastic tube was attached to the inlet valve, which was inserted down to the bottom of the jar to ensure uniform flushing of gas mixture. Each storage chambers was flushed with humidified air with gas mixture according to Table 1 until equilibrium archived. The changes in O_2/CO_2 concentrations inside the headspace of the glass jars were monitored over a period of 8 d storage using gas analyser (Checkmate 3, PBI Dansensor, Ringstead, Denmark).

Table 1
Simplex lattice experimental design layout for different gas mixtures.

Gas mixture	Uncoded variables (kPa)			Pseudo components		
	O_2	CO_2	N_2	O_2	CO_2	N_2
MA-1	18	2	80	1	0	0
MA-2	2	18	80	0	1	0
MA-3	2	2	96	0	0	1
MA-4	4.67	12.67	82.67	⅙	⅔	⅙
MA-5	4.67	4.67	90.67	⅙	⅙	⅔
MA-6	12.67	4.67	82.67	⅔	⅙	⅙
MA-7	7.33	7.33	85.33	⅓	⅓	⅓

2.2. Microbial analysis

Microbial quality of pomegranate arils was studied according to the method described by Caleb, Ilte, Fröhling, Geyer, and Mahajan (2016) using total plate count method. Total aerobic mesophilic bacterial count was determined using plate count agar (PCA), while yeast and mould counts were determined using rosebengal chloramphenicol agar (RBCA). Sample (10 g) of pomegranate arils was taken for each treatment and transferred into 90 mL peptone buffered water. Samples were homogenized for 2 min at speed 4 strokes s^{-1} in a lab blender (BagMixer1400CC1, Interscience, France). Thereafter, a ten-fold serial dilution up 10^{-3} was made after adding 30 µL of each diluent into 270 µL of PS Rotilabo1-microtest plates (96er U-profile, Carl Roth GmbH & Co KG, Germany) and 100 µL from each dilution was pour-plated on respective growth media. All analyses were done in duplicate for each package (n = 7 per treatment). PCA plates for aerobic mesophilic bacterial were incubated at 30 °C for 72 h, and RBCA plate for yeast and mould, respectively, were incubated at 25 °C for 5 d. After incubation, colonies (between 30 and 300 colonies) on each plate were counted, and the results were expressed as log colony forming unit per weight (log CFU g^{-1}).

2.3. Mathematical modelling

The results from the experiment data were further used to fit the linear model (Eq. (1)). However, once the significant effect of a single gas component (CO_2, O_2, and N_2) is known, design points can be increased in order to minimize the variance of the first order estimated parameters in the model to increase the precision of the estimated parameters in the second order model (Mahajan et al., 2014). Therefore, a cubical model (Eq. (2)) with interaction terms (binary and tertiary terms) was fitted to the experimental data.

$$Y = \beta_1 O_2 + \beta_2 CO_2 + \beta_3 N_2 \quad (1)$$

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